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Integrative bioinformatics analysis of miRNA and mRNA expression profiles and identification of associated miRNA-mRNA network in aortic dissection

Yiming Su, MD, Qiyi Li, MD, Zhiyong Zheng, MD, Xiaomin Wei, MD, Peiyong Hou, MD*

Abstract

Background: Aortic dissection (AD) is one of the most lethal cardiovascular diseases. The aim of this study was to identify core genes and pathways revealing pathogenesis in AD.

Methods: We screened differentially expressed mRNAs and miRNAs using mRNA and miRNA expression profile data of AD from Gene Expression Omnibus. Then functional and pathway enrichment analyses of differential expression genes (DEGs) was performed utilizing the database for annotation, visualization, and integrated discovery (DAVID). Target genes with differential expression miRNAs (DEMIs) were predicted using the miRWalk database, and the intersection between these predictions and DEGs was selected as differentially expressed miRNA-target genes. In addition, a protein–protein interaction (PPI) network and miRNA-mRNA regulatory network were constructed.

Results: In total, 130 DEGs and 47 DEMIs were identified from mRNA and miRNA microarray, respectively, and 45 DEGs were DEMI-target genes. The PPI and miRNA-mRNA network included 79 node genes and 74 node genes, respectively, while 23 hub genes and 2 hub miRNAs were identified. The DEGs, PPI and modules differential expression miRNA-target genes were all mainly enriched in cell cycle, cell proliferation and cell apoptosis signaling pathways.

Conclusion: Taken above, the study reveals some candidate genes and pathways potentially involving molecular mechanisms of AD. These findings provide a new insight for research and treatment of AD.

Abbreviations: AD = aortic dissection, DAVID = database for annotation, visualization, and integrated discovery, DEG = differential expression gene, DEMI = differential expression miRNA, GEO = gene expression omnibus, GO = gene ontology, PPI = protein–protein interaction, VC = vascular calcification, VSMC = vascular smooth muscle cell.

Keywords: aortic dissection, bioinformatical analysis, microarray, miRNA, mRNA

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1. Introduction

Aortic dissection (AD) is a severe, life-threatening aortic disease characterized by blood flow into the middle layer of the aorta through a tear in the intimal layer forming a true cavity and false cavity in the aorta or bleeding within aortic wall.[1,2] Accumulated evidence has shown the overall annual incidence of AD is between 4.58 and 6 per 100,000 people and up to 35 cases per 100,000 in those aged from 65 to 75 years.[3,4] Furthermore, the mortality rate of patients with AD prior to admission is 25%, total hospital mortality and all-cause 3-year mortality of such patients are 26.57% and 39.52%, respectively.[5] Even though AD usually has very poor outcomes, the development of endovascular repair has greatly improved treatment efficacy. A large number of studies have confirmed many congenital factors and acquired factors, for example, gene mutation, infectious disease and vascular inflammation, contribute to AD.[6–8] However, we still do not have good strategies or drugs to prevent and cure the early occurrence and development of AD because its molecular mechanisms are still unclear.

MiRNAs are endogenous small non-coding RNAs and regulate gene expression at the post-transcriptional level by inhibiting translation or promoting degradation of target mRNAs, playing an important physiological and pathophysiological functions. In recent years, the role of miRNAs have been confirmed to be closely involved in cardiovascular diseases and even can be regarded as a new strategy for diagnosis, therapy or prediction about the presence and progression of cardiovascular diseases.[9] For example, some miRNAs were viewed as biomarkers distinguishing patients with diabetic atherosclerosis from diabetic and healthy controls and showing severity of vascular calcification.[10,11] Furthermore, mimics and inhibitors

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Department of Vascular Surgery, Liuzhou Worker's Hospital, Fourth Affiliated Hospital of Guangxi Medical University, Liuzhou, Guangxi Province, China.

* Correspondence: Peiyong Hou, Department of Vascular Surgery, Liuzhou Worker's Hospital, Fourth Affiliated Hospital of Guangxi Medical University, Liuzhou 545005, Guangxi Province, China (e-mail: HPeiYong@126.com).

