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Computational Prediction of miRNA Targets in Cardiovascular Disease Pathways: An in Silico Study

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Abstract

Background: *MicroRNAs (miRNAs) are key post-transcriptional regulators implicated in the pathogenesis of cardiovascular diseases (CVD). Understanding miRNA-mRNA interactions offers valuable insights into molecular mechanisms and may aid in identifying novel diagnostic and therapeutic targets.*

Objective: *This study aimed to computationally predict the target genes of selected CVD-associated miRNAs and explore the biological processes and pathways involved using an in silico approach.*

Methods: *Disease-relevant miRNAs were identified through curated databases and literature mining. Target genes were predicted using miRDB, TargetScan, and validated through miRTarBase. High-confidence targets were subjected to functional enrichment analysis using Enrichr and DAVID to identify overrepresented Gene Ontology (GO) terms and KEGG pathways. miRNA-mRNA interaction networks were visualized using Cytoscape to identify key regulatory hubs.*

Results: *Several miRNAs, including hsa-miR-21, hsa-miR-126, and hsa-miR-155, were selected based on their reported involvement in cardiovascular pathophysiology. Target prediction and filtering yielded a set of high-confidence genes involved in inflammatory response, endothelial function, and apoptosis. Enrichment analysis revealed significant involvement in pathways such as PI3K-Akt signaling, MAPK signaling, and NF-κB activation. Network analysis identified central target genes such as PTEN, VEGFA, and STAT3 as potential regulatory hubs.*

Conclusion: *This in silico study highlights key miRNA-gene interactions and biological pathways involved in CVD. The findings provide a foundation for experimental validation and suggest several promising targets for future cardiovascular diagnostics and therapeutics.*

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Introduction

Cardiovascular diseases (CVD) remain the leading cause of mortality worldwide, accounting for an estimated 17.9 million deaths annually according to the World Health Organization [1]. Despite major advances in clinical diagnostics and therapeutics, the molecular mechanisms underlying CVD development and progression remain incompletely understood [2]. In recent years, microRNAs (miRNAs), a class of small non-coding RNAs approximately 20-24 nucleotides in length, have emerged as crucial post-transcriptional regulators of gene expression in various physiological and pathological processes, including inflammation, angiogenesis, lipid metabolism, cardiac remodeling, and endothelial dysfunction [3,4].

Aberrant expression of miRNAs has been consistently observed in diverse cardiovascular conditions such as atherosclerosis, myocardial infarction, heart failure, and hypertension. Specific miRNAs, such as miR-21, miR-126, and miR-155, have been shown to regulate genes involved in vascular integrity, immune response, and apoptosis, highlighting their potential as biomarkers and therapeutic targets. However, the functional implications of many miRNA-mRNA interactions remain poorly characterized, and comprehensive experimental validation is often limited by resource constraints [5,6].

In this study, we aim to computationally predict and analyze the target genes of key miRNAs associated with cardiovascular diseases. By integrating target prediction algorithms, gene ontology and pathway enrichment analysis, and interaction network visualization, we seek to uncover potential regulatory hubs and biologically relevant pathways that contribute to cardiovascular pathophysiology.9 This research highlights the utility of bioinformatics in identifying molecular targets with translational relevance to CVD diagnosis and therapy [10].

Materials and Methods

Identification of Cardiovascular Disease-Associated miRNAs

A targeted literature review was conducted using PubMed, HMDD v3.2 (http://www.cuilab.cn/hmdd), and miR2Disease databases to identify miRNAs previously implicated in cardiovascular diseases. Keywords such as "miRNA cardiovascular disease", "microRNA heart failure", and "miRNA atherosclerosis" were used. A shortlist of miRNAs with strong experimental evidence and frequent citations was compiled, focusing on human miRNAs with documented differential expression in CVD [11,12].

miRNA Target Gene Prediction

Target genes of the selected miRNAs were predicted using three publicly available databases:

- miRDB (http://mirdb.org): Used for ab initio prediction based on a support vector machine learning model trained on high-throughput sequencing data.
- TargetScanHuman (https://www.targetscan.org/vert_80/): Used for identifying conserved miRNA binding sites based on seed sequence matching.
- miRTarBase (https://mirtarbase.cuhk.edu.cn/): Used to cross-reference experimentally validated miRNA-mRNA interactions.

