Construction and Analysis of Immune Infiltration-Related ceRNA Network for Coronary Artery Disease

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Abstract: This study investigated the role of ceRNA networks in coronary atherosclerosis, focusing on genes co-expression with immune cells. Data from the GEO database was used for GO/KEGG analysis of DE-mRNA, and ceRNA networks were constructed via Cytoscape using predicted lncRNA-miRNA-mRNA regulatory pairs. The CIBERSORT algorithm assessed immune cell proportions, revealing significant correlations between specific immune cells and hub mRNAs in the ceRNA network, particularly involving ESR1. The findings offer new insights into the molecular mechanisms of atherosclerosis and potential diagnostic and therapeutic targets.

***Keywords*:** atherosclerosis, ceRNA, immune cell infiltration, GEO, co-expression

1. Introduction

Coronary Artery Disease (CAD), characterized by coronary artery narrowing due to atherosclerosis, presents a significant global health challenge[1]. The role of genetics in CAD suggests exploring genomics for potential pathogenesis, biomarkers, and therapeutic targets could be crucial for its diagnosis, treatment, and prevention[2].

Non-coding RNA (NcRNA) is the RNA that is ubiquitous in the body without the function of encoding proteins.[3] MicroRNA (miRNA), as a kind of ncRNA, is defined as a class of endogenous small RNA with about 20-24 nucleotides[4]. The rapid advancement of gene sequencing has shown that ncRNAs are significantly involved in CAD[5]. Understanding the specific ways miRNAs regulate gene expression and their targets is crucial for studying CAD's mechanisms[6].

This study investigates how ceRNAs, including lncRNAs and mRNAs, impact RNA transcription by competing for miRNAs, forming a crucial regulatory network[7]. The goal is to understand ceRNA-related CAD pathogenesis and identify immune gene signatures for improved early diagnosis and non-invasive monitoring.

2. Methods

2.1 Data preprocessing

We processed three GEO datasets from NCBI [https://www.ncbi.nlm.nih.gov/geo], averaging values for genes represented by multiple samples.

2.2 Differential expression analysis and enrichment

We employed the "limma" package for differential analysis, setting thresholds of |log2(fold change)|>1 and P-value <0.05 for lncRNA and mRNA, and P-value <0.05 for DEmiRNA. GO/KEGG enrichment analysis of mRNA was performed using "clusterProfiler".

2.3 Construction of ceRNA regulatory network

The study mapped miRNA-mRNA and miRNA-lncRNA networks for CAD by identifying miRNAs targeting differential lncRNAs and mRNAs using databases like mircode (http://www.mircode.org), mirtargetbase (http://mirtarbase.mbc.nctu.edu.tw), and TargetScan (http://www.targetscan.org). These networks were then integrated with differentially expressed miRNAs to construct a comprehensive ceRNA network by Cytoscape.

2.4 Immune infiltration and co-expression analysis

The study employed the CIBERSORT algorithm to assess the proportions of 22 immune cell types in atherosclerotic and normal artery samples, identifying hub immune cells and mRNAs related to CAD development through a spearman co-expression analysis.

3. Results

3.1 Filtering differentially expressed genes

Differentially expressed lncRNAs, miRNAs, and mRNAs were identified through a comparison between normal and pathological tissues. Table 1 depicted details of three datasets.

Table 1. Information on mRNA, miRNA, and lncRNA datasets in CAD patients

	GEO dataset	Platform	Sample source	Sample size		Number of differential genes	
				pathological tissues	Normal tissues	Up-regulated	Down-regulated
mRNA	GSE40231	GPL570	Coronary atherosclerotic tissue	80	40	102	126
miRNA	GSE59421	GPL10850	blood	36	63	68	38
lncRNA	GSE113079	GPL20115	blood	93	48	152	54

3.2 GO and KEGG enrichment analysis

The study involved GO and KEGG enrichment analyses to explore the functions of lncRNA-associated DE-mRNAs. Figure 1A showed biological process (BP) enrichment in phosphorylation, rRNA processing, and mRNA splicing, with cell component (CC) enrichment in vesicle membranes related to ER to Golgi transport. Molecular function (MF) was enriched in nucleoside triphosphate and ATP binding activities. KEGG (Figure 1B) revealed significant pathways including the Rap1 signaling pathway and the Apelin signaling pathway.