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of miRNAs were used as a potential therapeutic methods succeeding in limiting progression of atherosclerosis, aneurysm and myocardial infarction in animal models.[12–14] In AD, several serum miRNAs have been also considered to have potential as biomarkers for diagnosing AD, such as plasma miR-15a and miR-23a. The receiver operating characteristic curve analysis of miR-15a and miR-23a were 0.855 and 0.925 for detection of AD, respectively.[15] In another study, circulating miR-25, miR-29a, miR-155 and miR-26b had also the ability to predict AD, especially for patients with hypertension.[16] These studies showed great application value of crucial miRNAs on diagnosis and treatment of cardiovascular diseases. However, there are few studies focusing on the crucial miRNAs role in pathogenesis of AD.

Therefore, we attempted to identify core RNAs and crucial pathways that are involved in core molecular mechanism of AD using mRNA and miRNA expression profiles. Meanwhile, the miRNA-mRNA regulated axis for the occurrence and development of AD was constructed. This data will provide opportunities for future development of molecular targeted therapy for AD.

2. Materials and methods

2.1. Microarray data information

The mRNA expression profile GSE52093 based on GPL10558 (Illumina HumanHT-12 V4.0 expression beadchip) were download from the GEO database (https://www.ncbi.nlm.nih.gov/geo/), which included 7 Stanford type A aortic dissection samples and 5 normal aortic tissue samples. The miRNA expression data were obtained from Liao et al who published a microRNA profile comparison between thoracic aortic dissection and normal thoracic aorta. This study included 6 aortic dissection samples from thoracic aortic dissection patients undergoing surgical repair and 6 normal thoracic aorta samples from age-matched donors without aortic diseases. The miRNA microarray was analyzed with the miRCURY LNA Array (version 11.0; Exiqon, Vedbaek, Denmark).[17] Additional approval by an ethics committee was not necessary because the datasets included in the current study were downloaded from public databases, and data acquisition and application were performed according to GEO publication guidelines and data access policies.

2.2. Identification of differential expression RNAs and hierarchical clustering analysis

In the GSE52093 expression profile, differential expression genes (DEGs) were analyzed using GEO2R online tool and adjusted P value $< .05$ and $|\log FC| > 1.5$ were used as cut-off criteria. A volcano map and clustering analysis were executed in R software with the ggplot2 and vegan package, respectively. The miRNA expression profile, which were analyzed using GenePix 4000-B scanner and GenePix Pro 6.0 software (Axon Instruments, Union City, Calif),[17] these differential expression miRNAs (DEMIs) were identified with a P value $< .05$ and $|\log FC| > 1.5$ from the published miRNAs expression data.

2.3. Gene ontology and pathway enrichment analysis

Gene Ontology (GO) and pathway enrichment analysis of DEGs were used to show the functions and roles in biological pathways. DAVID (version 6.8, https://david.ncifcrf.gov) online database was applied to analyze GO terms and signaling pathways of candidate DEGs and P values $< .05$ were considered to statistical significance.

2.4. Protein–protein interaction (PPI) network and modular analysis

First, the STRING online tool (https://string-db.org) was used to constructed a PPI network of DEG-encoded proteins, which was visualization with Cytoscape software. The node gene that had greater or equal to 30° were defined as hub gene. In addition, module analysis of the PPI network was performed using Cytoscape software MCODE package and subsequently enriched analysis of the genes consisting of the modules with database for annotation, visualization and integrated discovery (DAVID) (version 6.8, https://david.ncifcrf.gov) online database.

2.5. Prediction of miRNA-targeted gene and DEMI-DEG regulatory network

Target genes of DEMIs were predicted by employing miRWalk online database (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk), which includes five different miRNA target genes predicted databases, miRanda (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk), miRDB (http://www.mirdb.org/), Targetscan (http://www.targetscan.org/vert_71/), RNA22 (https://cm.jefferson.edu/rna22/) and miRWalk. A gene was viewed as target gene of DEMI when the gene was predicted to be a target gene of the miRNA at least 4 databases. The intersection of predicted target genes of DEMIs and DEGs were regarded as significantly differential expression target genes. The Draw Venn Diagram online tool (http://bioinformatics.psb.ugent.be/webtools/Venn/) was employed to draw a Venn diagram showing the intersection of these genes. These significant differential expression target genes and corresponding miRNAs were used to construct the miRNA-mRNA regulatory network using the Cytoscape software. Then, the number of node miRNA interaction with its target genes was applied to screen hub miRNAs. The degree of each node miRNA was greater than or equal to 6, which were defined as hub miRNA.