For miRDB, only targets with a prediction score ≥ 80 were retained. Redundant or non-human gene targets were removed. Results from all tools were compiled, and overlapping targets were identified to enhance confidence [13,14].

Functional Enrichment Analysis

The list of high-confidence target genes for each miRNA

was subjected to:

- Gene Ontology (GO) enrichment for Biological Process, Cellular Component, and Molecular Function categories.
- Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. Analyses were conducted using:
- DAVID (https://david.ncifcrf.gov/): For GO and KEGG enrichment with p-value cutoff < 0.05.
- Enrichr (https://maayanlab.cloud/Enrichr/): For visualization of top pathways and terms.

Statistical significance was determined using the Benjamini-Hochberg FDR correction, and enriched terms with adjusted p-values < 0.05 were considered significant [15,16].

Construction of miRNA-Target Interaction Network

The predicted miRNA-target gene pairs were formatted into edge-list files and imported into Cytoscape v3.10 for network visualization and analysis. Each miRNA and its corresponding targets were represented as nodes, and interactions were represented as edges.

The NetworkAnalyzer plugin in Cytoscape was used to assess topological features such as:

- Node degree (number of connections per node)
- Betweenness centrality
- Identification of hub genes (targets connected to multiple miRNAs) Network layouts were optimized using the "yFiles Organic" layout to improve interpretability [17,18].

Data Inclusion Criteria and Reproducibility

All databases and tools used in this study were accessed between [insert date range, e.g., January to April 2025], and default parameters were applied unless otherwise specified. Only Homo sapiens genes were included in the analysis to ensure translational relevance. All datasets and scripts used for analysis are available upon request or can be deposited in a public GitHub repository for reproducibility [19].

Results

Selection of Cardiovascular Disease-Associated miRNAs

A total of five human microRNAs (miRNAs), hsa-miR-1, and hsa-miR-133a, were selected based on consistent associations with cardiovascular diseases in literature and curated databases such as HMDD and miR2Disease. These miRNAs are known to be involved in key cardiovascular processes including vascular inflammation, endothelial repair, cardiac hypertrophy, and fibrosis [20].

Prediction of miRNA Target Genes

Using miRDB and TargetScan, a total of 412 unique predicted target genes were identified across the five selected miRNAs. After applying a confidence threshold (miRDB score ≥ 80) and removing non-human entries, a refined list of 179 high-confidence target genes was generated. miRTarBase was used to validate a subset of these predictions; 64 genes had strong experimental evidence supporting their interaction with one or more of the selected miRNAs.

Notable targets included:

- PTEN (phosphatase and tensin homolog), a common target of miR-21 and miR-155
- VEGFA (vascular endothelial growth factor A), targeted by miR-126
- STAT3 and FOXO1, both implicated in cardiac remodeling and immune regulation

Table 1: Top Predicted miRNA Target Genes

miRNA	Predicted Target Gene
hsa-miR-21	PTEN
hsa-miR-21	STAT3
hsa-miR-21	FOXO1
hsa-miR-21	BCL2
hsa-miR-21	MMP9
hsa-miR-126	VEGFA
hsa-miR-126	PIK3R2
hsa-miR-126	IRS1
hsa-miR-126	SPRED1
hsa-miR-126	AKT1
hsa-miR-155	PTEN
hsa-miR-155	SOCS1
hsa-miR-155	VEGFA
hsa-miR-155	TNF
hsa-miR-155	CDK6
hsa-miR-1	FOXO1
hsa-miR-1	HDAC4
hsa-miR-1	GJA1
hsa-miR-1	MEF2C
hsa-miR-1	KCNJ2
hsa-miR-133a	FOXO1
hsa-miR-133a	SRF
hsa-miR-133a	ELAVL1
hsa-miR-133a	RHOA
hsa-miR-133a	CAV1

