Journal of Inflammation Research

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Identification of Necroptosis and Immune Infiltration in Heart Failure Through Bioinformatics Analysis

Yuanting Zhu[1,*], Qiang Zhang[2,*], Yanbo Wang[1,*], Wenqiang Liu[3], Shichen Zeng[4], Qinghua Yuan[1], Kun Zhang[1]

[1]Department of Cardiology, The Seventh Affiliated Hospital of Sun Yat-sen University, Shenzhen, People's Republic of China; [2]Department of Emergency Medicine, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, People's Republic of China; [3]Digestive Diseases Center, The Seventh Affiliated Hospital, Sun Yat-sen University, Shenzhen, People's Republic of China; [4]Clinical Medicine, Changsha Medical University, Changsha, People's Republic of China

*These authors contributed equally to this work

Correspondence: Kun Zhang; Qinghua Yuan, Department of Cardiology, The Seventh Affiliated Hospital of Sun Yat-sen University, Shenzhen, People's Republic of China, Email zhangk65@mail.sysu.edu.cn; yuanqinghua@sysush.com

Purpose: Heart failure (HF) remains a leading cause of mortality and morbidity in cardiovascular disease. Research has shown that necroptosis contributes to HF, and immune infiltration has been reported to be implicated in HF. However, the specific mechanisms by which necroptosis and immune infiltration promote HF remain poorly understood. This study aims to elucidate these mechanisms, thereby providing new insights for future therapeutic strategies.

Methods and Results: In the GSE21610 dataset, there were 1848 differentially expressed genes (DEGs), 14 of which related to necroptosis (NRDEGs) in HF. Gene Set Enrichment Analysis (GSEA) indicated that Th1 and Th2 cell differentiation, TGF-beta signaling, Renin secretion, and Wnt signaling pathways may be closely associated with HF. The NRDEGs may play a role in responding to mechanical stimuli, membrane rafts, cytokine receptor binding, or the necroptosis signaling pathway. The protein-protein interaction (PPI) network identified EGFR, TXN, FASLG, MAPK14, and CASP8 as hub NRDEGs. Furthermore, immune infiltration analysis of CIBERSORT algorithm suggested that M2 macrophages, memory B cells, monocytes, regulatory T cells (Tregs), follicular helper T cells, and gamma delta T cells may participate in the development of HF. The hub NRDEGs, including EGFR, FASLG, and TXN, exhibited significant correlations with various immune cell types. Finally, animal models confirmed that in the HF group, EGFR and FASLG were up-regulated, while TXN was down-regulated.

Conclusion: The present findings demonstrate that necroptosis and immune infiltration are associated with the development of HF. This study provides valuable insights and recommendations for the clinical management of HF.

Keywords: heart failure, differentially expressed genes, necroptosis, immune infiltration

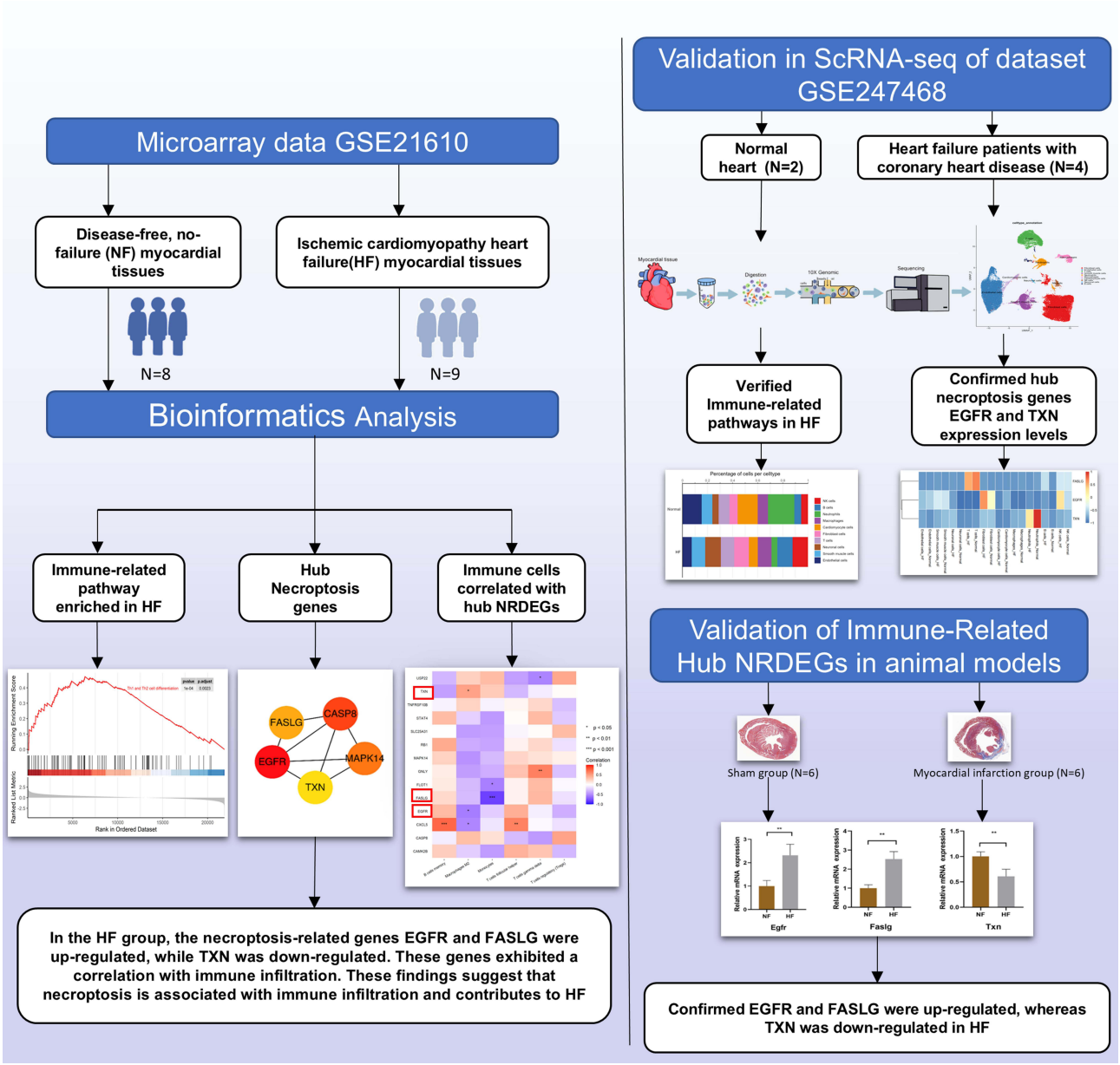
Introduction

Heart failure (HF) is an increasingly significant public health issue, currently affecting over 37.7 million individuals worldwide.[1] Lifestyle changes have contributed to the rising incidence of HF, which is associated with a five-year all-cause mortality rate of approximately 53%, leading to a considerable economic burden on individuals.[2] Therefore, it is crucial to implement novel strategies for the prevention and treatment of HF to reduce its morbidity, hospitalization, and mortality rates.

Ischemic cardiac injury induces an inflammatory response and the release of damage-associated molecular patterns (DAMPs), which subsequently activate the immune cells.[3] This inflammation-immune response can lead to the secretion of cytokines and growth factors that are crucial in regulating oxidative stress, fibrosis, scarring and matrix remodeling,

Received: 9 November 2024
Accepted: 10 February 2025
Published: 19 February 2025

Graphical Abstract



ultimately leading to HF.[4] Previous research has demonstrated that, M1 macrophages, monocytes, and natural killer (NK) cells are activated in the HF patients, highlighting the role of immune infiltration in heart failure.[5]

Necroptosis is a novel form of cell death distinguished by its pronounced inflammatory characteristics.[6] Death receptors mediate the formation of the necrosome, which plays a crucial role in initiating necroptosis. The core components of the necrosome are receptor-interacting serine/threonine kinase 1 (RIPK1) and receptor-interacting serine/threonine kinase 3 (RIPK3).[7] Within the necrosome, RIPK1 undergoes autophosphorylation and interacts with RIPK3, which subsequently phosphorylates mixed lineage kinase domain-like (MLKL) proteins.[8] This process leads to the oligomerization of MLKL and its translocation to the plasma membrane, ultimately causing membrane rupture and the destruction of cellular structure.[9] In the myocardial infarction induced HF models, RIP1/RIP3/MLKL pathway was

activated, suggesting that necroptosis is involved in the pathophysiology of HF.[10] Elevated levels of necroptosis markers (p-RIP1, p-RIP3, p-MLKL) were observed in doxorubicin (DOX)-induced HF, and treatment with the Nox2 inhibitor GSK2795039 reduced necroptosis, improving cardiac remodeling and function.[11] Similarly, failing hearts from HF patients exhibited increased staining for RIP1 and RIP3 compared to controls, confirming the activation of necroptosis in HF.[12] Additionally, hydrogen sulfide was found to suppressed RIP1/RIP3/MLKL-mediated necroptosis, providing protection against ischemic HF.[13] Furthermore, necroptosis was activated after apoptosis during the later stages of diabetes and was functionally responsible for cardiac dysfunction. Injection of Nec-1 significantly preserved left ventricular end-diastolic diameter, left ventricular end-systolic diameter, ejection fraction, and fractional shortening, while also attenuating heart pathology.[14] These findings emphasize the critical role of necroptosis in HF. Moreover, necroptosis exacerbates the immune-inflammatory response and mitochondrial oxidative stress, creating a vicious loop.[15] However, the role of necroptosis-mediated immune-inflammatory responses in HF remains underexplored.

In this study, we employed bioinformatics methods to analyze the infiltration of immune cell types and identify differentially expressed genes related to necroptosis (NRDEGs) in HF. Our objective was to investigate the relationship between necroptosis and immune infiltration in HF, with the aim of providing insights for the clinical prevention of HF.

Materials and Methods

Experimental Process

Figure 1 presents a flowchart outlining the overall experimental design. Initially, we obtained the expression matrix from the GSE21610 dataset. Next, we identified differentially expressed genes (DEGs) and NRDEGs between HF and no-failure (NF) groups. Subsequently, we conducted gene set enrichment analysis (GSEA) for both the HF and NF groups, followed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses for the NRDEGs. Fourth, we constructed a protein-protein interaction (PPI) network for the NRDEGs. Fifth, we analyzed immune infiltration in HF and examined the correlation between immune infiltration and the NRDEGs. Sixth, we predicted the transcription factors (TFs) associated with immune-related hub NRDEGs. Finally, we validated the expression levels of immune-related hub NRDEGs in animal models, as well as the immune infiltration and NRDEGs expressions in single-cell RNA sequencing (scRNA-seq).