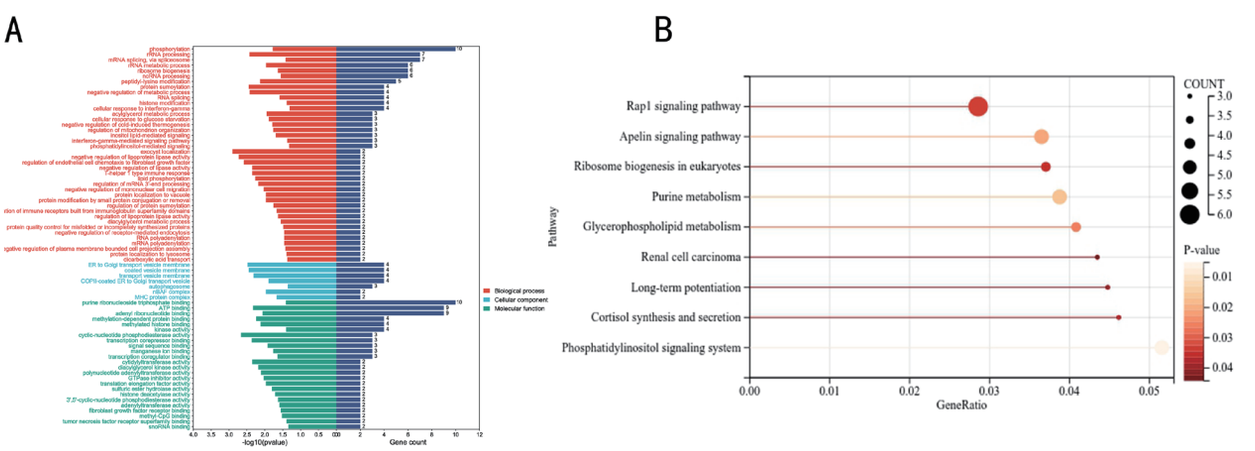

Figure 1. Enrichment analysis (A: GO analysis; B: KEGG analysis)

3.3 CeRNA Network construction

MiRcode datasets identified 160 interactions between 48 DE-lncRNAs and 11 DE-miRNAs. Mirtarbase and Targetscan datasets revealed these miRNAs target 1275 mRNAs, with a subset of 23 DE-mRNAs targeted by both up- and down-regulated DE-miRNAs, resulting in 29 interaction pairs (11 up, 18 down). A network with 11 miRNA nodes, 23 mRNA nodes, and 48 lncRNA nodes, illustrating 189 interactions, was visualized using Cytoscape in Figure 2.

3.4 Immune Infiltration Analysis

CIBERSORT revealed immune cell composition in coronary artery disease patients compared to controls, shown in Figure 3. Key findings include follicular helper T cells (Tfh) were negatively correlated with resting memory CD4T cells (R=-0.68), and activated mast cells negatively correlated with macrophage M2 (R=-0.56). In patients with coronary artery disease, naïve B cells, Tfh, neutrophils, and activated mast cells were significantly upregulated, whereas resting memory CD4T cells and T-cells γδ were downregulated.