3. Results

3.1. Identification of differential expression RNAs in aortic dissection and normal ascending aorta

Differentially expressed genes (DEGs) in GSE52094 were screened with $P < .05$ and $|\log FC| > 1.5$. A total of 130 DEGs were identified when aortic dissection samples were compared with normal ascending aorta samples, including 83 up-regulated genes and 47 down-regulated genes (Fig. 1A, S-table 1, http://links.lww.com/MD/D32). Furthermore, heatmaps of these DEGs were used in hierarchy cluster analysis suggested they can be distinguished between aortic dissection group and normal ascending aorta group (Fig. 1B). In addition, we identified 47 differentially expressed miRNAs (DEMIs) from miRNA profile comparison between aortic dissection and normal thoracic aorta published by Liao et al,[17] including 12 up-regulated miRNAs and 35 down-regulated miRNAs (S-table 2, http://links.lww.com/MD/D32).

3.2. Functional and pathway enrichment analysis of DEGs

GO and signaling pathway enrichment analysis were applied to explore the pathogenesis in aortic dissection. GO enrichment analysis of classified the DEGs into 3 groups, a biological process group, a cellular component group and a molecular function group, which are shown in Figure 2 (A–C). In the biological process

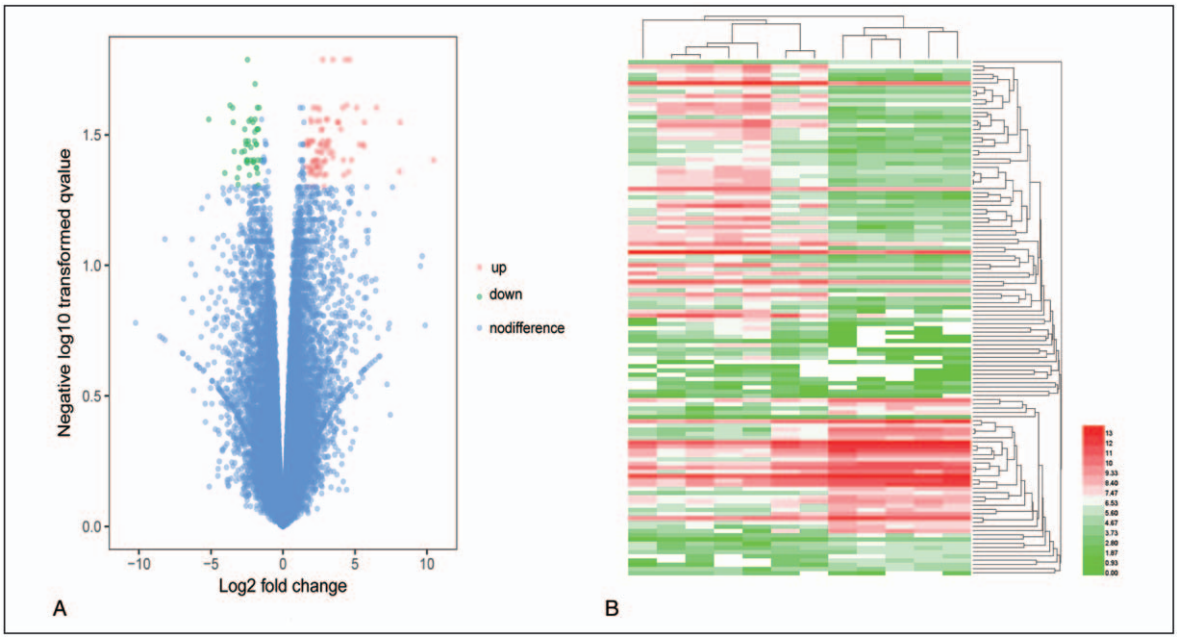

Figure 1. Expression profiles of differentially expressed genes (DEGs). (A) Volcano map of DEG expression levels between aortic dissection and control samples. The red nodes represent up-regulated DEGs with P value $< .05$ and $|\log FC| > 1.5$; the green nodes represent down-regulated DEGs with P value $< .05$ and $\log FC < -1.5$; the purple nodes represent genes with P value $> .05$ or $|\log FC| < 1.5$. (B) Hierarchical clustering of the DEGs. Each row in the heatmap represents a gene, and each column represents a sample. The color scale at the right of the heatmap represents the raw Z-score ranging from green (low expression) to red (high expression).