Microarray Data

We downloaded the microarray data GSE21610 from the GEO database (https://www.ncbi.nlm.nih.gov/geo). This dataset includes 8 samples of disease-free, NF myocardial tissues and 9 samples of ischemic cardiomyopathy HF myocardial tissues obtained prior to ventricular assist device (VAD) support for further investigation.

We searched the KEGG pathway database (https://www.kegg.jp/kegg/pathway.html) for necroptosis genes using the keyword “necroptosis”, and identified 159 genes. Subsequently, we incorporated additional necroptosis genes from previous studies.[16–19] After removing duplicate entries, we obtained a total of 239 unique necroptosis genes (Supplementary Materials S1).

Acquisition of Necroptosis Differential Expressed Genes

We employed the “limma” package in R to conduct differential gene analysis on GSE21610, with a threshold of |log2 Fold Change| > 1 and a P value < 0.05. NRDEGs were identified based on the overlap of DEGs and necroptosis genes. The “ggplot2” and “heatmap” R packages were utilized to generate violin and volcano plots, as well as heatmaps for DEGs and NRDEGs, respectively.

Functional Enrichment Analysis

GSEA based on GO and KEGG database was utilized to conduct function and pathway enrichment analysis for HF. The thresholds were established at normalized enrichment score (NES) >1, P value < 0.05, and FDR < 0.25. In addition, the

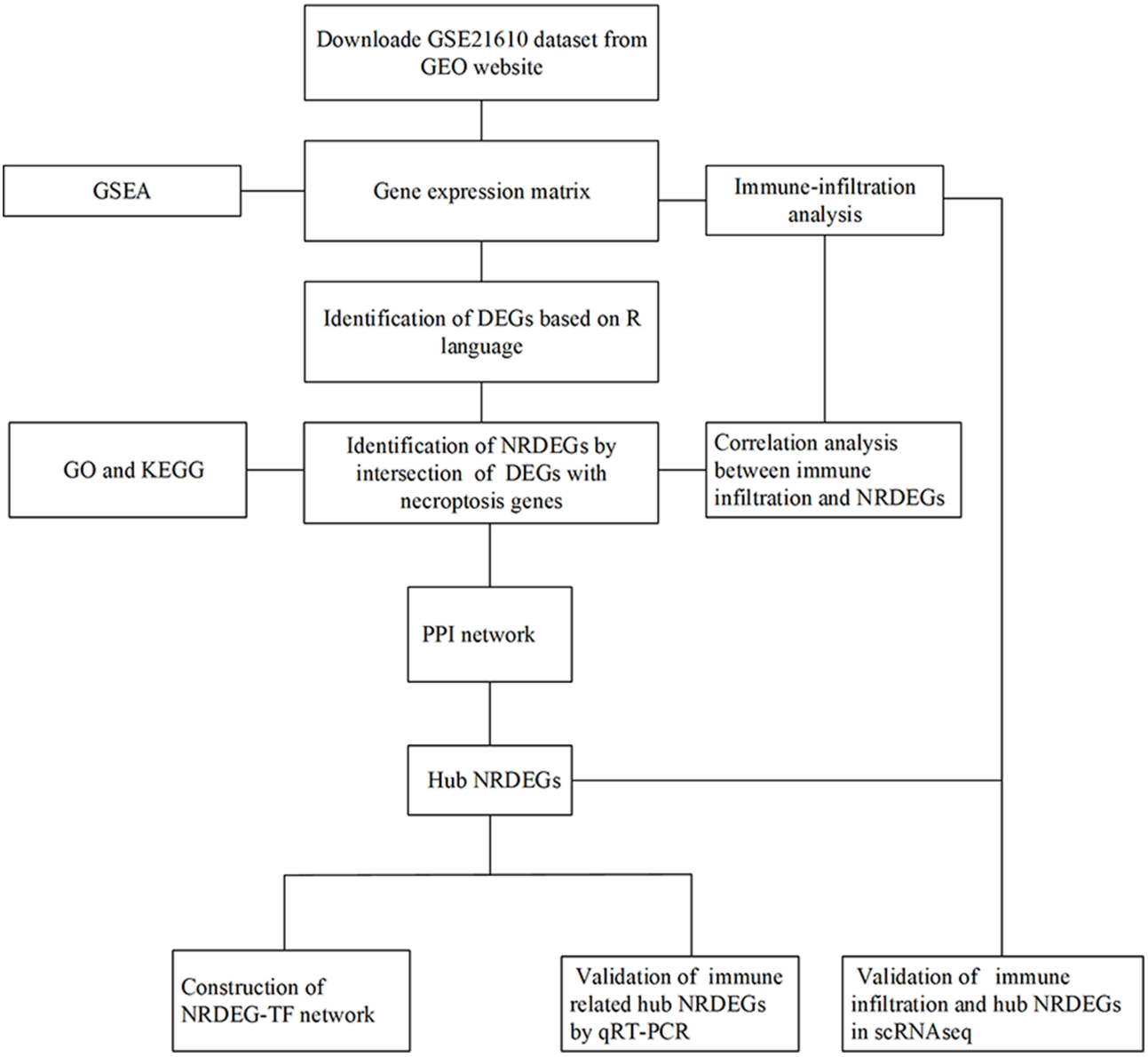


Figure 1 The experimental processes of this study.
Abbreviations: DEGs, differentially expressed genes; NRDEGs, differentially expressed genes of necroptosis; GSEA, gene set enrichment analysis; GO, gene ontology; KEGG, Kyoto encyclopedia of genes and genomes; PPI, protein-protein interaction; TF, transcription factor; qRT-PCR, quantitative Real-time PCR.

"cluster profile" R package was used for GO and KEGG enrichment analyses of NRDEGs, P value < 0.05 was set as a screening criterion, which were visualized by the "ggplot2" R package accordingly.

Identification of Hub NRDEGs by PPI Network

The "corrplot" R package was utilized to assess the correlation among NRDEGs. PPI network was analyzed using the online STRING database (https://string-db.org). Subsequently, the cytoHubba plug-in of Cycloscape software was employed to calculate the maximum clique centrality (MCC), degree, and edge-perched component (EPC) of the PPI network, while also identifying the top five hub genes.

Analysis of Immune Infiltration in HF and NF

The CIBERSORT package in R provided a thorough analysis of immune cell infiltration in both the HF and NF groups. A differential expression matrix of immune cells was integrated with the expression matrix of NRDEGs to perform Spearman correlation analysis, thereby elucidating the relationship between NRDEGs and immune infiltration in HF.

Construction of NRDEGs - TFs Network

To investigate the upstream molecules involved in necroptosis, the KnockTF database (www.licpathway.net/KnockTFv1) was employed to predict the transcription factors linked with the knockdown or knockout of immune-related hub NRDEGs, using a cutoff value of $|\log2FC| > 1$. The regulatory network of NRDEGs-TFs was visualized with Cytoscape software.

ScRNA-seq Analysis

ScRNA-seq of dataset GSE247468 was employed to validate immune infiltration and expression levels of NRDEGs in the HF and normal groups, utilizing the R package Seurat version 5.1.0. This dataset comprises two samples from normal donor hearts and four samples from HF patients with a history of coronary heart disease. Cells with a unique Molecular Identifier (UMI) count of fewer than 500 or more than 5000, as well as those with mitochondrial gene expression greater than 10%, were excluded from the analysis as low-quality cells. The LogNormalize function was applied to standardize the expression data, followed by the removal of batch effects. The FindClusters function, set to a resolution of 0.2, was utilized to identify cell clusters, which were visualized using two-dimensional uniform manifold approximation and projection (UMAP). The FindAllMarkers function was used to detect marker genes for each cluster, applying a log-fold change threshold of 1 and a minimum expression percentage of 0.1. Finally, the cell types within each cluster were defined based on prior research and the public CellMarker database (http://bio-bigdata.hrbmu.edu.cn/CellMarker).[20,21]

Construction of HF Model

We established an ischemic cardiomyopathy HF model based on permanent coronary ligation. Male C57BL/6J mice, supplied by GemPharmatech (Jiangsu, China), which were divided into two groups: sham and myocardial infarction (MI) groups. The surgical procedures for both the MI and sham groups were adapted from previous research. After four weeks, cardiac tissues were collected for further analysis.[22,23]

Echocardiography

The cardiac function of HF and NF was assessed using the Vevo 3100 animal ultrasound imaging system. After 4 weeks of operation, the mice were anesthetized using 1.5% isoflurane inhalation, and M-mode echocardiography was performed to capture the left ventricular graphics. The VevoLab quantification software was utilized to determine the left ventricular ejection fraction (LVEF) and fractional shortening (FS).[24]

Masson Trichrome Staining

Myocardial tissues were obtained from HF and NF groups after 4 weeks of operation, and subsequently were fixed in a 4% paraformaldehyde solution for 24 hours. The myocardial tissue was then dehydrated, infiltrated with paraffin, embedded, and sectioned, followed by Masson trichrome staining (Servicebio, G1006). The infarct size was observed under a microscope, and the quantification of infarct size was measured as the ratio of the circumference of the infarct region to the circumference of the left ventricle.[25]

Validation of Immune-Related Hub NRDEGs

Real-time PCR was employed to evaluate the gene expression levels of immune-related hub NRDEGs in myocardial tissues from HF and NF animals. GAPDH served as the internal reference, and the $2^{-\Delta\Delta Ct}$ method was utilized to quantify the expression levels of the target genes. The primers for each gene are detailed in Supplementary Materials S2.

Statistical Methods

The gene expression levels between the HF and NF groups were analyzed using an independent samples *t*-test, which revealed a statistically significant difference with a P-value less than 0.05. Graphs were generated using GraphPad Prism 8.0 software.

Results

Differential Expression Analysis Between HF and NF

A total of 1848 DEGs were identified between the NF and HF groups, with 770 genes being up-regulated and 1078 genes down-regulated. The top DEGs are illustrated in the heatmap and volcano plot (Figure 2A and B).

By intersecting the DEGs with necroptosis genes, 14 NRDEGs were identified (Figure 2C and Supplementary Materials S3). Among these, 11 NRDEGs were upregulated, while 3 NRDEGs were downregulated. The NRDEGs are presented in violin plots, heatmaps, and volcano plots (Figure 2D–F).

