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Bioinformatics Identification of Conserved microRNAs and miRNA-targeted Biomarkers for Cardiovascular Disease

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Abstract

Background: Cardiovascular diseases (CVD) are a leading cause of global mortality. MicroRNAs (miRNAs) regulate gene networks involved in endothelial dysfunction, vascular remodeling, and atherosclerosis as demonstrated in previous cardiovascular studies, yet conserved miRNAs and their target genes in CVD remain incompletely defined. Methodology: Two Gene Expression Omnibus (GEO) datasets, GSE118578 and GSE89188, were analyzed using GEO2R with quantile normalization to identify differentially expressed miRNAs (DEmiRNAs). Overlapping miRNAs were determined using Venny 2.0.2. Target genes were predicted via miRTarBase, TargetScan, and miRDB. Protein-protein interaction (PPI) networks were constructed using STRING, and hub genes were identified with Cytoscape and CytoHubba employing eleven topological algorithms. Results: Seven conserved DEmiRNAs: hsa-miR-1225-5p, hsa-miR-483-5p, hsa-miR-296-5p, hsa-miR-188-5p, hsa-miR-630, hsa-miR-557, and hsa-miR-1246 were consistently dysregulated. A total of 352 high-confidence target genes were initially predicted, of which 270 were retained for downstream PPI and enrichment analysis. PPI network analysis revealed five hub genes: *NOTCH1*, *RHOA*, *BCL2*, *GSK3B*, and *PTEN*, which are involved in key cardiovascular pathways including FoxO, mTOR, and Wnt signaling. Conclusion: This study systematically identifies conserved miRNAs and hub genes that regulate oxidative stress, inflammation, and cardiovascular dysfunction, offering potential biomarkers for early diagnosis and promising targets for therapeutic intervention.

Keywords

Cardiovascular disease, microRNA, Hub genes, Bioinformatics, Protein-protein interaction, Biomarkers

Article History

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1. Introduction

Cardiovascular disease (CVD) includes a wide range of problems that affect the heart, brain, and blood vessels. These conditions are often caused by high blood pressure, high cholesterol, and the buildup of fatty plaques in the arteries [1]. CVD covers illnesses such as acute myocardial infarction (AMI), acute coronary syndrome (ACS), stroke, heart failure (HF), unstable angina, valvular heart disease, arrhythmias, and congenital heart defects [2]. Unhealthy habits like smoking, poor diet, obesity, and lack of physical activity are major reasons for the rising number of CVD cases worldwide [3].

CVD remains the leading cause of death globally. In 2021, it caused about 20.5 million deaths, which was much higher than the 12.1 million deaths reported in 1990. More than 80 percent of these deaths occurred in low and middle income countries [4]. The number of people living with CVD also continues to rise, with 612 million cases recorded in 2021. In the United States, 941,652 deaths were linked to CVD in 2022, which was higher than the previous year. Although diagnostic tools and treatments have improved, CVD still places a heavy burden on health systems around the world [5].

Biomarkers play an important role in diagnosing CVD, predicting outcomes and guiding treatment decisions. Commonly used clinical biomarkers include cardiac troponins, creatine kinase MB (CK-MB), B-type natriuretic peptide (BNP), and C-reactive protein (CRP), whose circulating levels increase in response to myocardial injury, ischemia, or inflammation. These biomarkers are released into the bloodstream when the heart is stressed or damaged [6]. They help detect heart attacks, ischemia and other heart problems early [7]. However, many biomarkers also change in other diseases, which makes it difficult to find markers that are reliable and specific to CVD [8]. To improve biomarker discovery, researchers are now using genomics, proteomics and metabolomics [9,10]. Proteomics plays a critical role in exploring the composition and variation of cellular, tissue or organismal proteomes, elucidating the underlying mechanisms of physiological and pathological changes in organisms [9]. Proteomics studies have identified promising biomarkers such as GDF 15, KIM and WFDC2 [11]. Large genetic studies have also shown that genes like *APOA1*, *APOB*, *TP53* and *PPARG* play important roles in lipid disorders and atherosclerosis [12]. While these biomarkers are valuable for early detection of heart attacks, ischemia, and HF, many of them also show altered levels in other disease conditions, limiting their specificity for CVD. To improve biomarker discovery, researchers are now using genomics, proteomics, and metabolomics.

Recent years have also seen progress in new therapies, including gene therapy, protein therapy, cell therapy and exosome-based treatments. These therapies target important pathways involved in inflammation, cell death, fibrosis and the formation of new blood vessels [13,14]. Pathways such as TLR4, MyD88, NF-κB, TGF-β, SMADs, Notch, Hippo YAP, RhoA ROCK, Wnt, β catenin and PI3K, Akt are known to play major roles in how the heart responds to injury [15,16].

