



Identification and characterization of necroptosis-related differentially expressed genes in acute myocardial infarction: Insights into immune-related pathways and protein-protein interactions

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ABSTRACT

Acute myocardial infarction (AMI) is a serious cardiovascular medical emergency that can lead to death. Necroptosis, a programmed cell death pathway, has been implicated in the development and progression of AMI. Our study aimed to identify necroptosis-related differentially expressed genes (NRDEGs) in AMI and investigate their interactions and functions. The GSE66360 dataset was screened for NRDEGs using the 'limma' R package, with a threshold of $p < 0.05$. A set of 159 necroptosis-related genes (NRGs) was retrieved from the KEGG database. The protein-protein interactions (PPI) network was constructed using the STRING data resource. Molecular Complex Detection (MCODE) and cytoHubba plugin was applied to find the major modules and genes. Gene ontology (GO) and KEGG pathway analyses were performed using the R 'clusterProfiler' package. The enrichment scores for immune cell types and associated biological pathways or functions were gained using the ssGSEA method. Our study identified 5 down-regulated and 16 up-regulated NRDEGs in AMI. The PPI network analysis revealed several important modules and hub genes, including TNF, IL1B, TLR4, STAT3, NLRP3, TNFAIP3, CYBB, IFNGR1, FADD, and IL33. GO analysis revealed that NRDEGs were enriched in multiple biological processes, cellular components, and molecular functions, including those related to cytokine production, response to cytokine stimulus, and necroptotic process. NRDEGs were found to be particularly abundant in a number of non-disease pathways, such as necroptosis and immune-related pathways like cytokine-cytokine receptor interaction and TNF signaling pathway, according to KEGG pathway analysis. The ssGSEA analysis revealed a correlation between immune cells and NRDEGs in AMI. The study identified NRDEGs and their interactions in AMI, providing insights into the potential function of necroptosis in the pathological process of AMI. The results imply that immune-related pathways and cytokines may be crucial in the initiation and development of AMI. The study provides a foundation for further research on the underlying mechanisms of necroptosis in AMI and the potential for developing novel therapies.

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Introduction

Acute myocardial infarction (AMI) is a serious cardiovascular event characterized by the abrupt occlusion of coronary arteries, leading to ischemia and necrosis of the myocardial tissue (1). According to the World Health Organization (WHO), cardiovascular diseases (CVDs), including AMI, are the leading cause of death globally, accounting for approximately 17.9 million deaths annually (2). In the United States, the American Heart Association (AHA) reports that about 805,000 Americans suffer from AMI each year, and it is responsible for approximately one in every seven deaths in the country (3-5). Furthermore, the incidence of AMI increases with age, and men are more likely to experience AMI than women (6).

Necroptosis is a form of programmed necrosis mimicking features of apoptosis and necrosis (7). It is primarily regulated by receptor-interacting protein kinase 1 (RIPK1)- and RIPK3 and its substrate MLKL (8). Other genes and proteins are also involved in the regulation of necropto-

sis, including caspase-8, Fas-associated protein with death domain (FADD), a cellular inhibitor of apoptosis proteins (cIAPs), and TNF receptor-associated factor (TRAF) proteins. Dysregulation of these genes and proteins can lead to various pathological conditions associated with necroptosis, such as inflammation, autoimmune diseases, and cancer (9-11). It has also been shown to play a critical role in the pathogenesis of AMI (12). Zhu *et al.* (13) found that the necroptosis-related gene RIPK3 was upregulated in a mouse model of AMI and that ablation of RIPK3 protected against AMI-induced cardiac injury. Luedde *et al.* (14) revealed that overexpression of RIPK3 was able to induce necroptosis of cardiomyocytes. It implies that necroptosis might contribute to the pathogenesis of AMI. Interestingly, a study on the inhibition of RIP1 prevents adverse cardiac remodeling (15). Despite the identification of numerous necroptosis-related genes (NRGs), the underlying mechanisms of necroptosis in AMI remain poorly understood.