Gene Set Enrichment Analysis Between HF and NF

According to the results of GSEA, a total of 1650 GO terms and 134 KEGG pathways were found to be enriched in the HF group (Supplementary Materials S4 and S5). Notably, Th1 and Th2 cell differentiation, TGF-beta signaling, Renin secretion, and Wnt signaling pathways were significantly upregulated and enriched in the HF group (Figure 3A–D).

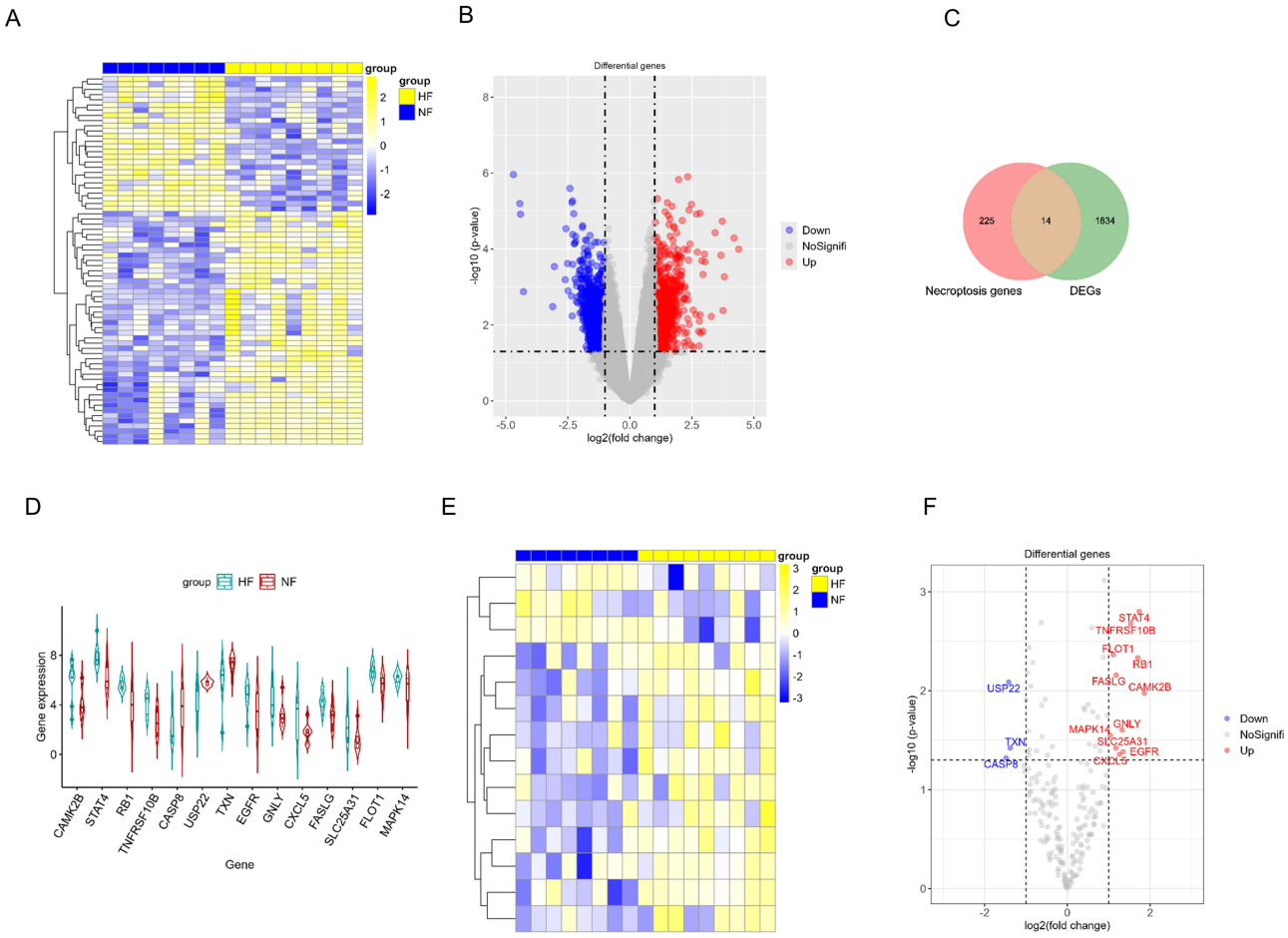


Figure 2 Differential expression analysis between HF and NF. (**A**) Heatmap of differentially expressed genes (DEGs). (**B**) Volcano plot of DEGs. (**C**) Venn diagram of 14 NRDEGs (**D**) Violin chart of NRDEGs. (**E**) Heatmap of NRDEGs. (**F**) Volcano plot of NRDEGs.

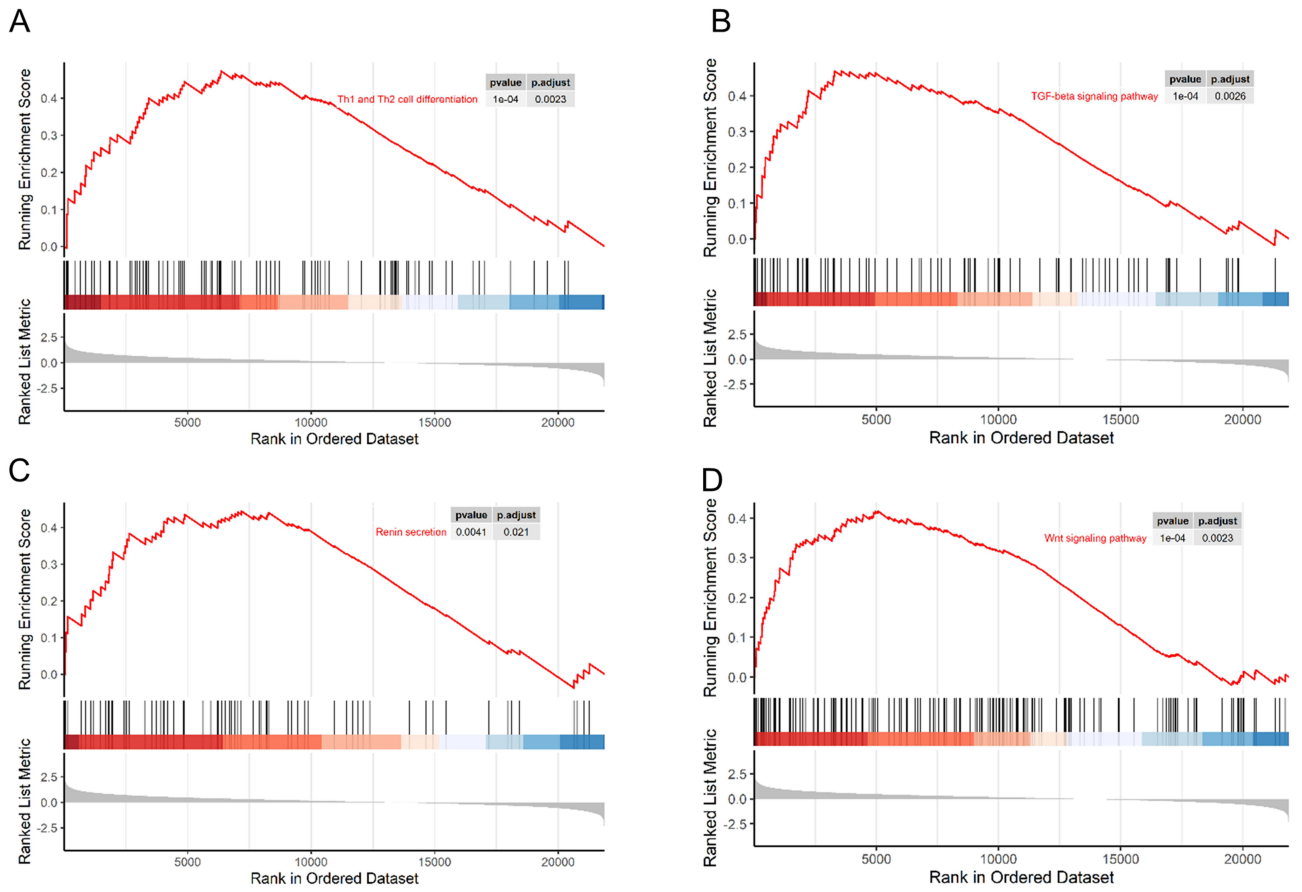


Figure 3 GSEA between HF and NF. (**A**–**D**) Th1 and Th2 cell differentiation, TGF-beta signaling pathway, Renin secretion, and Wnt signaling pathway are enriched in the HF group.

GO and KEGG Enrichment Analyses of NRDEGs

According to the GO functional annotation of 14 NRDEGs, the main terms enriched in biological processes (BP) included response to mechanical stimulus, cellular response to abiotic stimulus et al (Figure 4A). Cellular components (CC) were mainly distributed in membrane raft and membrane microdomain et al (Figure 4B). Molecular functions (MF) were mainly related to cytokine receptor binding, ubiquitin protein ligase binding et al (Figure 4C). Furthermore, KEGG analysis revealed 14 NRDEGs implicated in necroptosis, hepatitis B, lipid and atherosclerosis et al (Figure 4D). The Kegg suggested that NRDEGs were enriched in Chagas disease, Endocrine resistance, Glioma, GnRH signaling pathway et al (Figure 4E).

Identification of Hub NRDEGs by PPI Network

We performed a correlation analysis among the NRDEGs, which revealed a certain degree of linkage among them, however, minimal correlation was observed between EGFR, CXCL5, and the NRDEGs (Figure 5A). Utilizing the STRING database, we constructed a PPI network for the NRDEGs (Figure 5B). Subsequently, the cytoHubb plugin of Cytoscape 3.7.2 was employed to identify the top five hub NRDEGs, which included EGFR, TXN, FASLG, MAPK14, and CASP8 (Figure 5C).