Although progress has been made, several important gaps remain. Many studies focus on a single biomarker or a single dataset, which makes the findings difficult to repeat in different populations. Only a few studies try to identify miRNAs that are consistently changed across more than one dataset. There is also limited strong validation of miRNA and mRNA interactions, since many studies depend on just one prediction tool. Another major gap is that we still do not fully understand how these miRNAs and their target genes work together in networks or how they affect key pathways related to inflammation, fibrosis, oxidative stress and cell survival. Without this network level information, it is challenging to develop reliable diagnostic tools or targeted treatments.

To help close these gaps, this study uses an integrated bioinformatics approach to identify miRNAs that are consistently dysregulated in CVD across two datasets. We then predict their target genes using the three main databases miRTarBase, TargetScan and miRDB to improve confidence in the results. Protein-protein interaction (PPI) networks are built, and several algorithms in CytoHubba are used to identify the most important hub genes. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses are performed to understand the biological functions and pathways related to these genes. Finally, disease association analysis is carried out to see how these genes may be linked to other health conditions. By combining all these steps, this study provides a clearer understanding of miRNA gene networks in CVD and offers potential pathways for improving early diagnosis, risk prediction and treatment strategies.

2. Methodology

2.1 Materials and Resources

All datasets analyzed in this study were obtained from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). Differential expression analysis of miRNAs was performed using the GEO2R web-based tool (https://www.ncbi.nlm.nih.gov/geo/geo2r/), which is based on the limma R package. Target gene prediction for the conserved miRNAs was conducted using three widely used databases: miRTarBase (https://mirtarbase.cuhk.edu.cn/), which provides experimentally validated miRNA-target interactions; TargetScan (https://www.targetscan.org/), which predicts targets based on sequence complementarity and evolutionary conservation; and miRDB (https://mirdb.org/), which uses a machine learning-based prediction algorithm. PPI networks of target genes

were constructed using the STRING database (https://string-db.org/) and visualized with Cytoscape software (version 3.10.3; https://cytoscape.org/). Hub gene identification was performed using the CytoHubba plugin within Cytoscape. Functional enrichment analyses, including GO and KEGG pathway analyses, were carried out using the Database for Annotation, Visualization and Integrated Discovery (DAVID; https://david.ncifcrf.gov/). All analyses were conducted using publicly available tools and databases to ensure transparency, reproducibility, and accessibility of the computational workflow.

2.2 Data Collection

MicroRNA (miRNA) expression data were obtained from the GEO (https://www.ncbi.nlm.nih.gov/gds), a public repository maintained by NCBI [17]. Only Homo sapiens datasets profiling non-coding RNAs were included, while synthetic construct datasets were excluded. We focused on CVD GEO Series (GSEs) related to each. Based on the presence of differentially expressed miRNAs (DEmiRNAs), two datasets were selected: GSE118578, and GSE89188. GSE118578 profiles miRNAs from peripheral whole blood, which reflects systemic cardiovascular and inflammatory changes and is commonly used for biomarker discovery. GSE89188 profiles miRNAs from bone marrow, a key hematopoietic tissue involved in immune regulation, inflammation, and vascular repair processes that are closely linked to CVD progression. Dataset details, including sample size, tissue or cell source, and case/control distribution, are provided in Table 1. These datasets include peripheral whole blood, and Bone marrow offering diverse insights into CVD-associated miRNA regulation.

Table 1. Summary of selected GEO datasets for miRNA analysis in CVD.

GSE Number	Sample Size	Source of Cell/Tissue	Case Sample	Control Sample	Data Type	Species
GSE118578	8	Whole blood	4	4	miRNA	Human
GSE89188	8	Bone marrow	6	2	miRNA	Human

2.3 Data Preprocessing, Normalization and Differential Expression Analysis

Differential miRNA expression analysis was performed using GEO2R online tool, which uses the limma package to compare case and control groups [18]. Raw expression data were first processed using background correction and quantile normalization to make the samples comparable. Log2 transformation was applied automatically where required. To control for false positive results caused by multiple testing, Benjamini-Hochberg false discovery rate (FDR) correction was used. We used adjusted $p < 0.05$ and |log fold change |log2 fold change| >1 as thresholds for identifying significantly DEmiRNAs. miRNAs with log2 fold change >2 were considered upregulated, and log2 fold change < -2 were considered downregulated [19]. Before statistical analysis, duplicate entries and missing values were removed to improv data quality and ensure reliable results.