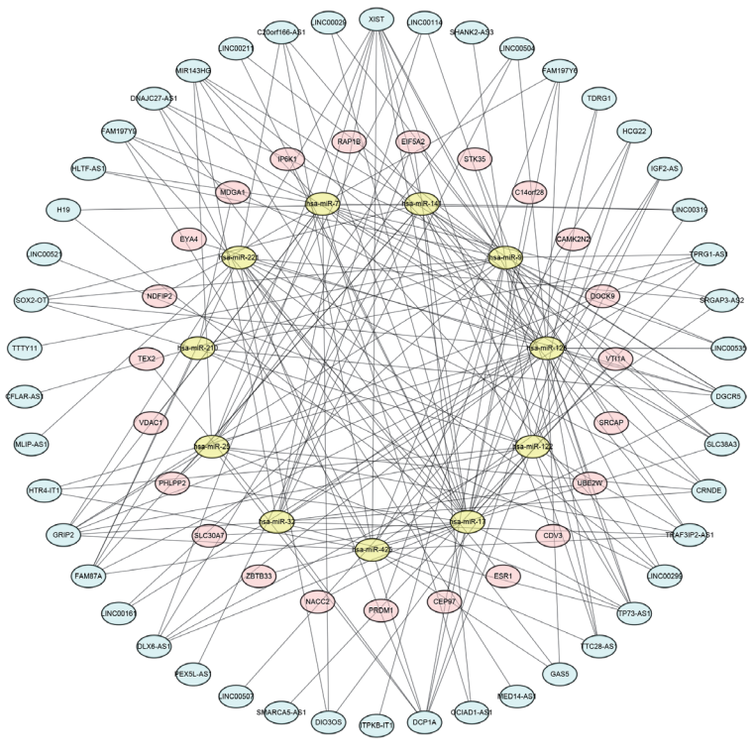

Figure 2. Construction of gene diagnosis model related ceRNA network

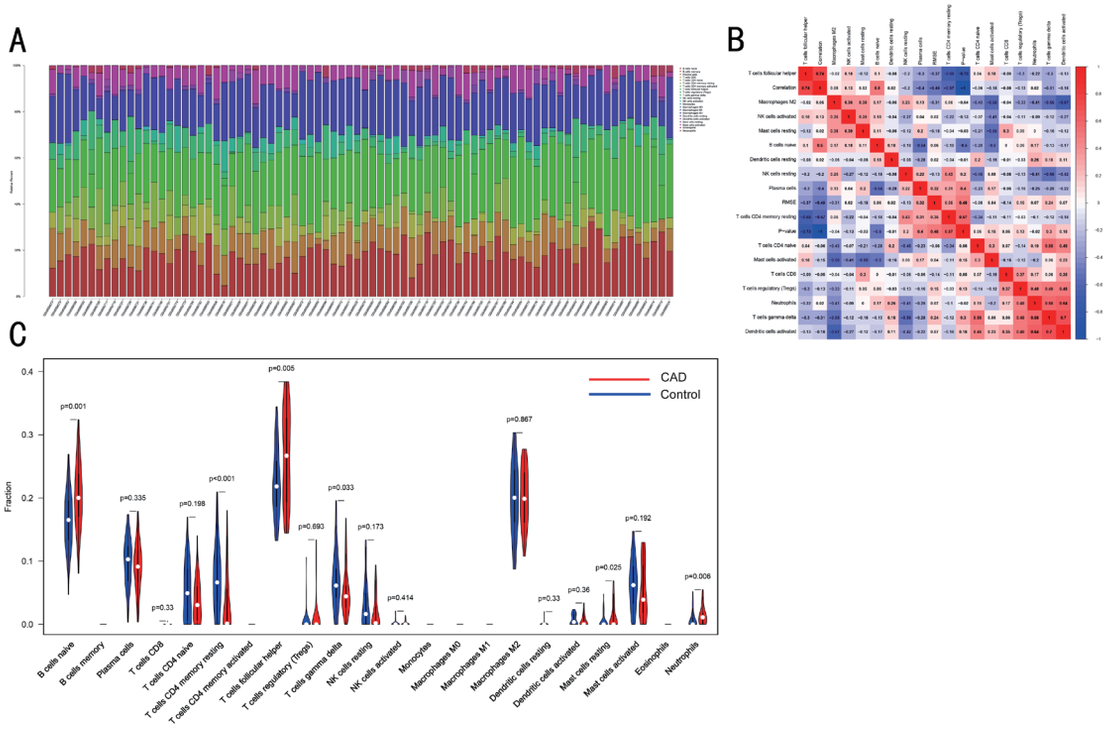

Figure 3. (A: Immune cell distribution per sample; B: Correlation among immune cells; C: Violin plot of infiltrating immune cells)

3.5 Immune Co-expression Analysis

The study revealed notable correlations between hub mRNAs and immune cells, including a negative correlation of Tfh cells with C14orf28 (R=-0.53), positive correlations of plasma cells with ESR1 (R=0.55), and a negative correlation of naïve CD4T cells with DOCK9 (R=-0.5). These correlations, indicating co-expression relationships, were visualized in Figure 4.