Analysis of Immune Infiltration in HF and NF

In the enrichment analyses, NRDEGs were found to be abundant in immune-related pathways. Consequently, we analyzed immune infiltration in the HF and NF groups by CIBERSORT algorithm. As illustrated in Figure 6A–D, six types of immune cells exhibited significant differences between the HF and NF groups, with each type showing a degree

https://doi.org/10.2147/JIR.S502203

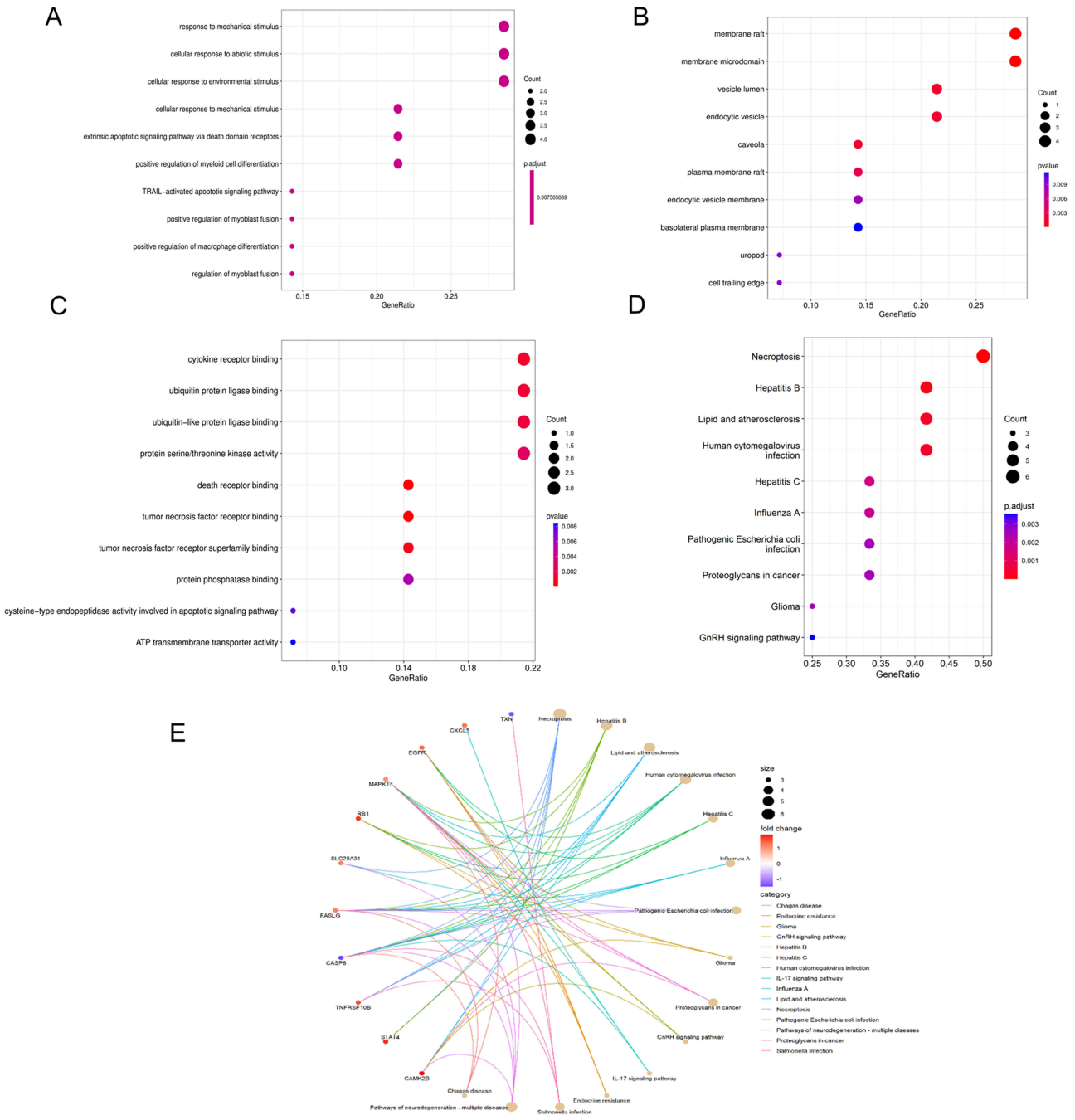


Figure 4 GO and KEGG Enrichment Analyses of NRDEGs. (**A**–**C**) GO enrichment analysis of NRDEGs. (**D** and **E**) KEGG enrichment analysis of NRDEGs.

of correlation. Among the six types of differential immune cells, the macrophages M2, monocytes, and T cells regulatory (Tregs) were down-regulated in the HF group. However, B cells memory, T cells follicular helper, and T cells gamma delta were up-regulated in the HF group. Additionally, as shown in Figure 6E, the hub NRDEGs, including EGFR, FASLG, and TXN, demonstrated significant correlations with the differential immune cells.

Construction of NRDEG-TFs Regulatory Network

To elucidate the upstream molecular mechanisms of necroptosis, the KnockTF database was utilized to predict TFs associated with immune-related hub NRDEGs. Target genes were either knocked down or knocked out, with

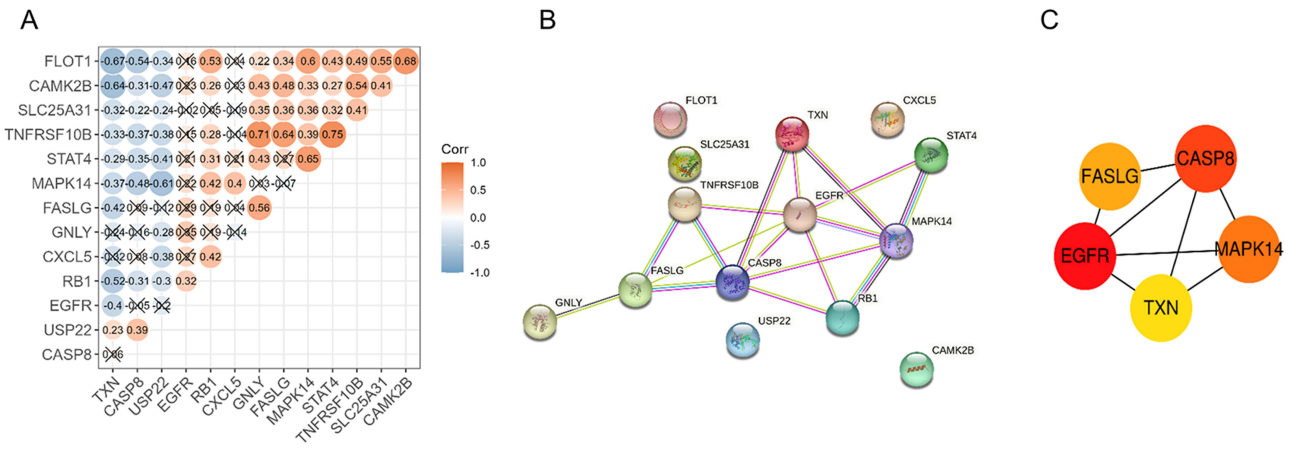


Figure 5 Identification of Hub NRDEGs by PPI Network. (**A**) Correlation analysis between 14 NRDEGs. (**B**) The NRDEGs PPI regulatory network. (**C**) Top five hub NRDEGs.

transcription factors selected based on a |log2FC| > 1 cutoff criterion. The interactions between the transcription factors and NRDEGs are illustrated in Figure 7 and further detailed in Supplementary Materials S6.

Validation of Immune Infiltration and Hub NRDEGs in scRNA-seq

The scRNA-seq dataset GSE247468 was utilized to analyze the differences between normal and HF groups. After quality control, we obtained a total of 61860 cells for analysis, which included 40490 cells from HF group and 21370 cells from normal group. These cells were classified into 15 clusters using UMAP and cluster analysis. According to established markers, T cells (CD3D, CD3E, and CD3G), natural killer (NK) cells (FCGR3A and KLRB1), B cells (CD79A, CD79B), endothelial cells (ECs) (VWF, TAGLN, and CLDN5), fibroblast cells (FBs) ((LUM and DCN), smooth muscle cells (SMCs) (MYH11), cardiomyocyte cells (MYH7 and MYL2), neuronal cells (PLP1), neutrophils (S100A8), macrophages (CD68), resulting in the identification of 10 distinct cell clusters (Figure 8A). Violin plot showing the expression of selected cell marker gene to define cell clusters from integrated data (Figure 8B). The cell distribution revealed differences between the normal and HF groups, with HF samples exhibiting a significantly lower percentage of neutrophils, cardiomyocyte cells and a significantly higher percentage of smooth muscle cells, fibroblast cells, macrophages, B cells, and T cells (Figure 8C). GO enrichment analysis of top marker genes indicated that immune-related pathways are implicated in HF (Figure 8D). As shown in Figure 8E, the EGFR is predominantly expressed in fibroblast cells, FASLG is primarily expressed in T cells, and TXN is expressed across all cell types. Figure 8F indicates that the expression of EGFR in fibroblast cells was increased in HF compared to the normal group, while the expression of FASLG in T cells decreased in HF relative to the normal group, and TXN expression was significantly reduced in HF compared to the normal group.

Validation of Immune-Related Hub NRDEGs in HF and NF Models

We constructed the HF and NF models. As illustrated in Figure 9A, the EF and FS were significantly decreased in the HF group compared to the NF group. Additionally, the infarct size was increased in the HF group relative to the NF group (Figure 9B). Subsequently, we performed qRT-PCR on HF and NF animal models to validate the immune-related hub NRDEGs. Figure 9C demonstrates that the mRNA expression levels of Egfr and Faslg were up-regulated in the HF group, whereas Txn was down-regulated.