2.4 Identification of Common miRNAs

To identify overlapping miRNAs across datasets, we used the Venny 2.0.2 web tool [20]. Out of the 336 DEmiRNAs identified in GSE118578, 7 were also found among the 71 DEmiRNAs from GSE89188.

2.5 Biomarkers Prediction of Targeted miRNAs

To identify reliable target genes of common DEmiRNAs, we utilized three databases: miRTarBase [21], TargetScan [22], and miRDB [23]. In miRTarBase, only targets with functional miRNA-target interactions (MTIs) validated by qRT-PCR or Western blot were selected. From TargetScan, we included targets with a cumulative weighted context ++ score < 0 and binding site types (8mer, 7mer-m8, 7mer-A1, 6mer) with values ≥1. In miRDB, we selected targets with a prediction score >90 to ensure high confidence. After merging and removing duplicates across all datasets, we identified 352 unique high-confidence target genes, of which 270 genes with STRING-supported interactions were retained for downstream PPI and enrichment analyses. These high-confidence target genes were subsequently used to construct an integrated miRNA-mRNA regulatory network, linking conserved miRNAs to prioritized hub gene targets.

2.6 PPI Network Analysis and Potential Hub Genes Identification

A PPI network of the predicted target genes was constructed using the STRING database with a confidence score of 400 [24]. STRING database using a medium confidence interaction score threshold of 0.4, with disconnected nodes hidden. The network was visualized and analyzed in Cytoscape v3.10.3. Hub genes were identified using the CytoHubba plugin [25], applying eleven topological algorithms including MCC, MNC, DMNC, Degree, EPC, Eccentricity, Bottleneck, Closeness, Radiality, Betweenness, and Stress [26]. In CytoHubba, genes were ranked independently by each of the eleven algorithms, and genes appearing within the top 10 ranked genes in at least eight of the eleven methods were selected as hub genes. Overlaps across ranking methods were visualized with an UpSet plot generated using SRplot [27].

2.7 Functional Enrichment Analysis

Functional annotation of the identified genes was performed using the DAVID v6.8 database, with homo sapiens selected as the background. P-values were adjusted using the Benjamini-Hochberg method, and terms with adjusted $p < 0.05$ were considered significant [28]. We analyzed GO terms, including Biological Process (BP), Molecular Function (MF), and Cellular Component (CC), and KEGG pathways [29]. Statistical significance was determined using an adjusted $p < 0.05$.

3. Results

3.1 DEmiRNA Recognition in CVD

After normalization of the GSE118578 and GSE89188 datasets, differential expression analysis was performed to identify miRNAs associated with CVD. The volcano plot in Figure 1 shows the distribution and expression patterns of the identified DEmiRNAs. In GSE118578, a total of 336 miRNAs were differentially expressed, including 6 upregulated and 330 downregulated miRNAs. In GSE89188, 78 DEmiRNAs were identified, with 27 upregulated and 51 downregulated. The total number of profiled miRNAs and the corresponding DEmiRNAs for each dataset are summarized in Table 2. The complete differential expression results for each dataset are provided in Supplementary Data File 1 (GSE89188) and Supplementary Data File 2 (GSE118578).