group, GO terms were mainly associated with cell cycle, cell division, cell proliferation and apoptotic process. In the cellular component group, GO terms were mainly included nucleus, cytoplasm, focal adhesion and cytosol. In the molecular function group, GO terms mainly involved protein binding, ATP binding and kinase activity. Pathway enrichment analysis showed these DEGs were mainly involved in cell cycle pathway, such as DNA replication, separation of sister chromatids and cell division.

3.3. DEG protein-protein interaction (PPI) Network and modular analysis

A PPI network was constructed with STRING online database (https://string-db.org) to study the relationship during these DEGs and to find hub genes. The PPI network consisted of 79 nodes and 613 edges, including 54 up-regulated genes and 25 down-regulated genes (Fig. 3). Among the 79 nodes, there are 23 nodes were hub genes which had greater or equal to 30° and the most significant 10 node degree genes were *CDK1, MELK, CDC20, TOP2A, BUB1, CENPF, PBK, KIF20A, CDKN3, and CHEK1*. There was one significant module based on modules analysis of PPI network, which contained 32 nodes and 458 edges (Fig. 3B). All node genes in the modules were up-regulated genes. Functional enrichment analysis of these genes in the modules showed they were mainly related with cell cycle, cell division, cell proliferation and apoptotic process (Fig. 3C).

3.4. Differential expression miRNA predicted target genes (DEMIGs) and miRNA–mRNA regulatory network

In order to further identify important miRNA/mRNA regulated axes in aortic dissection progression, target genes of differential expression miRNAs (DEMIs) were predicted using miRWalk online database (http://zmf.umm.uni-heidelberg.de). There were 5745 genes predicted as target genes of DEMIs, which had 45 commonly genes with DEGs in GSE52094 (Fig. 4A, S-table3, http://links.lww.com/MD/D32). These 45 common DEGs (26 up-regulated and 19 down-regulated) and DEMIs were used to constructed miRNA–mRNA network, which consisted of 74 nodes and 84 edges, including 29 miRNAs (5 up-regulated and 24 down-regulated) (Fig. 4B). In the network, hsa-miR-93 and hsa-miR-485-3p had a higher degree and their target genes were enriched in cell proliferation or apoptotic processes; genes included *MCM10, MCM4, PBK, MELK, CDCA7, and CKAP2*. Functional enrichment analysis of these target genes in the miRNA-mRNA network suggested they were mainly associated with cell cycle, cell proliferation and the apoptotic process (Table 1).

4. Discussion

Aortic dissection (AD) is a catastrophic aortic disease with a high mortality rate. However, the pathogenesis of AD has not been completely elucidated and important genes and pathways involved in AD need to be further uncovered. In this study, mRNA and miRNA expression profiles are used to identify differential expression mRNAs (DEMs) and miRNAs (DEMIs). A total of 130 DEGs (including 83 upregulated and 47 downregulated mRNAs) and 47 DEMIs (including 12 upregulated and 35 downregulated miRNAs) were screened, respectively. In the protein–protein interaction (PPI) network, 10 hub genes had a higher degree, including *CDK1, MELK, CDC20, TOP2A* and so on, which were all identified from the cluster. Additionally, the miRNA-mRNA network, hsa-miR-93 and