The objective of this study was to identify differentially expressed necroptosis-related genes (NRDEGs) in

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acute myocardial infarction (AMI) and investigate their potential roles in the disease. We conducted bioinformatic analysis of publicly available gene expression datasets and identified 21 NRDEGs that showed significant dysregulation in AMI. Additionally, we performed protein-protein interaction (PPI) network analysis and gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis to examine the interactions and functions of these NRDEGs. The findings from our study offer valuable insights into the molecular mechanisms underlying necroptosis in AMI, which may contribute to the development of effective diagnostic and therapeutic strategies for this disease.

Materials and Methods

Data source

A set of 159 necroptosis-related genes were obtained by downloading from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The mRNA expression profile dataset GSE66360 was downloaded from the GEO database (<http://www.ncbi.nlm.nih.gov/geo/>), which contains sequencing data in circulating endothelial cells from 50 healthy individuals versus 49 patients with acute myocardial infarction. The data were processed on the GPL570 platform ((HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array).

Necroptosis-related differentially expressed genes screening

Necroptosis-related differentially expressed genes (NRDEGs) were screened by the 'limma' R package with a screening threshold of $P < 0.05$. The visualization of NRDEGs was generated by using the R packages 'heatmap' and 'ggplot2'.

Protein-protein interaction network and correlation analysis of NRDEGs

The STRING database (<https://string-db.org/>) was used to predict protein-protein interactions between proteins. Then, hub genes and important modules in the PPI network were found using Cytoscape's (<https://cytoscape.org>) cytoHubba and MCODE plug-ins. The correlation analysis of NRDEGs was identified using Spearman correlation in the R software 'corrplot' package.

Functional enrichment analysis

GO enrichment and KEGG pathway analysis of NRDEGs were performed using the R 'clusterProfiler' package, and P values were corrected using the BH method, with a corrected P-value of < 0.05 as the threshold.

Immune infiltration analysis

Enrichment scores for immune cell types, biological pathways and functions were obtained using 'single-sample Gene Set Enrichment Analysis (ssGSEA)' method. Spearman's correlation was used to explore the association of NRDEGs and the composition and quantity of infiltrating immune cells, which were visualised using the R package 'ggcorrplot'.

Statistical analysis

Based on the data distribution features, the statistical significance of differences between the two groups was

determined using a non-parametric test or a t-test. All analyses were carried out with the R3.5.3 program, and a P-value of 0.05 was considered statistically significant.

Results

Identification of differentially expressed genes

We retrieved 159 necroptosis-related genes (NRGs) from the KEGG database. The R 'limma' package was used to screen necroptosis-related differentially expressed genes (NRDEGs) in the GSE66360 dataset. NRDEGs were screened out based on the threshold of $P < 0.05$. There were 16 genes upregulated and 5 genes downregulated in acute myocardial infarction (AMI). A volcano plot was generated from all 159 NRGs in the dataset (Figure 1A). We highlighted the ten most up-regulated and down-regulated genes in the dataset, including IL1B, GLUL, PYGL, NLRP3, CHMP4B, USP21, PPID, FADD, AIFM1, and PARP. Furthermore, a heat map (Figure 1B) was created to represent the expression levels of the 21 NRDEGs across the AMI and control groups. The average expression levels of these genes were also presented in Figure 1C.

NRDEGs interactions analysis

To explore the interactions between NRDEGs, we utilized the STRING database (<https://string-db.org/>) to conduct protein-protein interactions (PPI) analysis of NRDEGs, as depicted in Figure 2A. The outcome was subsequently subjected to MCODE analysis, which identified key modules including IL33, NLRP3, FADD, TNFAIP3, IL1B, CYBB, IFNGRI, TNF, and RARPI in the PPI network (Figure 2B). Additionally, we employed