Discussion

Recent research has focused on the role of necroptosis in cardiovascular disease, yet its involvement in HF remains poorly understood. Our findings suggest that necroptosis is associated with immune infiltration, which plays a crucial role in the progression of HF.

https://doi.org/10.2147/JIR.S502203

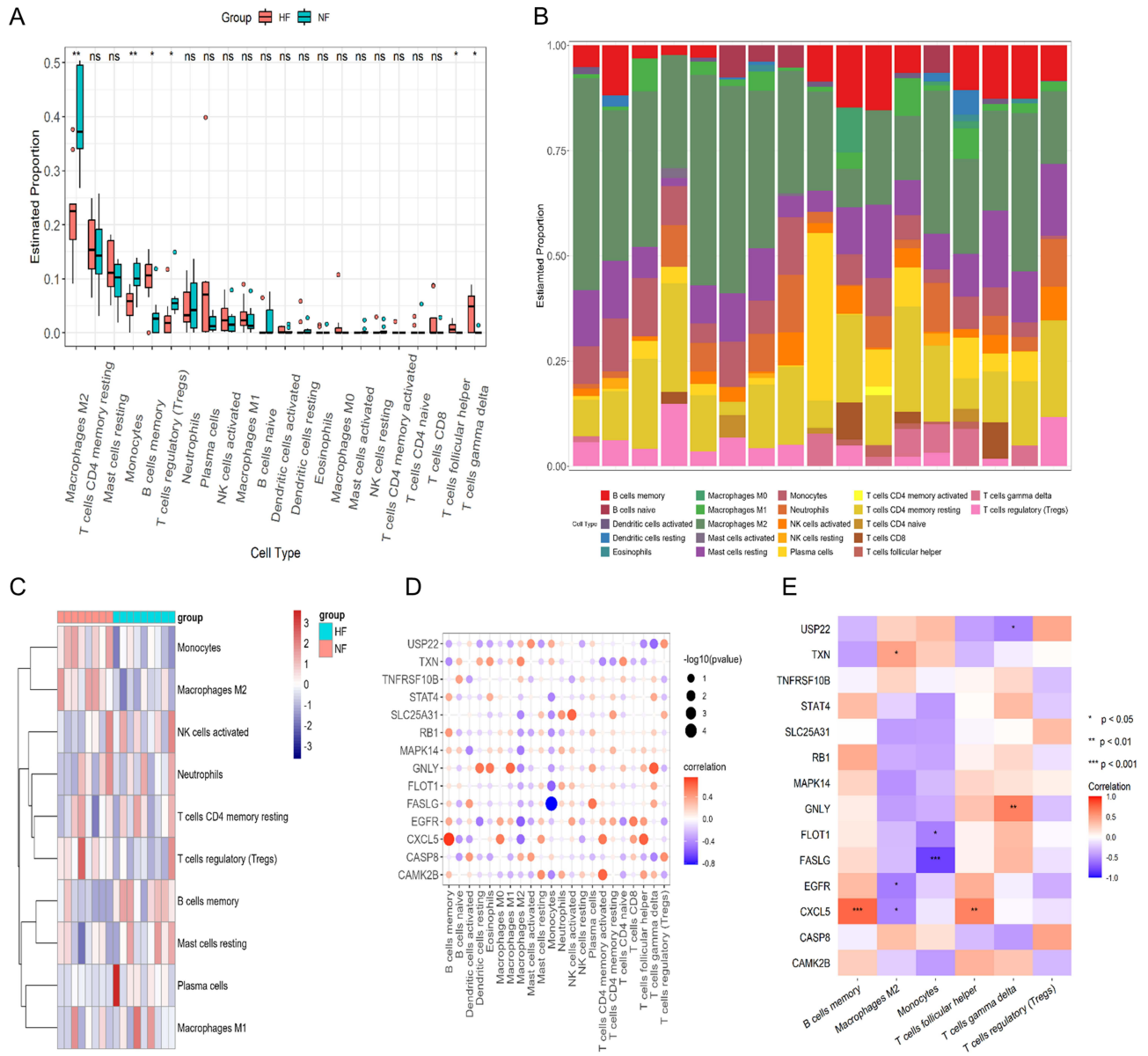


Figure 6 Analysis of Immune Infiltration in HF and NF. (**A**) Violin plot illustrating the distribution of immune cells. (**B**) Histogram depicting the proportions of various immune cell types. (**C**) Heatmap showing the expression levels of immune cells. (**D**) Heatmap representing the correlations among immune cells. (**E**) Heatmap of NRDEGs and their correlation with differential immune cell populations. *P < 0.05, **P < 0.01, ***P < 0.001.

In this study, we identified 1848 DEGs between the two groups. GSEA revealed that Th1 and Th2 cell differentiation, TGF-beta signaling pathway, Renin secretion, and Wnt signaling pathways were significantly elevated in the HF group. Previous research has reported that T cells are activated during cardiac remodeling and HF.[26] Additionally, prior studies have indicated that the differentiation between Th1 and Th2 cells may be associated with HF.[27] The TGF- beta signaling pathway deactivates inflammatory macrophages while promoting myofibroblast transdifferentiation and matrix synthesis, both of which are involved in HF.[28] Furthermore, Wnt signaling is activated during inflammation, neovascularization, and fibrosis, all of which are related to HF.[29] Moreover, NRDEGs also enriched in the GnRH signaling pathway, which is traditionally associated with the reproductive functions, however, recent study found that GnRH immunization in rats alters T cell populations, and elevates cytokine levels.[30] In glycine N-methyltransferase knockout (GNMT−/−) mice, RIPK1 expression was elevated compared to wild-type mice, with differential genes also enriched in the GnRH pathway.[31] Additionally, GnRH antagonists have been shown to reduce atherosclerotic plaque formation in mouse

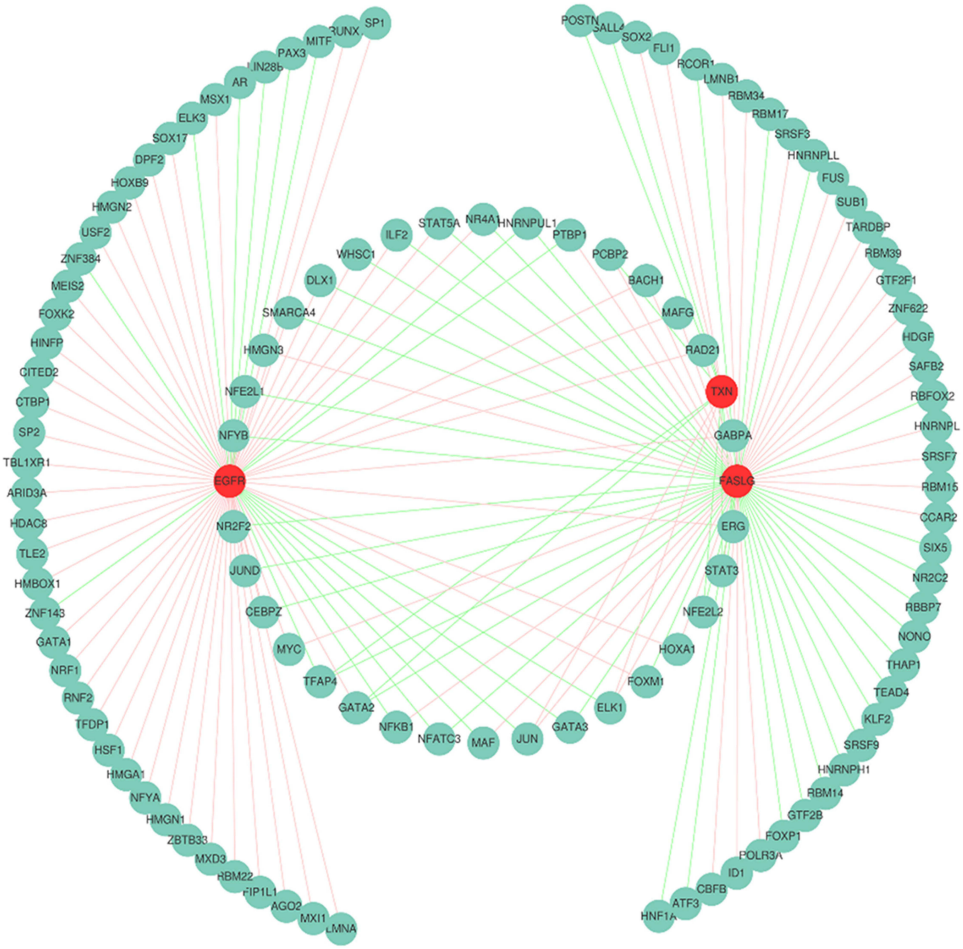


Figure 7 NRDEGs–TFs Regulatory Network. Red nodes, immune related hub NRDEGs; Green nodes, predicted transcription factors; Red edge lines, up-regulated TFs upon NRDEG knockdown or knockout; Green edge lines, down-regulated TFs upon NRDEG knockdown or knockout.

models of cardiovascular disease.[32] These findings demonstrated that the GnRH signaling pathway may be involved in the necroptosis, fibrosis, immune activation/infiltration, or HF. Furthermore, previous studies have suggested that cAMP signaling pathways contribute to the HF by suppressing cardiac immune infiltration, inflammation and fibrosis.[33–35] In the Supplementary Materials S5 of the GSEA results, several differential genes between HF and NF, including AKT2 and PTGER3 are associated with the cAMP signaling pathway and may have a regulatory effect in cardiac inflammation and fibrosis. Further research will elucidate the potential functions of these genes.

Necroptosis is characterized as a form of programmed cell death initiated by the RIPK1-RIPK3-MLKL phosphorylation signaling pathway. The oligomerization of MLKL leads to the rupture of the cell membrane, lysis of the nucleus, and the subsequent release of cellular contents, which in turn triggers secondary inflammatory responses, mitochondrial dysfunction, and oxidative stress.[36–38] These processes can activate hypertrophic signaling pathways, resulting in myofibrillar proliferation and ventricular dysfunction, ultimately leading to HF.[39,40] In this study, we investigated 14 NRDEGs between the HF and NF groups. Among these, 3 NRDEGs were found to be down-regulated, while 11 NRDEGs were up-regulated in the HF group. We identified EGFR, TXN, FASLG, MAPK14, and CASP8 as the top 5 hub NRDEGs within the PPI network. Epidermal growth factor receptor (EGFR) is a member of the ErbB family of the tyrosine kinase receptors, which are implicated in cancer, diabetes, and cardiovascular diseases. Studies have shown that EGFR siRNA can alleviate HF by inhibiting the renin-angiotensin-aldosterone system, inflammation and oxidative stress.[41–43] Sustained activation of ErbB contributes to neurodegenerative by inducing necroptosis in neurocytes. Animal studies have demonstrated that early intervention in ErbB activation effectively prevents necroptosis,[44] indicating