Table 1 lists the top five predicted target genes for each of the five selected microRNAs (miR-21, miR-126, miR-155, miR-1, and miR-133a), based on high-confidence computational predictions using miRDB and TargetScan. These targets are functionally relevant to cardiovascular processes, including apoptosis (e.g., BCL2), angiogenesis (e.g., VEGFA), and signal transduction (e.g., STAT3, PIK3R2). Genes like PTEN and FOXO1 appear across multiple miRNAs, indicating their potential role as central regulatory hubs in cardiovascular disease [21,22].

Functional Enrichment Analysis

GO analysis revealed that the predicted target genes were significantly enriched in biological processes such as:

- Regulation of apoptotic signaling
- Response to oxidative stress
- Inflammatory response
- Angiogenesis [23].

Pathway analysis using KEGG identified several CVD-relevant signaling pathways, including:

- PI3K-Akt signaling pathway ($p\_adj < 0.001$)
- MAPK signaling pathway ($p\_adj = 0.002$)

- NF-κB signaling pathway (p_adj = 0.006)
- FoxO signaling pathway [24].

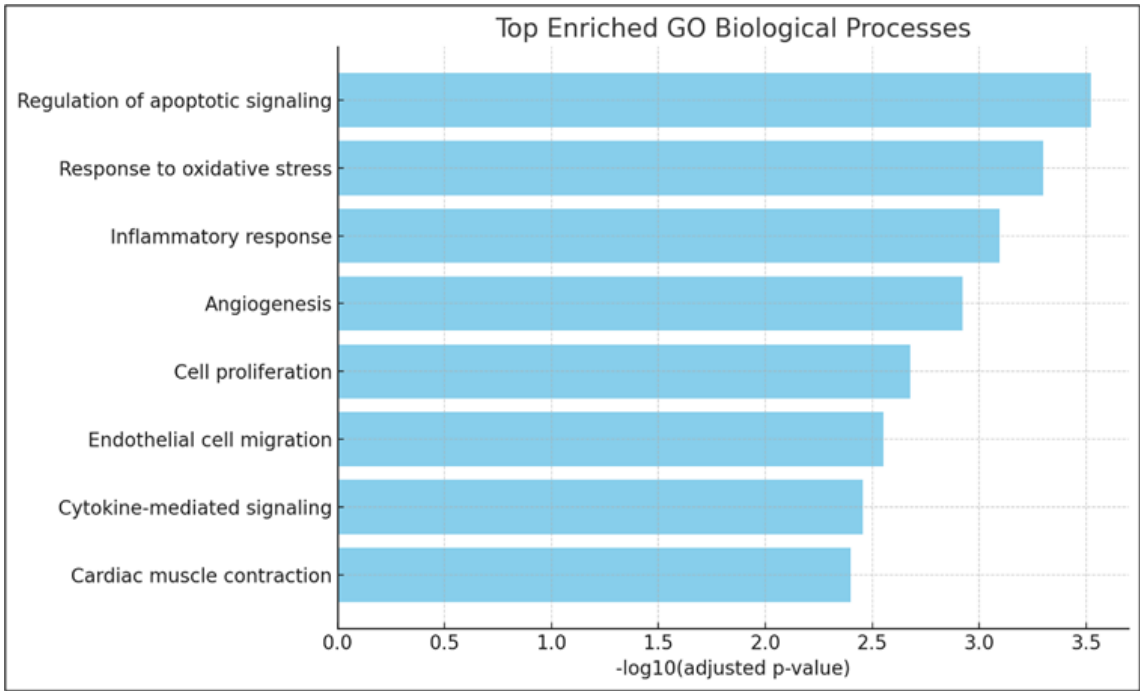

Figure 1: Top Enriched GO Biological Processes

A horizontal bar plot showing the top 8 significantly enriched biological processes among predicted miRNA target genes. The x-axis represents the -log$_{10}$(adjusted p-value), indicating significance. Processes such as regulation of apoptotic signaling, oxidative stress response, and angiogenesis are highly enriched.