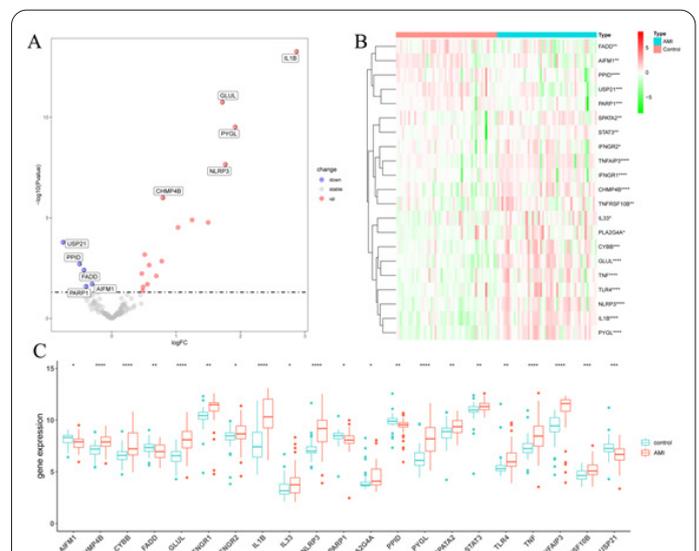


Figure 1. (A) Volcano plot of 159 NRGs with the five most significantly down-regulated genes and the five most significantly up-regulated genes highlighted and red dots denoting significantly up-regulated genes, blue dots denoting significantly down-regulated genes, and grey dots denoting genes with no differential expression. (B) Heat map of expression of NRDEGs, red indicates higher expression and green indicates lower expression. (C) The box-line plot of 21 differentially expressed autophagy-related genes in AMI samples versus healthy control samples, including 16 highly expressed genes and 5 down-regulated genes. Student t-test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. NRGs, necroptosis-related genes; NRDEGs, necroptosis-related differentially expressed genes.

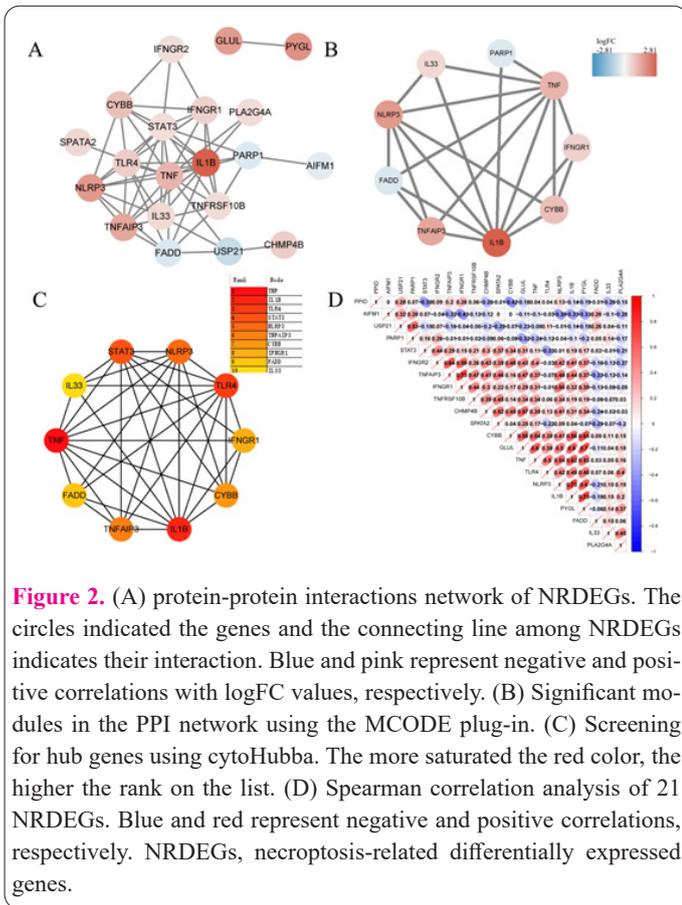


Figure 2. (A) protein-protein interactions network of NRDEGs. The circles indicated the genes and the connecting line among NRDEGs indicates their interaction. Blue and pink represent negative and positive correlations with logFC values, respectively. (B) Significant modules in the PPI network using the MCODE plug-in. (C) Screening for hub genes using cytoHubba. The more saturated the red color, the higher the rank on the list. (D) Spearman correlation analysis of 21 NRDEGs. Blue and red represent negative and positive correlations, respectively. NRDEGs, necroptosis-related differentially expressed genes.

cytoHubba to screen the top ten hub genes, which were ranked based on their importance in the network, namely TNF, IL1B, TLR4, STAT3, NLRP3, TNFAIP3, CYBB, IFNGR1, FADD, and IL33 (Figure 2C). Additionally, as shown in Figure 2D, we used the "corrplot" R package to perform Spearman correlation analysis among NRDEGs.