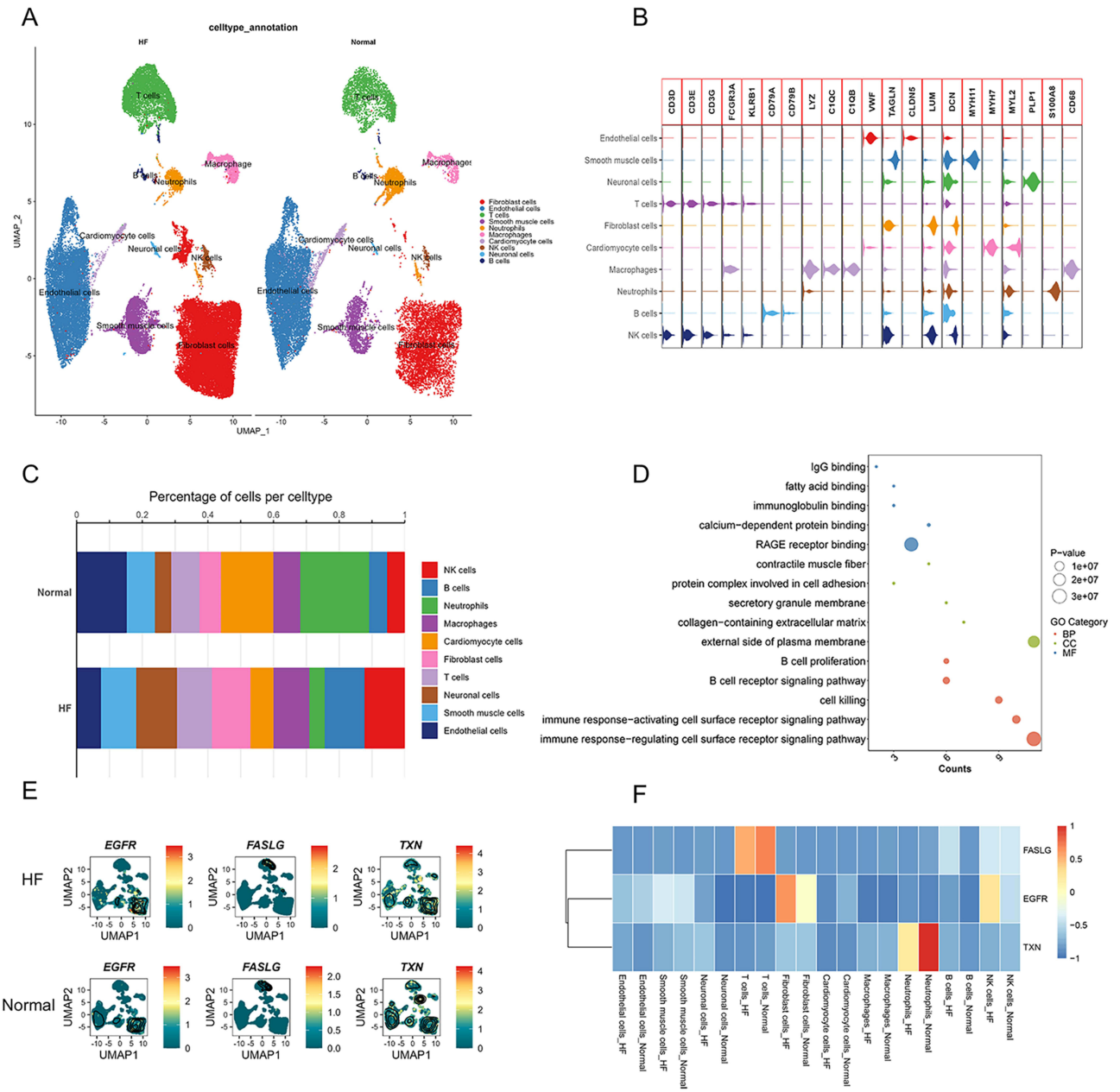


Figure 8 Validation of immune infiltration and hub NRDEGs in scRNA-seq. (**A**) The UMAP plot of scRNA-seq data depicts 10 distinct cell types. (**B**) A violin plot displays the expression of selected cell marker gene to define cell clusters from integrated data. (**C**) Differences in cell distribution between the Normal and HF groups. (**D**) GO enrichment analysis of top marker genes. (**E**) The expression distribution featureplot of EGFR, FASLG and TXN. (**F**) Expression levels of EGFR, FASLG and TXN in cell types between HF and Normal groups.

a strong relationship between EGFR and necroptosis. Thioredoxin (TXN) is an oxidoreductase that cleaves oxidized substrate proteins and thereby prevents heart failure. TXN knockout mice demonstrate deteriorating cardiac phenotypes.[45] A previous study indicated that TXN suppress necroptosis by preventing the formation and polymerization of MLKL disulfide bonds, suggesting that TXN functions as an upstream molecule in the necroptosis pathway.[46] Fas ligand (FASLG) is a component of the FAS system that induces cell death and contributes to the pathogenesis of cardiovascular disease. The interaction between Fas and FasL leads to cardiomyocyte apoptosis, which has been associated with the progression of HF.[47] Furthermore, interactions in the FAS/FASL signaling pathway can also trigger necroptosis in an Ang II-treated cellular model.[48] Mitogen-activated protein kinase 14 (MAPK14) is a member of the MAPK family, whose activation is typically associated with cardiac hypertrophy and contractile dysfunction, both of

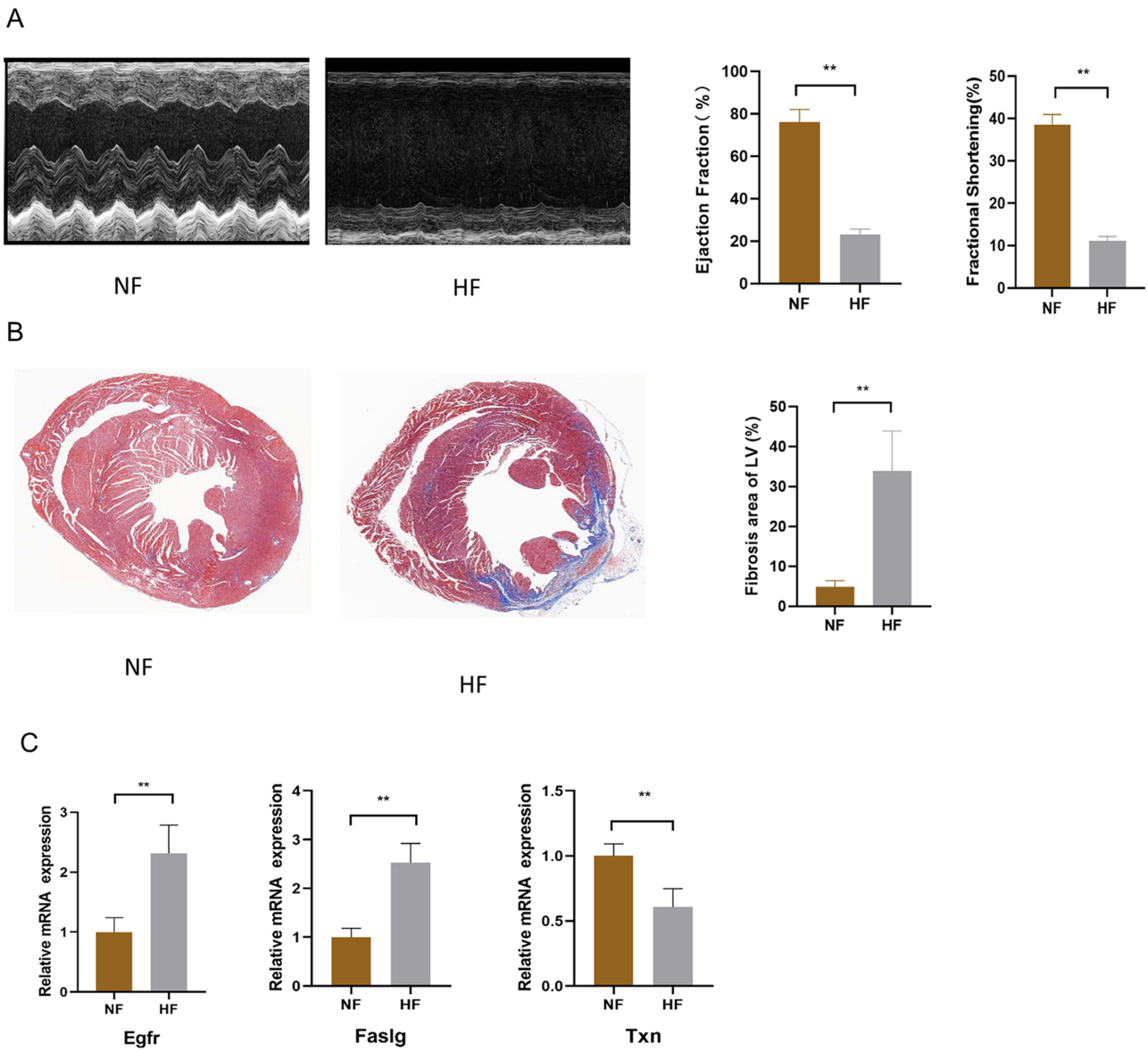


Figure 9 Validation of Immune-Related Hub NRDEGs. (**A**) Echocardiography in HF and NF animal models, (**B**) Masson trichrome staining in HF and NF animal models. (**C**) Egfr, Faslg, and Txn mRNA expression levels in HF and NF animal models. ** P < 0.01.

which contribute to the progression of HF.[49] Previous research has demonstrated that MAPK activation can induce necroptosis in the adrenal gland and trigger inflammatory responses,[50] indicating that MAPK is implicated in the necroptotic process. Cystine protease 8 (Casp8) is a well-established key regulator of apoptosis and necroptosis. Inhibition of caspase 8 activates necroptosis, which contributes to the progression of HF.[51] These findings suggest that NRDEGS may be involved in necroptosis and contribute to the development of HF. In our validation using the scRNA-seq database, we found that the expression levels of EGFR were increased and TXN levels were decreased in HF compared to the normal group. These findings align with the microarray data and results from animal models. However, the expression level of FASLG was decreased in HF compared to the normal group, which differs from the microarray data. In the animal models, we confirmed that FASLG was upregulated in the HF group, consistent with the microarray data, we speculate that the observed disparity in FASLG expression detected through scRNA-seq may be attributed to variations across samples.

https://doi.org/10.2147/JIR.S502203

To further investigate the role of immune cell infiltration in HF, we conducted a comprehensive assessment of immune infiltration using the CIBERSORT method. Our analysis revealed that M2 macrophages, monocytes, memory B cells, Tregs, follicular helper T cells, and gamma delta T cells are all involved in HF. Macrophage polarization imbalance between the pro-inflammatory M1 and anti-inflammatory M2 phenotypes can lead to excessive inflammation and cardiac damage.[52] A previous study demonstrated that increased M2 macrophage polarization in heart tissue can alleviate HF.[53] These findings suggest that a decrease in M2 macrophages may accelerate the HF process, which aligns with our result. M2 macrophages release enzymes, cytokines, and peptides, that can enhance cardiac function.[54,55] A reduction in M2 macrophages leads to lower levels of anti-inflammatory cytokines, chemokines, and growth factors, resulting in diminished cell proliferation, angiogenesis, and increased fibroblast stroma, ventricular remodeling, and dysfunction.[56] Monocytes serve as a crucial source of both pro-inflammatory and anti-inflammatory cytokines, playing a significant role in the inflammatory cascade and the pathophysiology of HF. In the end stages of myocardial infarction, monocytes are recruited to the myocardium, where they amplify inflammatory processes and promote healing by increasing angiogenesis and collagen deposition.[57] Memory B cells are a differentiated subset of B cells. The upregulation of these cells leads to the production of antibodies, cytokines, and chemokines, which are involved in HF.[58] Tregs are a subset of CD4+ T effector cells. A reduction in Tregs has been observed in the peripheral blood of patients with HF.[59–61] Gamma delta T cells secrete cytokines that promote apoptosis. While previous studies have reported significant elevations of gamma delta T cells in cases of myocardial infarction and atherosclerosis; however, no studies have yet demonstrated a close relationship between gamma delta T cells and heart failure. Further exploration of the underlying mechanisms is warranted.[62,63] ScRNA-seq has confirmed the role of immune infiltration in the development of HF. The existing literature, combined with our analytical findings, suggests that immune infiltration is associated with both the incidence and progression of HF.