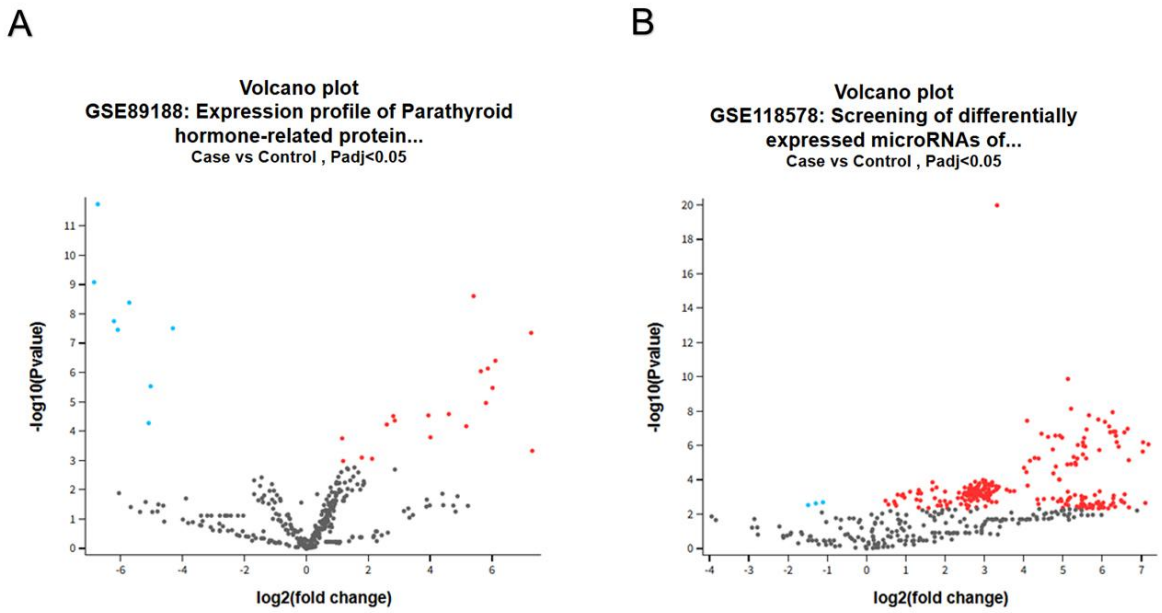

Figure 1. Volcano plots showing differentially DEmiRNAs associated with CVD in (A) GSE89188 and (B) GSE118578.

Table 2. Overview of miRNA expression profiles and demirna counts.

GSE Number	Total miRNA	DEmiRNA	Upregulated	Downregulated
GSE 118578	2570	336	6	330
GSE 89188	936	78	27	51

3.2 The Identification of Common DEmiRNAs

To identify shared miRNAs between the datasets, we used a Venn diagram analysis. As shown in Figure 2, seven common DEmiRNAs were found in both datasets. These include hsa-miR-1225-5p, hsa-miR-483-5p, hsa-miR-296-5p, hsa-miR-188-5p, hsa-miR-630, hsa-miR-557, and hsa-miR-1246. Figure 3 illustrates the expression changes of these miRNAs. Several additional miRNAs, such as hsa-miR-501-214-5p, hsa-miR-654-3p, and hsa-miR-324-3p, were also consistently downregulated. Heatmaps illustrating the expression patterns of the seven conserved miRNAs in each dataset are provided in Supplementary Figure S1A and S1B.

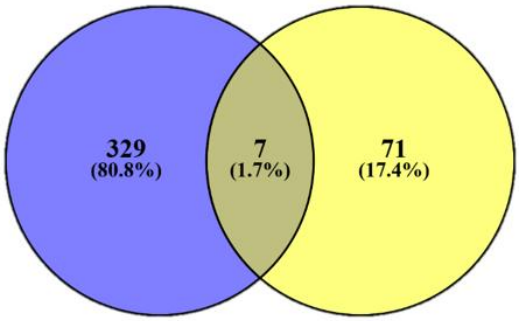

Figure 2. Venn diagram showing common DEmiRNAs in GSE118578 and GSE89188.