Functional enrichment analysis of NRDEGs

To enhance our understanding of NRDEGs, we utilized the R 'clusterProfiler' package to perform GO enrichment and KEGG pathway analysis. Our GO analysis showed significant enrichment of NRDEG in 1,028 biological processes (BP), 1 cellular component (CC) and 41 molecular functions (MF). Notably, the enriched terms included positive regulation of protein secretion, positive regulation of peptide secretion, positive regulation of cytokine production, regulation of protein secretion, regulation of response to cytokine stimulus, necroptotic process (BPs), phagocytic cup (CCs), interferon-gamma receptor activity, interferon receptor activity, steroid hormone receptor binding, oxidoreductase activity, acting on NAD(P)H, oxygen as acceptor, cytokine receptor binding (MFs), among others (Figure 3A).

Additionally, our KEGG pathway analysis results, presented in Figure 3B, revealed that NRDEGs exhibited high enrichment in these pathways, including necroptosis, NOD-like receptor signaling pathway, NF-kappa B signaling pathway, HIF-1 signaling pathway, and apoptosis. Importantly, rich enrichment of NRDEGs was also observed in immune-related pathways, including cytokine-cytokine receptor interaction, IL-17 signaling pathway, Th17 cell differentiation, and TNF signaling pathway.

Since our study primarily focused on BP terms, we further examined the relationships between BP terms, which are illustrated in Figure 4A. Notably, we identified 12 genes

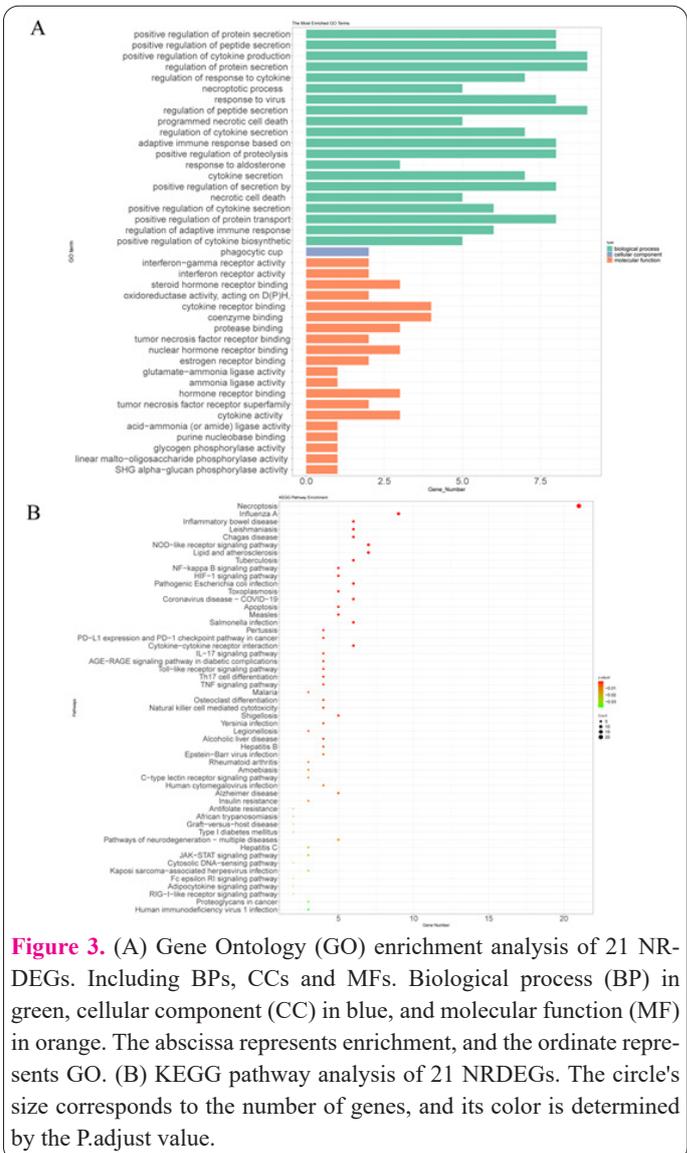


Figure 3. (A) Gene Ontology (GO) enrichment analysis of 21 NRDEGs. Including BPs, CCs and MFs. Biological process (BP) in green, cellular component (CC) in blue, and molecular function (MF) in orange. The abscissa represents enrichment, and the ordinate represents GO. (B) KEGG pathway analysis of 21 NRDEGs. The circle's size corresponds to the number of genes, and its color is determined by the P.adjust value.