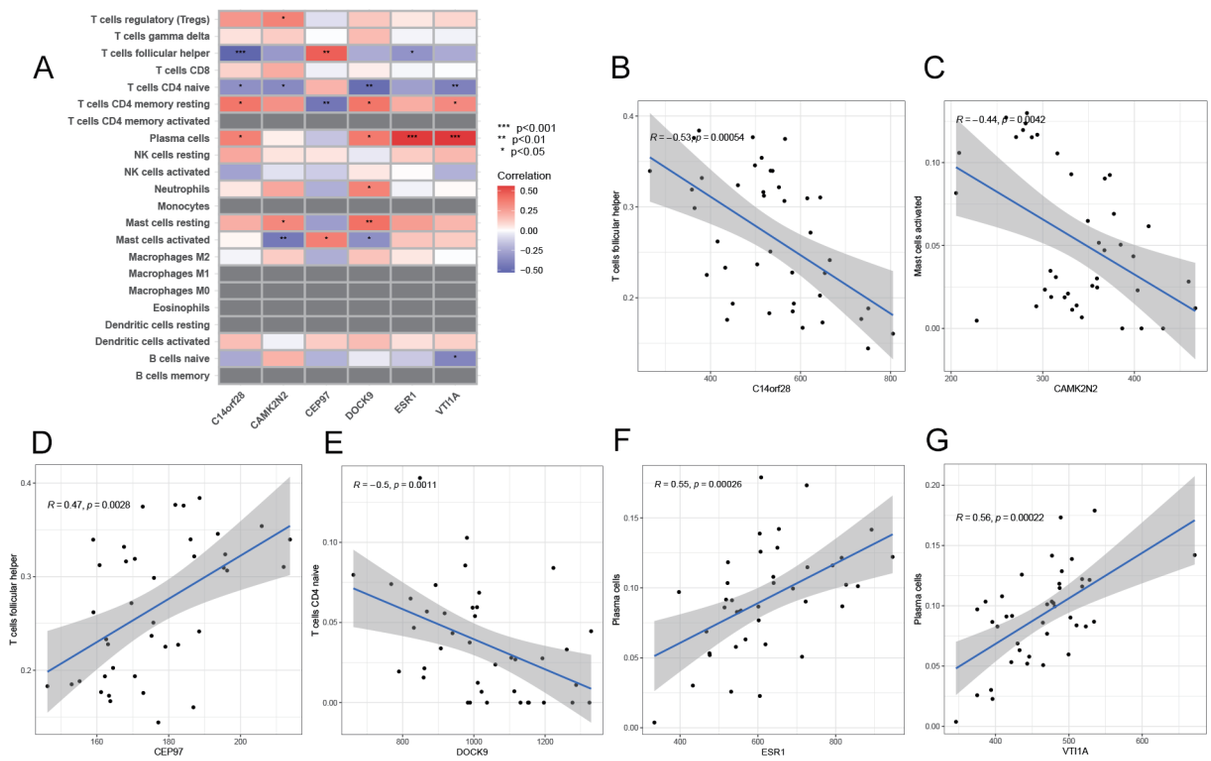

Figure 4. (A: The correlation between hub mRNA and immune cells; B-G: The correlation coefficient was significantly correlated with>0.4, P<0.05)

4. Discussion

CAD, a leading cause of cardiovascular disease globally and notably in China[8], is diagnosed via symptoms, exams, ECG, biomarkers, and angiography[9].Molecular research on CAD, particularly the roles of ncRNAs, RNA interactions, and the ceRNA network (involving mRNAs, ncRNAs, and lncRNAs in miRNA sponging and mRNA expression) [7], is advancing but still lacks clarity. Additionally, exploring the immune response, especially inflammatory cell infiltration in atherosclerosis[10], presents a further area for CAD research.

The study identified CAD-related miRNAs and target genes, constructing a ceRNA network. Significant variations in neutrophils, dendritic cells, and mast cells were found in CAD patient tissues versus normal tissues. A correlation between ESR1 and miR-221 and their link to Tfh cells was also noted.

Research links the ESR1 gene, encodes estrogen receptor α (ERα), to CAD, suggesting estrogen's protective effects in premenopausal women reduce CAD incidence[11]. Targeting ESR1 to modulate estrogen levels could be a viable treatment for coronary atherosclerosis. Additionally, mir-21 has been proposed as both a novel diagnostic marker for coronary artery stenosis severity and a potential therapeutic target[12].

Patients with CAD have higher levels of pro-inflammatory Tfh cells in their blood compared to healthy individuals. Tfh cells, crucial for B cell activation and germinal center formation, are also involved in atherosclerotic plaque regulation through interactions with Treg and B cells[13]. Significant correlations were found between Tfh cells and the expression of C14orf28, resting Neutrophils or Mast cells, and DOCK9, but direct evidence of these associations is yet unexplored. Further research is necessary to determine if these ceRNA hub genes influence CAD pathogenesis by affecting immune cells in the artery wall.

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