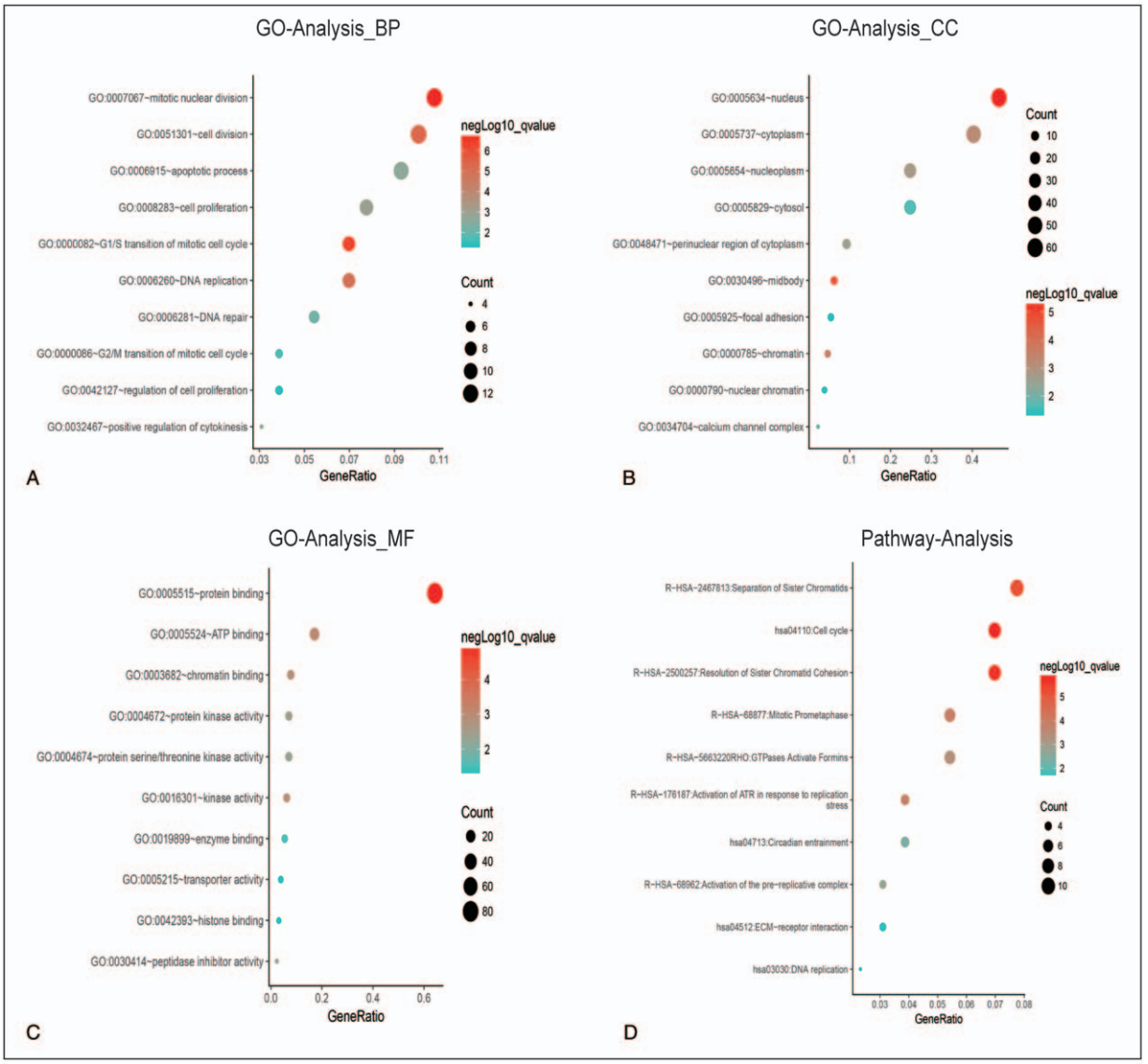

Figure 2. Gene Ontology (GO) and signaling pathway enrichment analysis of DEGs. GO enrichment analysis of DEGs showed (A) biological process (BP), (B) cellular component (CC), (C) molecular function (MF). (D) Signaling pathways enrichment of DEGs.

hsa-miR-485-3p had higher degrees. Functional and pathway enrichment analysis of DEGs, module genes originated from PPI network and target genes of DEMIs in miRNA-mRNA network were all associated with cell cycle, cell proliferation, and apoptotic process. These results therefore showed core genes and pathways involved in molecular mechanism of AD initiation and progression, which is useful for the development of new therapeutic strategies.