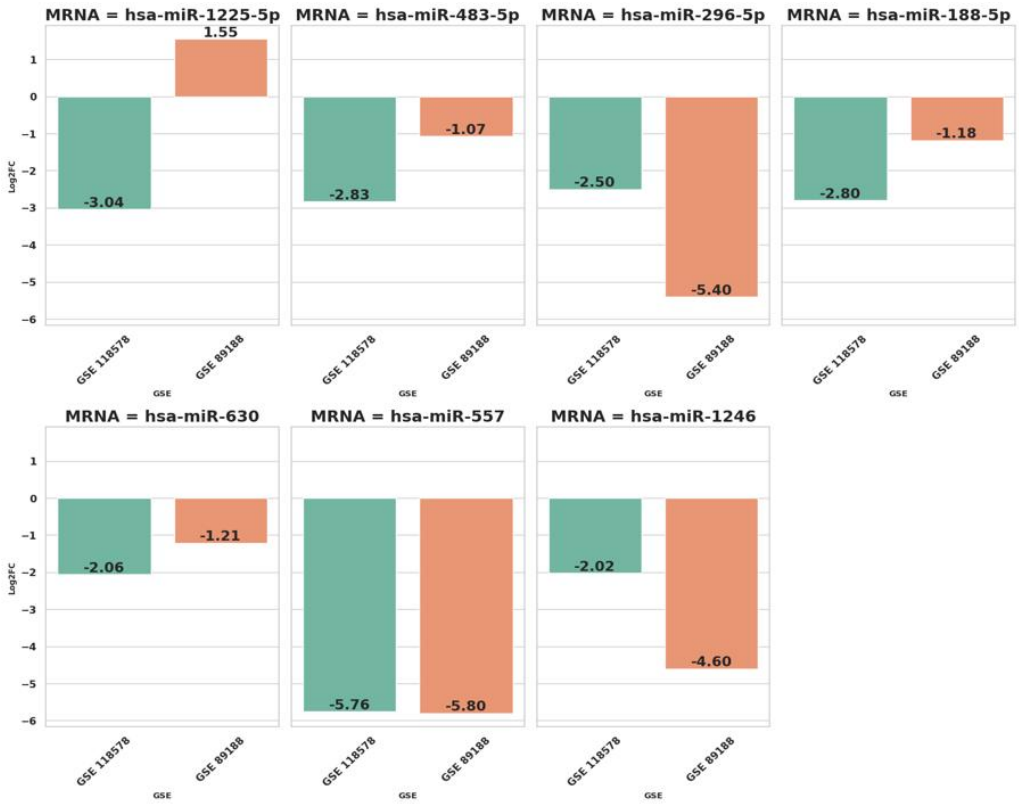

Figure 3. Expression patterns of the seven common dysregulated miRNAs.

3.3 miRNA Targeted Gene Prediction, PPI Analysis, and Hub Gene Selection

Target genes of the seven common DEmiRNAs were predicted using miRTarBase, TargetScan, and miRDB. After removing duplicate entries and excluding genes without STRING-supported interactions, a total of 270 high-confidence target genes were retained across all databases from an initial pool of 352 predicted targets (Table 3). For example, hsa-miR-483-5p had 10 predicted targets, hsa-miR-1225-5p had 11, hsa-miR-296-5p had 29, and hsa-miR-557 had the highest number with 116.

Table 3. Predicted target genes of common DEmiRNAs.

Common DEmiRNAs	miRTarBase	Targetscan	miRDB	Total Target Genes
hsa-miR-1225-5p	1	2	8	11
hsa-miR-483-5p	8	1	1	10
hsa-miR-296-5p	19	1	9	29
hsa-miR-188-5p	4	1	22	27
hsa-miR-630	12	15	21	48
hsa-miR-557	0	24	92	116
hsa-miR-1246	4	11	14	29

A PPI network was created using the STRING database, resulting in a network containing 254 nodes and 476 edges (Figure 4A). The CytoHubba tool was used to identify hub genes based on eleven topological algorithms. Genes consistently ranked among the top results across most algorithms were selected as hub genes. As shown in Figure 4B, the final hub genes included *NOTCH1*, *RHOA*, *BCL2*, *GSK3B*, *PTEN*, *FBXW7*, and *IGF1*.

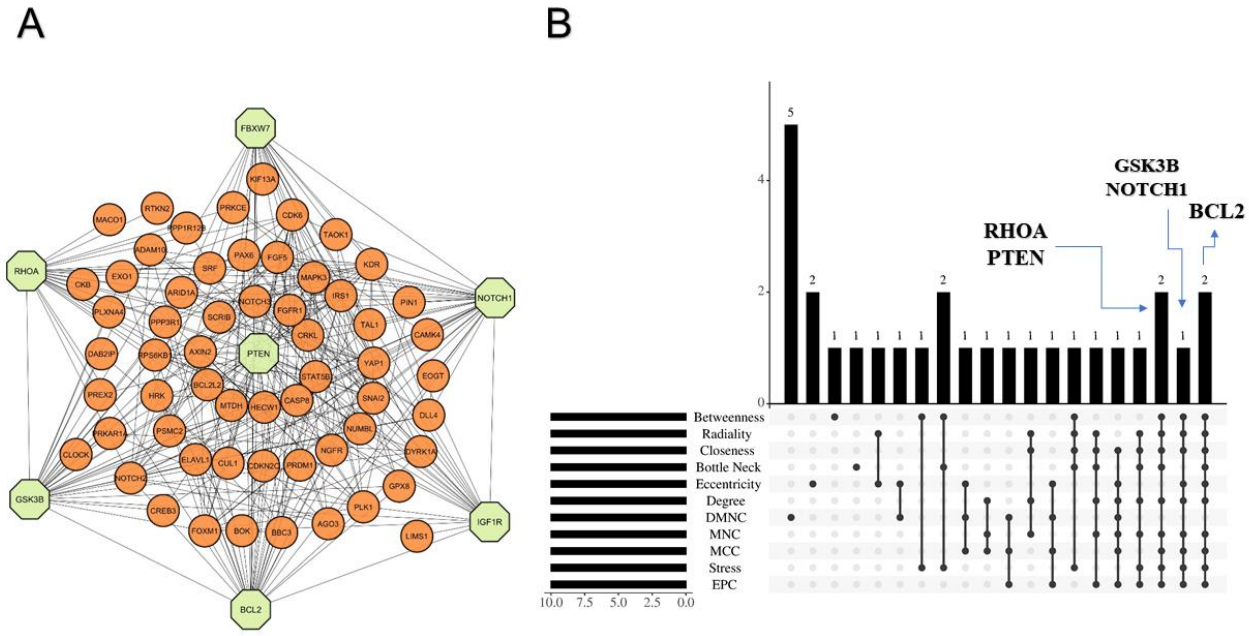

Figure 4. Hub-gene identification. (A) PPI network analysis of hub genes, (B) Upsetplot of 11 topological algorithms.