To better understand the significance of necroptosis-mediated immune infiltration in HF, we examined the relationship between NRDEGs and immune cells. Our findings indicate that NRDEGs are associated with immune infiltration. Specifically, among the key NRDEGs, EGFR, FASLG, and TXN exhibited correlations with various immune cell types. Previous studies have demonstrated that caspase-8-deficient mice exhibit necroptosis, which disrupts T cell homeostasis, decreases T cell populations, and influences T cell proliferation.[64] Inhibition of necroptosis also shifts the M1/M2 macrophage ratio toward an anti-inflammatory M2 phenotype, thereby alleviating the inflammatory response.[65] Our study illustrates that necroptosis is correlated with immune infiltration and plays significant roles in the development of HF. Further investigation into the mechanisms underlying necroptosis-mediated immune infiltration in HF may help identify potential therapeutic targets and enhance treatment efficacy for HF patients.

This study has several limitations. First, the sample size was relatively small, potentially leading to biased results, additional HF models, such as Ang II, TAC, or diabetes-induced myocardial injury models, are needed to validate our results in future research. Second, the analysis was limited to mRNA microarray data, therefore, additional functional validation of NRDEGs in HF is necessary to gain deeper insights into the underlying mechanisms. Third, although there is a relationship between immune infiltration and necroptosis, the cause-and-effect relationship remains unclear. Future studies should be conducted to further investigate the mechanisms connecting necroptosis and immune infiltration in HF.

Conclusion

In this study, we screened for novel NRDEGs and potential pathways associated with HF, analyzed immune infiltration in HF. We identified immune-related hub NRDEGs, and constructed NRDEG – TFs regulatory network. Our findings suggest that immune infiltration plays a significant role in the progression of HF. Furthermore, the hub NRDEGs EGFR, FASLG, and TXN exhibit a correlation with immune infiltration in HF. Overall, our results may offer an innovative theoretical foundation for future therapies targeting HF.

Institutional Review Board Statement

The animal study protocol was approved by the Institutional Animal Care and Use Committee of Sun Yat-sen University and followed the standards of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (SYSU-IACUC-2022-001210).

According to the item 1 and 2 of Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects dated February 18, 2023, China, we used legally obtained public data or data generated through observation without interfering with public behavior for research and we conducted research using anonymized information data.

Data Sharing Statement

The original data can be obtained from the corresponding author if it is permitted by all authors.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

The study was supported by the grants from the Clinical Research Fund of the Seventh Affiliated Hospital of Sun Yat-sen University, ZSQYLCKYJJ202302, and the Project of Henan Province Medical Science and Technology Research Program (Grant Number: LHGJ20240194).

Disclosure

The authors declare no competing interests in this work.

References

1. Ziaeian B, Fonarow GC. Epidemiology and aetiology of heart failure. *Nat Rev Cardiol*. 2016;13(6):368–378. doi:10.1038/nrcardio.2016.25
2. McDonagh TA, Metra M, Adamo M, et al. 2021 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J*. 2021;42(36):3599–3726. doi:10.1093/eurheartj/ehab368
3. Kologrivova I, Shtatolkina M, Suslova T, Ryabov V. Cells of the immune system in cardiac remodeling: main players in resolution of inflammation and repair after myocardial infarction. *Front Immunol*. 2021;12:664457. doi:10.3389/fimmu.2021.664457
4. Ayoub KF, Pothineni N, Rutland J, Ding Z, Mehta JL. Immunity, inflammation, and oxidative stress in heart failure: emerging molecular targets. *Cardiovasc Drugs Ther*. 2017;31(5–6):593–608. doi:10.1007/s10557-017-6752-z
5. Liu W, Li Y, Zhang Y, et al. Identification of biomarkers and immune infiltration in acute myocardial infarction and heart failure by integrated analysis. *Biosci Rep*. 2023;43(7): BSR20222552. doi:10.1042/BSR20222552
6. Dhuriya YK, Sharma D. Necroptosis: a regulated inflammatory mode of cell death. *J Neuroinflammation*. 2018;15(1):199. doi:10.1186/s12974-018-1235-0
7. Choi ME, Price DR, Ryter SW, Choi A. Necroptosis: a crucial pathogenic mediator of human disease. *JCI Insight*. 2019;4(15): e128834. doi:10.1172/jci.insight.128834
8. Sun L, Wang H, Wang Z, et al. Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. *Cell*. 2012;148 (1–2):213–227. doi:10.1016/j.cell.2011.11.031
9. Cai Z, Jitkaew S, Zhao J, et al. Plasma membrane translocation of trimerized MLKL protein is required for TNF-induced necroptosis. *Nat Cell Biol*. 2014;16(1):55–65. doi:10.1038/ncb2883
10. Marunouchi T, Ito T, Onda S, et al. Effects of 17-AAG on the RIP1/RIP3/MLKL pathway during the development of heart failure following myocardial infarction in rats. *J Pharmacol Sci*. 2021;147(2):192–199. doi:10.1016/j.jphs.2021.06.009
11. Zhang XJ, Li L, Wang AL, et al. GSK2795039 prevents RIP1-RIP3-MLKL-mediated cardiomyocyte necroptosis in doxorubicin-induced heart failure through inhibition of NADPH oxidase-derived oxidative stress. *Toxicol Appl Pharmacol*. 2023;463:116412. doi:10.1016/j.taap.2023.116412
12. Corsetti G, Chen-Scarabelli C, Romano C, et al. Autophagy and oncosis/necroptosis are enhanced in cardiomyocytes from heart failure patients. *Med Sci Monit Basic Res*. 2019;25:33–44. doi:10.12659/MSMBR.913436
13. Ma F, Zhu Y, Chang L, et al. Hydrogen sulfide protects against ischemic heart failure by inhibiting RIP1/RIP3/MLKL-mediated necroptosis. *Physiol Res*. 2022;71(6):771–781. doi:10.33549/physiolres.934905
14. Gao P, Cao M, Jiang X, et al. Cannabinoid receptor 2-centric molecular feedback loop drives necroptosis in diabetic heart injuries. *Circulation*. 2023;147(2):158–174. doi:10.1161/CIRCULATIONAHA.122.059304
15. Yang Y, Wu M, Cao D, et al. ZBP1-MLKL necroptotic signaling potentiates radiation-induced antitumor immunity via intratumoral STING pathway activation. *Sci Adv*. 2021;7(41):eabf6290. doi:10.1126/sciadv.abf6290
16. Liu F, Wei T, Liu L, et al. Role of necroptosis and immune infiltration in human Stanford type A aortic dissection: novel insights from bioinformatics analyses. *Oxid Med Cell Longev*. 2022;2022:6184802. doi:10.1155/2022/6184802
17. Nie S, Huili Y, He Y, et al. Identification of bladder cancer subtypes based on necroptosis-related genes, construction of a prognostic model. *Front Surg*. 2022;9:860857. doi:10.3389/fsurg.2022.860857