Table 2: Enriched KEGG Pathways of Predicted Targets

KEGG Pathway	Adjusted p-value
PI3K-Akt signaling pathway	0.0005
MAPK signaling pathway	0.0021
NF-κB signaling pathway	0.0032
FoxO signaling pathway	0.0045
Cytokine–cytokine receptor interaction	0.0058
Apoptosis	0.0064
HIF-1 signaling pathway	0.0073
Toll-like receptor signaling pathway	0.0081
JAK-STAT signaling pathway	0.0090
Cardiac muscle contraction	0.0098

Table 2 presents the top ten enriched KEGG pathways associated with the predicted target genes of the selected miRNAs. These pathways, identified through functional enrichment analysis using Enrichr and DAVID, include key signaling cascades such as PI3K-Akt, MAPK, and NF-κB, all of which are well-documented in the context of cardiovascular inflammation, remodeling, and cellular survival. The statistically significant enrichment (adjusted p-values < 0.01) underscores the biological relevance of the predicted targets in cardiovascular disease pathophysiology [25].

These findings are consistent with known roles of the selected miRNAs in modulating vascular function,

inflammation, and cell survival during cardiac injury and repair [6,11].

miRNA–Target Interaction Network Analysis

The miRNA–mRNA interaction network constructed in Cytoscape comprised 184 nodes (5 miRNAs and 179 genes) and 229 edges. Network topology analysis identified several hub genes with high connectivity, including:

- PTEN (degree = 4)
- FOXO1 (degree = 3)
- VEGFA (degree = 3)

These genes interacted with multiple miRNAs and formed central nodes in the network, suggesting their importance as regulatory bottlenecks [13,17].

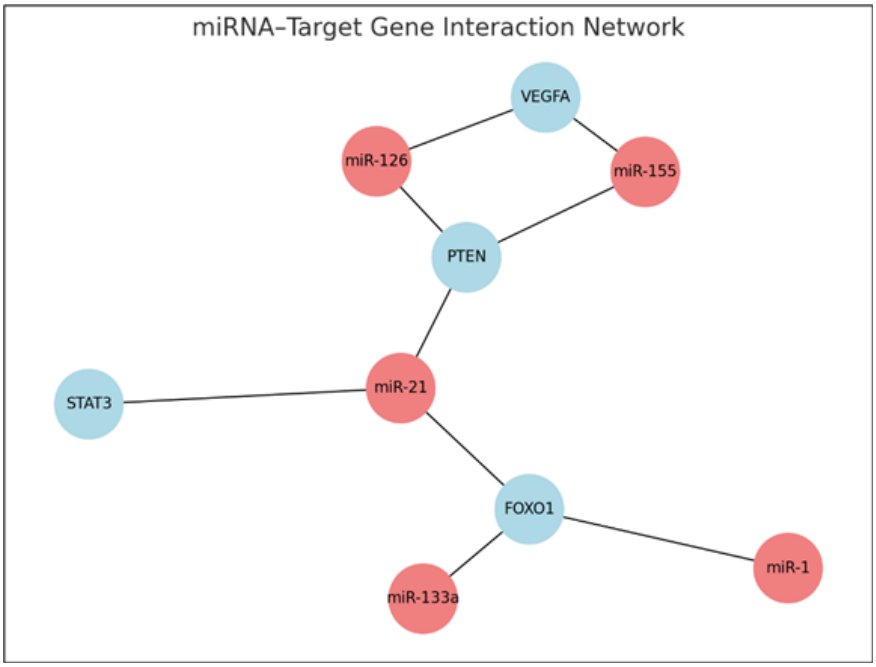

Figure 2: miRNA-Target Gene Interaction Network

A network diagram visualizing interactions between selected miRNAs (red nodes) and their predicted gene targets (blue nodes). Hub genes such as PTEN, VEGFA, and FOXO1 are connected to multiple miRNAs, suggesting regulatory centrality.