3.4 GO Enrichment and KEGG Pathway Analysis

Functional enrichment analysis was carried out for the identified hub genes. GO enrichment revealed 97 significant terms, including 52 biological processes, 23 CC, and 22 MF ($p < 0.05$). The main BP involved neuron differentiation, protein dephosphorylation, and regulation of RNA polymerase II transcription. CC terms were enriched in nuclear structures, intracellular organelles, synapses, and dendritic spines. MF terms mainly included protein tyrosine phosphatase activity, DNA binding, and protein binding. The top GO terms are shown in Figure 5A.

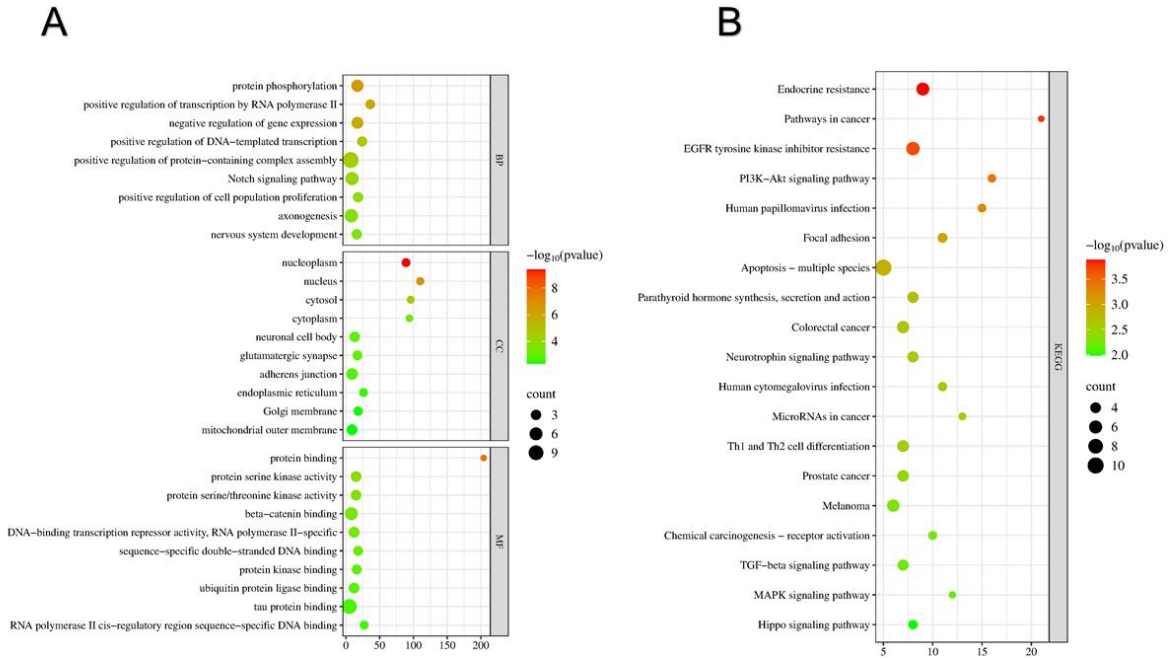

Figure 5. Functional enrichment analysis. (A) GO enrichment of hub genes across BP, CC, and MF categories and (B) KEGG pathway enrichment showing key signaling pathways associated with the hub genes.

KEGG pathway analysis showed in Figure 5(B) that the hub genes were significantly enriched in key regulatory pathways related to aging, cell survival, and disease. The FoxO signaling pathway, mTOR signaling pathway, and Wnt signaling pathway were strongly represented. Several cancer-related pathways, including hepatocellular, breast, and prostate cancer pathways, were also enriched, highlighting shared mechanisms between aging, cancer, and cardiovascular dysfunction. Additional pathways such as the p53 signaling pathway and long-term depression were also identified. Many of the enriched pathways, including FoxO, mTOR, Wnt, and p53 signaling, are well established regulators of oxidative stress, inflammatory responses, and cellular survival in CVD, providing mechanistic context for the observed enrichment patterns.