18. Hu T, Zhao X, Zhao Y, et al. Identification and verification of necroptosis-related gene signature and associated regulatory axis in breast cancer. *Front Genet*. 2022;13:842218. doi:10.3389/fgene.2022.842218
19. Ding C, Yu Z, Zhu J, et al. Construction and validation of a necroptosis-related gene signature for predicting prognosis and tumor microenvironment of pancreatic cancer. *Dis Markers*. 2022;2022:9737587. doi:10.1155/2022/9737587
20. Kong Y, Yang N, Luo Z, Huang R, Li Q. Key cell types and biomarkers in heart failure identified through analysis of single-cell and bulk RNA sequencing data. *Mediators Inflamm*. 2023;2023:8384882. doi:10.1155/2023/8384882
21. Du L, Wang X, Guo Y, et al. Altered lipid metabolism promoting cardiac fibrosis is mediated by CD34(+) cell-derived FABP4(+) fibroblasts. *Exp Mol Med*. 2024;56(8):1869–1886. doi:10.1038/s12276-024-01309-9
22. Mathiyalagan P, Adamiak M, Mayourian J, et al. FTO-dependent N(6)-methyladenosine regulates cardiac function during remodeling and repair. *Circulation*. 2019;139(4):518–532. doi:10.1161/CIRCULATIONAHA.118.033794
23. Humeres C, Shinde AV, Hanna A, et al. Smad7 effects on TGF-beta and ErbB2 restrain myofibroblast activation and protect from postinfarction heart failure. *J Clin Invest*. 2022;132(3): e146926. doi:10.1172/JCI146926
24. Huang CK, Dai D, Xie H, et al. Lgr4 governs a pro-inflammatory program in macrophages to antagonize post-infarction cardiac repair. *Circ Res*. 2020;127(8):953–973. doi:10.1161/CIRCRESAHA.119.315807
25. Li Q, Xu Y, Lv K, et al. Small extracellular vesicles containing miR-486-5p promote angiogenesis after myocardial infarction in mice and nonhuman primates. *Sci Transl Med*. 2021;13(584): eabb0202. doi:10.1126/scitranslmed.abb0202
26. Bansal SS, Ismahil MA, Goel M, et al. Activated T lymphocytes are essential drivers of pathological remodeling in ischemic heart failure. *Circ Heart Fail*. 2017;10(3):e003688. doi:10.1161/CIRCHEARTFAILURE.116.003688
27. Liang X, Bai Z, Wang F, et al. Full-length transcriptome sequencing: an insight into the dog model of heart failure. *Front Cardiovasc Med*. 2021;8:712797. doi:10.3389/fcvm.2021.712797
28. Dobaczewski M, Chen W, Frangogiannis NG. Transforming growth factor (TGF)-beta signaling in cardiac remodeling. *J mol Cell Cardiol*. 2011;51 (4):600–606. doi:10.1016/j.yjmcc.2010.10.033
29. Methatham T, Tomida S, Kimura N, Imai Y, Aizawa K. Inhibition of the canonical Wnt signaling pathway by a beta-catenin/CBP inhibitor prevents heart failure by ameliorating cardiac hypertrophy and fibrosis. *Sci Rep*. 2021;11(1):14886. doi:10.1038/s41598-021-94169-6
30. Pan F, Du H, Tian W, et al. Effect of GnRH immunocastration on immune function in male rats. *Front Immunol*. 2022;13:1023104. doi:10.3389/fimmu.2022.1023104
31. Yang MH, Chen YA, Tu SC, et al. Utilizing an animal model to identify brain neurodegeneration-related biomarkers in aging. *Int J Mol Sci*. 2021;22(6):3278. doi:10.3390/ijms22063278
32. Hopmans SN, Duivenvoorden WC, Werstuck GH, Klotz L, Pinthus JH. GnRH antagonist associates with less adiposity and reduced characteristics of metabolic syndrome and atherosclerosis compared with orchiectomy and GnRH agonist in a preclinical mouse model. *Urol Oncol*. 2014;32 (8):1126–1134. doi:10.1016/j.urolonc.2014.06.018
33. Stoicovy RA, Cora N, Perez A, et al. Cyclic adenosine monophosphate critically modulates cardiac GLP-1 receptor's anti-inflammatory effects. *Inflamm Res*. 2024;73(11):2043–2056. doi:10.1007/s00011-024-01950-0
34. Pollard CM, Desimine VL, Wertz SL, et al. Deletion of osteopontin enhances beta(2)-adrenergic receptor-dependent anti-fibrotic signaling in cardiomyocytes. *Int J Mol Sci*. 2019;20(6):1396. doi:10.3390/ijms20061396
35. Insel PA, Murray F, Yokoyama U, et al. cAMP and Epac in the regulation of tissue fibrosis. *Br J Pharmacol*. 2012;166(2):447–456. doi:10.1111/j.1476-5381.2012.01847.x
36. Kaczmarek A, Vandenabeele P, Krysko DV. Necroptosis: the release of damage-associated molecular patterns and its physiological relevance. *Immunity*. 2013;38(2):209–223. doi:10.1016/j.immuni.2013.02.003
37. Khoury MK, Gupta K, Franco SR, Liu B. Necroptosis in the Pathophysiology of Disease. *Am J Pathol*. 2020;190(2):272–285. doi:10.1016/j.ajpath.2019.10.012
38. Tonnus W, Meyer C, Paliege A, et al. The pathological features of regulated necrosis. *J Pathol*. 2019;247(5):697–707. doi:10.1002/path.5248
39. Chi RF, Li L, Wang AL, et al. Enhanced oxidative stress mediates pathological autophagy and necroptosis in cardiac myocytes in pressure overload induced heart failure in rats. *Clin Exp Pharmacol Physiol*. 2022;49(1):60–69. doi:10.1111/1440-1681.13583
40. Zhang T, Zhang Y, Cui M, et al. CaMKII is a RIP3 substrate mediating ischemia- and oxidative stress-induced myocardial necroptosis. *Nat Med*. 2016;22(2):175–182. doi:10.1038/nm.4017
41. Yu Y, Wei SG, Weiss RM, Felder RB. Silencing epidermal growth factor receptor in hypothalamic paraventricular nucleus reduces extracellular signal-regulated kinase 1 and 2 signaling and sympathetic excitation in heart failure rats. *Neuroscience*. 2021;463:227–237. doi:10.1016/j.neuroscience.2021.01.025
42. Amodio V, Yaeger R, Arcella P, et al. EGFR blockade reverts resistance to KRAS(G12C) inhibition in colorectal cancer. *Cancer Discov*. 2020;10 (8):1129–1139. doi:10.1158/2159-8290.CD-20-0187
43. Sugimoto M, Cutler A, Shen B, et al. Inhibition of EGF signaling protects the diabetic retina from insulin-induced vascular leakage. *Am J Pathol*. 2013;183(3):987–995. doi:10.1016/j.ajpath.2013.05.017
44. Hu X, Xiao G, He L, et al. Sustained ErbB activation causes demyelination and hypomyelination by driving necroptosis of mature oligodendrocytes and apoptosis of oligodendrocyte precursor cells. *J Neurosci*. 2021;41(48):9872–9890. doi:10.1523/JNEUROSCI.2922-20.2021
45. Oka SI, Chin A, Park JY, et al. Thioredoxin-1 maintains mitochondrial function via mechanistic target of rapamycin signalling in the heart. *Cardiovasc Res*. 2020;116(10):1742–1755. doi:10.1093/cvr/cvz251
46. Reynoso E, Liu H, Li L, et al. Thioredoxin-1 actively maintains the pseudokinase MLKL in a reduced state to suppress disulfide bond-dependent MLKL polymer formation and necroptosis. *J Biol Chem*. 2017;292(42):17514–17524. doi:10.1074/jbc.M117.799353
47. Rodrigues VJ, Agrelli GS, Leon SC, et al. Fas/Fas-L expression, apoptosis and low proliferative response are associated with heart failure in patients with chronic Chagas' disease. *Microbes Infect*. 2008;10(1):29–37. doi:10.1016/j.micinf.2007.09.015
48. Zhu Y, Cui H, Lv J, et al. Angiotensin II triggers RIPK3-MLKL-mediated necroptosis by activating the Fas/FasL signaling pathway in renal tubular cells. *PLoS One*. 2020;15(3):e0228385. doi:10.1371/journal.pone.0228385
49. Rose BA, Yokota T, Chintalgattu V, et al. Cardiac myocyte p38alpha kinase regulates angiogenesis via myocyte-endothelial cell cross-talk during stress-induced remodeling in the heart. *J Biol Chem*. 2017;292(31):12787–12800. doi:10.1074/jbc.M117.784553

50. Qi X, Zhang Y, Liu H, et al. Cadmium exposure induces inflammation and necroptosis in porcine adrenal gland via activating NF-kappaB/MAPK pathway. *J Inorg Biochem*. 2021;223:111516. doi:10.1016/j.jinorgbio.2021.111516
51. Xiao P, Wang C, Li J, et al. COP9 signalosome suppresses RIPK1-RIPK3-mediated cardiomyocyte necroptosis in mice. *Circ Heart Fail*. 2020;13 (8):e006996. doi:10.1161/CIRCHEARTFAILURE.120.006996
52. Mouton AJ, Li X, Hall ME, Hall JE. Obesity, hypertension, and cardiac dysfunction: novel roles of immunometabolism in macrophage activation and inflammation. *Circ Res*. 2020;126(6):789–806. doi:10.1161/CIRCRESAHA.119.312321
53. Lv J, Chen J, Wang M, Yan F. Klotho alleviates indoxyl sulfate-induced heart failure and kidney damage by promoting M2 macrophage polarization. *Aging*. 2020;12(10):9139–9150. doi:10.18632/aging.103183
54. Wang D, Xiong M, Chen C, et al. Legumain, an asparaginyl endopeptidase, mediates the effect of M2 macrophages on attenuating renal interstitial fibrosis in obstructive nephropathy. *Kidney Int*. 2018;94(1):91–101. doi:10.1016/j.kint.2017.12.025
55. Podaru MN, Fields L, Kainuma S, et al. Reparative macrophage transplantation for myocardial repair: a refinement of bone marrow mononuclear cell-based therapy. *Basic Res Cardiol*. 2019;114(5):34. doi:10.1007/s00395-019-0742-1
56. Peet C, Ivetic A, Bromage DI, Shah AM. Cardiac monocytes and macrophages after myocardial infarction. *Cardiovasc Res*. 2020;116 (6):1101–1112. doi:10.1093/cvr/cvz336
57. Wrigley BJ, Lip GY, Shantsila E. The role of monocytes and inflammation in the pathophysiology of heart failure. *Eur J Heart Fail*. 2011;13 (11):1161–1171. doi:10.1093/eurjhf/hfr122
58. Garcia-Rivas G, Castillo EC, Gonzalez-Gil AM, et al. The role of B cells in heart failure and implications for future immunomodulatory treatment strategies. *ESC Heart Fail*. 2020;7(4):1387–1399. doi:10.1002/ehf2.12744
59. Tang TT, Ding YJ, Liao YH, et al. Defective circulating CD4CD25+Foxp3+CD127(low) regulatory T-cells in patients with chronic heart failure. *Cell Physiol Biochem*. 2010;25(4–5):451–458. doi:10.1159/000303050
60. Tang H, Zhong Y, Zhu Y, et al. Low responder T cell susceptibility to the suppressive function of regulatory T cells in patients with dilated cardiomyopathy. *Heart*. 2010;96(10):765–771. doi:10.1136/hrt.2009.184945
61. Wei Y, Yu K, Wei H, et al. CD4(+) CD25(+) GARP(+) regulatory T cells display a compromised suppressive function in patients with dilated cardiomyopathy. *Immunology*. 2017;151(3):291–303. doi:10.1111/imm.12728
62. Chen XM, Zhang T, Qiu D, et al. Gene expression pattern of TCR repertoire and alteration expression of IL-17A gene of gammadelta T cells in patients with acute myocardial infarction. *J Transl Med*. 2018;16(1):189. doi:10.1186/s12967-018-1567-7
63. Gil-Pulido J, Amezaga N, Jorgacevic I, et al. Interleukin-23 receptor expressing gammadelta T cells locally promote early atherosclerotic lesion formation and plaque necrosis in mice. *Cardiovasc Res*. 2021; 118(14):2932–2945. doi:10.1093/cvr/cvab359
64. Ch'En IL, Tsau JS, Molkentin JD, Komatsu M, Hedrick SM. Mechanisms of necroptosis in T cells. *J Exp Med*. 2011;208(4):633–641. doi:10.1084/jem.20110251
65. Bai L, Kong M, Duan Z, et al. M2-like macrophages exert hepatoprotection in acute-on-chronic liver failure through inhibiting necroptosis-S100A9-necroinflammation axis. *Cell Death Dis*. 2021;12(1):93. doi:10.1038/s41419-020-03378-w