Accumulated evidences have shown cystic medial degeneration is the most common histopathological alteration in aortic dissection, which includes mainly extracellular matrix degradation and loss of vascular smooth muscle cells, leading to aortic wall instability and weakening of the mechanical strength of the vessel wall.[18,19] In this study, GO terms and pathway enrichment analysis of all DEGs or modules of the PPI network were enriched for biology associated with cellular characteristics, especially for the cell cycle, cell proliferation, apoptosis and cell division. This finding suggests arterial wall cell activities alterations affecting aortic structure play an important role in AD. A similar result was also found in a study by Wang et al study, who reported apoptosis, cell cycle, extracellular matrix and other related to inflammatory response GO were enriched in acute Stanford type B aortic dissection.[20] What was more, histopathological study also showed cell apoptosis in media was significantly increased both in human AD tissue and in a rat AD model and many genes contributed to AD by regulating aortic smooth muscle cell apoptosis and proliferation.[21–24] Moreover, cell biology activities, such as apoptosis, proliferation, and migration, were closely correlated with hypertension, atherosclerosis, and cocaine, which are all commonly risk factors for AD.[25,26] These results demonstrated that vascular cell biological function plays an important role for stability of aortic wall and AD formation.

In the current study, many differential expression RNAs had a higher degree in PPI and miRNA-mRNA networks suggesting they may play a very important role in pathogenesis of AD.