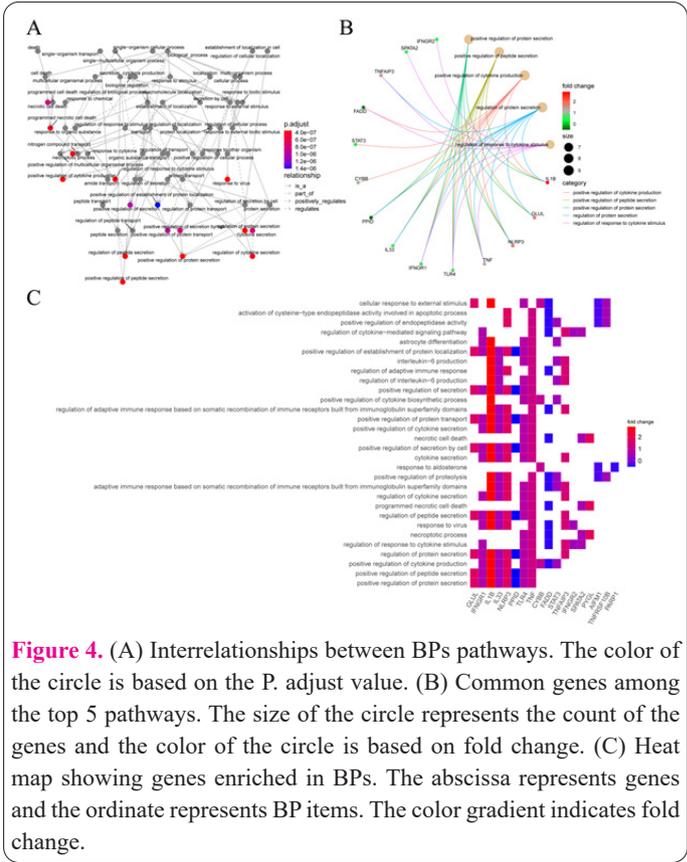


Figure 4. (A) Interrelationships between BPs pathways. The color of the circle is based on the P. adjust value. (B) Common genes among the top 5 pathways. The size of the circle represents the count of the genes and the color of the circle is based on fold change. (C) Heat map showing genes enriched in BPs. The abscissa represents genes and the ordinate represents BP items. The color gradient indicates fold change.

that were common to the top 5 BP enrichment pathways, including IFNGR2, SPATA2, TNFAIP3, FADD, STAT3, CYBB, PPID, IL33, IFNGR1, TLR4, TNF, NLRP3, GLUL, and IL1B (Figure 4B). Furthermore, we visualized the genes enriched in BP terms using a heatmap, as shown in Figure 4C.

Correlations of NRDEGs and immune cells

From the above results, NRDEGs were strongly enriched in immune pathways. The correlation between immune cells and NRDEGs was then assessed, by ssGSEA and the results are presented in Figure 5. Additionally, we examined the correlation between hub genes and immune cells.

Discussion

The present study aimed to investigate necroptosis-related differentially expressed genes (NRDEGs) in acute myocardial infarction (AMI) and their association with immune-related pathways and protein-protein interactions. To achieve this goal, we downloaded 159 necroptosis-related genes (NRGs) from the KEGG database and screened NRDEGs using the R 'limma' package in the GSE66360 dataset. Our results showed that 5 down-regulated and 16 up-regulated NRDEGs were identified in AMI.