3.5 Integrated miRNA-mRNA Regulatory Network

An integrated miRNA-mRNA regulatory network was constructed to visualize the interactions between the conserved DEmiRNAs and their predicted hub gene targets. The network illustrates regulatory relationships between miRNAs and key hub genes and shows how multiple miRNAs converge on central genes involved in CVD related pathways. This systems level representation integrates differential expression analysis, target prediction, and network-based prioritization to support the biological relevance of the identified miRNAs. The miRNA-mRNA regulatory network is shown in Figure 6. The miRNA-hub gene interaction pairs used to construct the regulatory network are provided in Supplementary Data File 3.

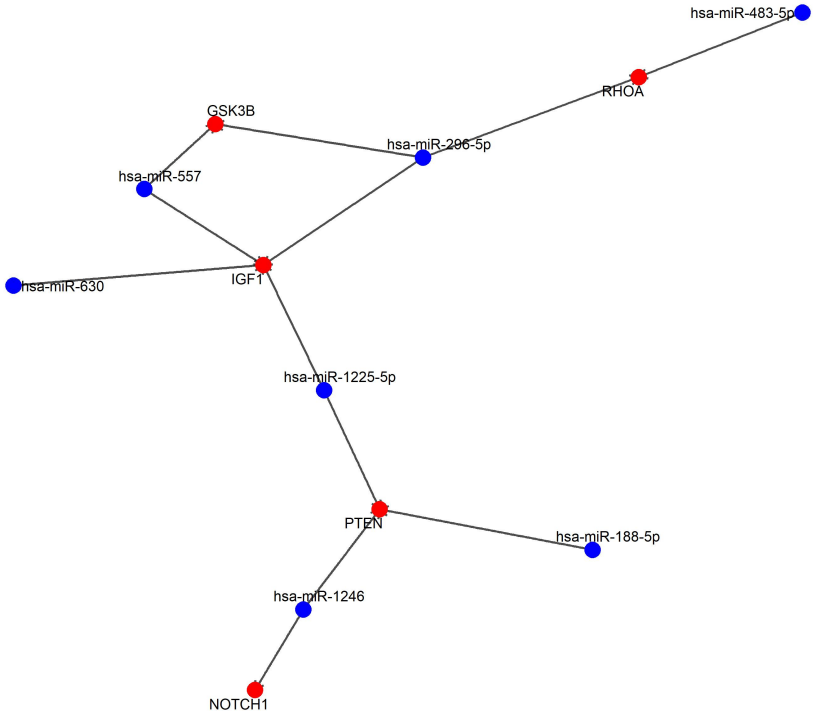

Figure 6. Integrated miRNA-mRNA regulatory network linking conserved miRNAs to hub genes.

3.6 Sensitivity Analysis of Conserved miRNAs

Sensitivity analysis was performed to evaluate the robustness of conserved miRNA identification under different statistical thresholds. Adjusted p value and absolute log2 fold change cutoffs were systematically varied, and the overlap of conserved miRNAs between datasets was re-evaluated. The majority of conserved miRNAs remained stable across reasonable threshold variations, indicating that the findings were not driven by a single arbitrary cutoff. A heatmap summarizing the overlap patterns across threshold combinations is shown in Supplementary Figure S2, and the corresponding quantitative results are provided in Supplementary Table S1.

4. Discussion

This study used an integrative bioinformatics approach to identify key miRNAs and hub genes involved in CVD. By integrating differential expression analysis across independent datasets, multi-database target prediction, and network-based prioritization, we aimed to improve the robustness and biological relevance of candidate biomarkers. The results show that seven conserved miRNAs such ashsa-miR-1225-5p, hsa-miR-483-5p, hsa-miR-296-5p, hsa-miR-188-5p, hsa-miR-630, hsa-miR-557 and hsa-miR-1246 are consistently dysregulated in cardiovascular conditions. Many of these miRNAs have been linked to heart injury, inflammation and vascular dysfunction in earlier studies, which supports their biological relevance in

CVD [30]. Their consistent appearance across two independent GEO datasets highlights their potential use as reliable biomarkers for early detection or monitoring of CVD.