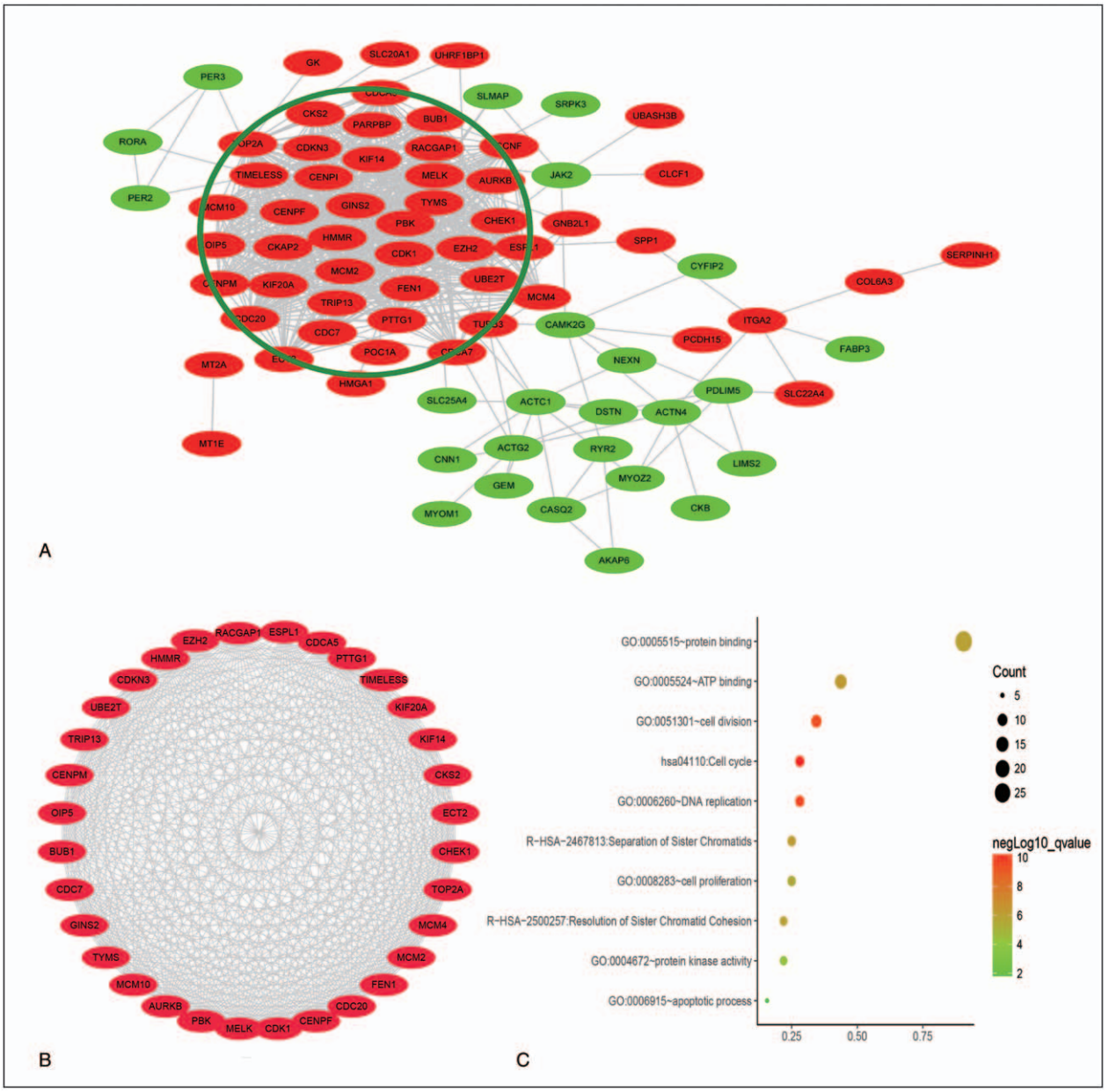

Figure 3. Protein–protein interaction (PPI) network of DEGs and modular analysis. A total of 79 DEGs and 613 edges constituted this PPI network. (A) The genes in red represent upregulation and the genes in green represent downregulation. The circled areas stand for the most significant module. (B) A total of 32 DEGs and 458 edges constituted this module, which was mainly associated with cell cycle, cell division, cell apoptosis, and cell proliferation pathways (C).

Cyclin-dependent kinase-1 (CKD1) had the highest degree in PPI network, and has been previously identified as a regulator of the cell cycle by interacting with cyclin A and cyclin B to affect cell proliferation and apoptosis. Furthermore, disorder of CKD1 expression was shown to be closely associated with cardiovascular diseases. It was reported that overexpression of CKD1 in adult mouse cardiac fibroblast lead to cardiac fibrosis.[27] Li et al found that inhibition of cell cycle by reducing expression of cyclin-dependent kinases and cyclin with myricitrin suppressed vascular smooth muscle cell proliferation and migration thereby weakening atherosclerosis.[28] Park et al reported also that rubiarbonone C could inhibit vascular smooth muscle cell proliferation and migration blocking mouse carotid intima thickening partly by inhibiting cyclin-dependent kinases and cyclin expression.[29] These researches suggested inhibition of CKD1 or cyclin would to be a possibility for treatment of AD.

Additionally, minichromosome maintenance family (MCM) 2, MCM4 and MCM10, which are essential for DNA replication limiting cell cycle,[30] were all upregulated and were all hub genes in our PPI network indicating they may play a very important role in AD. In recent studies, minichromosome maintenance family have also been closely related to cardiovascular diseases. For instance, MCM4 was predicted as a transcription factor and had a high degree in transcription factor-target-miRNA networks involving in intracranial aneurysm.[31] This may be a potential therapeutic target for the treatment of intracranial aneurysm.