We used the STRING database and cytoHubba to perform protein-protein interaction (PPI) analysis and identified 10 hub genes (TNF, IL1B, TLR4, STAT3, NLRP3, TNFAIP3, CYBB, IFNGR1, FADD, and IL33) in the PPI network. Moreover, we performed GO enrichment and KEGG pathway analysis using the R 'clusterProfiler' package to gain a deeper understanding of NRDEGs. It was indicated by GO analysis that NRDEGs were mainly enriched in immune pathways. NRDEGs were found to be particularly rich in pathways involved in necroptosis, NOD-like receptor signaling pathway, NF- κ B signaling pathway and other various pathways, according to KEGG pathway analysis.

The association of NRDEGs and immune cells was also demonstrated by ssGSEA, which indicated that NRDEGs appeared to be highly associated with immune-related pathways. Furthermore, IL1B, STAT3, TNF, and IFNGR1 hub genes were positively related to immune cell infiltration, whereas TNF6A, CYBB, and FADD hub genes were negatively related. It indicated that necroptosis may be associated with the immune response in AMI. Overall, our findings add to our understanding of how necroptosis and genes associated with it contribute to the development of AMI and suggest that inhibiting these genes may be a promising therapeutic strategy for the management of AMI (16,17).

This study emphasizes the potential of necroptosis-related genes as biomarkers for acute myocardial infarction (AMI). However, further investigation is necessary to validate these findings in a larger patient cohort and determine their clinical utility. Additionally, targeting necroptosis-related genes as a therapeutic strategy may hold promise for AMI treatment. Nevertheless, certain limitations should be acknowledged. Firstly, the modest sample size may limit the generalizability of the results. Secondly, the study solely focused on gene expression levels, without examining protein expression or activity, which could have provided a more comprehensive understanding of necroptosis-related

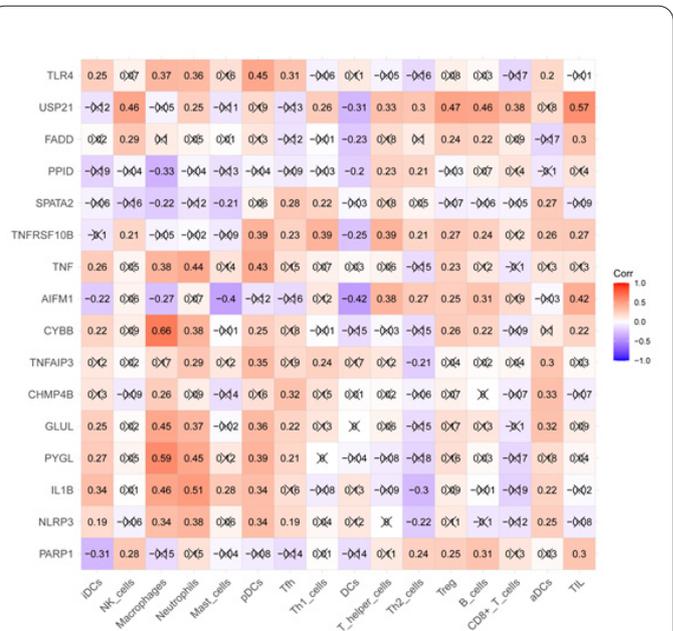


Figure 5. Correlation of immune cells and NRDEGs. The abscissa represents immune cell types and the ordinate represents genes. Red represents a positive correlation and blue represents a negative correlation.

gene function in AMI. Furthermore, exploring additional necroptosis-related genes in AMI is warranted, as this study only examined a small subset. Future research could delve into the mechanisms underlying the association between necroptosis-related genes and AMI. For instance, investigating whether these genes are involved in regulating inflammation, oxidative stress, or apoptosis—known contributors to AMI pathogenesis—would be worthwhile. Moreover, exploring potential interactions between necroptosis-related genes and other pathways implicated in AMI development would be of interest.

In conclusion, our study provides novel insights into the role of necroptosis-related genes in acute myocardial infarction and suggests potential therapeutic targets for the disease. However, further experimental studies are warranted to validate the findings and to elucidate the underlying mechanisms of necroptosis-related gene dysregulation in acute myocardial infarction.

Conflict of Interests

The authors declared no conflict of interest.

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