Several of these miRNAs have well-defined roles in cardiovascular biology. Hsa-miR-483-5p has been reported to influence cardiac dysfunction [31] Hsa-miR-296-5p shows diagnostic value in HFpEF and coronary artery disease [32]. Hsa-miR-188-5p affects the renin-angiotensin system and angiogenesis, suggesting a possible role in heart repair and remodeling [33]. Hsa-miR-630 has been associated with aortic stenosis and metabolic-cardiovascular overlap [34] Although hsa-miR-557 and hsa-miR-1246 have been studied mainly in cancer and COPD [35]. and hsa-miR-1246 was significantly downregulated in COPD patients with emphysema after long-term follow-up, their roles in CVD remain unclear [36], their dysregulation in our datasets points to possible new functions in CVD that should be examined further. These miRNAs may represent novel regulatory elements linking inflammation, metabolic stress, and cardiovascular remodeling, warranting further investigation.

The predicted target genes of these miRNAs formed a PPI network from which seven hub genes were identified. These genes include *NOTCH1*, *RHOA*, *BCL2*, *GSK3B*, *PTEN*, *FBXW7* and *IGF1*. These hub genes occupy central positions within the interaction network, indicating their potential role as key regulatory nodes mediating miRNA-driven effects in CVD. *PTEN* regulates vascular smooth muscle cell behavior and is reduced in atherosclerotic or remodeled arteries [37]. *NOTCH1* mutations contribute to congenital heart defects and valvular disease [38]. *FBXW7* affects cardiomyocyte hypertrophy and endothelial barrier function [39]. *RHOA* is involved in oxidative stress and is elevated in coronary artery disease [40]. Reduced *RHOA* expression has also been associated with altered mitophagy in cardiomyopathy [41]. *BCL2* protects cardiomyocytes from apoptosis and is elevated in HF patients [42]. *IGF1* levels show a U-shaped relationship with cardiovascular risk, where both low and high levels predict greater disease burden [43] Collectively, these findings provide strong support for the involvement of the identified hub genes in CVD progression.

Pathway analysis indicated enrichment in PI3K/Akt, FoxO, mTOR, and Wnt signaling pathways, which regulate cell survival, apoptosis, autophagy, and oxidative stress. PI3K/Akt is crucial for cardiomyocyte survival and remodeling post-myocardial infarction and in diabetic cardiomyopathy [44,45]. Also, EGFR-TKI resistance and melanoma-related pathways suggest shared mechanisms between cancer biology and cardiovascular stress, consistent with increased cardiac events in patients receiving targeted cancer therapies [46]. GO analysis further highlighted processes like protein phosphorylation, transcriptional regulation, and kinase activity, central to cardiac hypertrophy, arrhythmias, and structural remodeling [47].

Overall, the identified miRNAs and hub genes are key regulatory elements in CVD, influencing inflammation, fibrosis, oxidative stress, cardiomyocyte survival, and vascular remodeling. They hold promise as biomarkers for early diagnosis, disease severity indicators, or therapeutic targets. The robustness of the identified conserved miRNAs was supported by sensitivity analyses across multiple statistical thresholds and by consistency with previously reported CVD related studies.

Despite these strengths, several limitations should be acknowledged. First, the analysis relied on publicly available GEO datasets, which provide limited clinical annotation, restricting adjustment for patient-specific factors such as age, sex, comorbidities, and medication use. Second, this study was entirely computational in nature, and experimental validation of the predicted miRNA-mRNA interactions was beyond its scope. Therefore, in vitro and in vivo studies, including qRT-PCR, luciferase reporter assays, and functional knockdown experiments, are necessary to confirm the regulatory roles of these miRNAs and hub genes. Future studies incorporating larger, well-annotated cohorts and tissue-specific validation will be essential to translate these findings into clinical applications.

Although this study integrated two independent GEO datasets, the sample sizes were small and the tissue sources differed, which may introduce biological variability and limit generalizability. In addition, the findings are based on computational inference and database-supported interactions; therefore, independent cohort validation and experimental confirmation of the prioritized miRNAs and hub genes will be required before clinical translation. Because detailed clinical metadata were not available for these datasets, correlation of candidate biomarkers with disease severity, subtype, or outcomes could not be assessed and should be addressed in future clinically annotated cohorts. Finally, enrichment analyses provide functional hypotheses; deeper mechanistic studies are needed to define how the conserved miRNA-hub gene network regulates cardiovascular pathophysiology.

5. Conclusion

In conclusion, this study identifies miRNAs and hub genes closely linked to CVD mechanisms, enhancing our understanding of CVD biology and providing a foundation for developing new diagnostic biomarkers and targeted therapies. Further validation may enable translation of these molecular signatures into clinical tools for improving cardiovascular health.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

Conflict of Interest

The authors declare no conflict of interests.

Generative AI Statement

The authors declare that no Gen AI was used in the creation of this manuscript.

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