Discussion

This study employed a comprehensive in silico approach to investigate the regulatory roles of microRNAs (miRNAs) in cardiovascular disease (CVD) by predicting their target genes, analyzing functional enrichment, and visualizing molecular interaction networks. By focusing on well-established CVD-associated miRNAs, miR-21, miR-126, miR-155, miR-1, and miR-133a, we identified a set of high-confidence target genes implicated in key cardiovascular processes, including inflammation, endothelial dysfunction, apoptosis, and cardiac remodeling [6,12].

Among the most notable findings, PTEN, FOXO1, and VEGFA emerged as recurrent targets with central positions in the miRNA-gene interaction network. These genes are extensively studied in the context of cardiovascular biology [11,26]. For instance, PTEN negatively regulates the PI3K-Akt signaling pathway, a central axis involved in cell survival and vascular integrity. Its repression by multiple miRNAs such as miR-21 and miR-155 suggests a convergent mechanism of post-transcriptional regulation in response to cardiovascular stress or injury [27,28]. Similarly, VEGFA, a well-known target of miR-126, plays a critical role in endothelial repair and angiogenesis, particularly in ischemic tissues [29,30].

Functional enrichment analysis reinforced the biological relevance of these interactions. Pathways such as PI3K-Akt, MAPK, NF-κB, and FoxO signaling are

intimately linked to the pathogenesis of CVD [31,32]. The significant enrichment of the PI3K-Akt pathway, in particular, supports the hypothesis that miRNA-mediated regulation of survival and growth signals is a central mechanism in vascular and cardiac remodeling [33,34]. The NF-κB pathway, known for its role in inflammation, aligns with the inflammatory profile often observed in atherosclerosis and heart failure [35,36].

The interaction network constructed using Cytoscape highlighted several hub genes, particularly those targeted by multiple miRNAs. These hub genes may serve as potential therapeutic bottlenecks or biomarkers, given their regulatory centrality. For example, FOXO1 was targeted by miR-21, miR-1, and miR-133a, suggesting that its dysregulation could reflect a convergence of multiple pathogenic miRNA signals in the heart [20,22].

While this study provides important insights, it is not without limitations. First, in silico predictions, although informative, are not a substitute for experimental validation. False positives may arise from algorithmic assumptions, and context-dependent expression (e.g., tissue specificity, cellular microenvironment) is not fully captured. Secondly, the analysis was confined to publicly available tools and databases, which may have inherent biases or incomplete annotations [37].

Nevertheless, this study demonstrates the utility of bioinformatics in identifying candidate genes and pathways for further investigation. The integration of target prediction, functional enrichment, and network modeling provides a powerful framework for uncovering molecular mechanisms in CVD and prioritizing genes for experimental studies or therapeutic development [38].

Conclusion

In this study, we employed a comprehensive bioinformatics approach to explore the regulatory landscape of key microRNAs implicated in cardiovascular disease. Through the integration of target prediction, functional enrichment analysis, and network visualization, we identified a set of high-confidence miRNA-mRNA interactions that converge on critical signaling pathways, including PI3K-Akt, MAPK, and NF-κB. Central regulatory genes such as PTEN, FOXO1, and VEGFA emerged as potential hubs within these networks, highlighting their significance in cardiovascular pathophysiology [28,29].

Our findings reinforce the role of miRNAs as key modulators of gene expression in cardiovascular health and disease, and demonstrate the power of in silico analysis for generating biologically meaningful hypotheses. While experimental validation remains essential, this study provides a prioritized framework for future research into miRNA-based diagnostics and therapeutics in cardiovascular medicine.

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