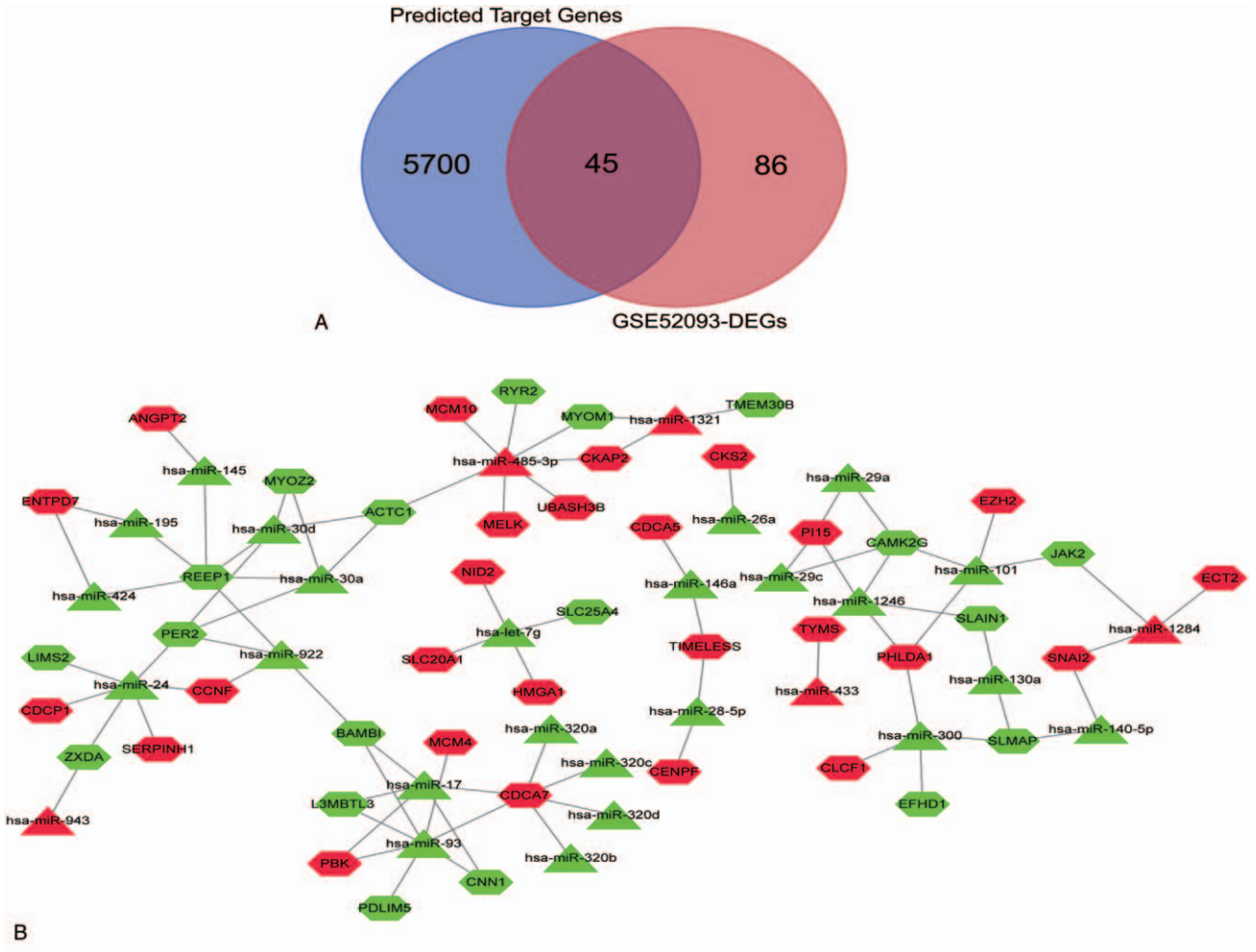

Figure 4. Venn diagram and DEMI-DEG regulatory network. (A) Venn diagram showing overlap genes both in the differential expression miRNAs (DEMIs) predicted target genes and DEGs. The overlap areas represent differentially expressed target genes (DETG). (B) DEMI-DEG regulatory network. The genes in red signify upregulation and the genes in green represent downregulation; the triangles represented DEMIs and the hexagons represented DETGs.

Moreover, peroxisome proliferator-activated receptor γ coactivator 1β can inhibit vascular smooth muscle cell proliferation thereby suppressing vascular lesion formation by decreasing MCM4 expression.[32] Additionally, Noseda et al confirmed that the Notch protein inhibited human microvascular endothelial cell, umbilical vein endothelial cell and human foreskin fibroblast DNA replication by blocking cell cycle exerting antiproliferation effect by decreasing MCM2 and MCM6 expression.[33] However, Notch-mediated signal pathways were closely associated with atherosclerosis and other proliferative vascular diseases.[34] These results suggest regulation of vascular wall cellular activity may be a treatment strategy for AD.

Remarkably, in the miRNA-mRNA network, hsa-miR-93 and hsa-miR-485-3p had the highest degree indicated they play possibly an important role in AD. Actually, some predicted target genes of hsa-miR-93, such as *L3MBTL3* and *CNN1*, were

Table 1

Pathway enrichment analysis of the intersection between miRNAs predicted target genes and DEGs in GSE52093.

Term	Description	Count	*P* value	Genes
GO:0000082	G1/S transition of mitotic cell cycle	5	.000109337	*TYMS, CAMK2G, MCM10, CDCA5, MCM4*
GO:0051301	cell division	6	.001546991	*CDCA7, TIMELESS, CCNF, CKS2, CENPF, CDCA5*
GO:0007067	mitotic nuclear division	5	.003062644	*TIMELESS, CCNF, CENPF, PBK, CDCA5*
GO:0042127	regulation of cell proliferation	4	.010316342	*CDCA7, TIMELESS, EZH2, JAK2*
GO:0006915	apoptotic process	6	.01185228	*CKAP2, ACTC1, CDCA7, JAK2, MELK, PHLDA1*
GO:0008283	cell proliferation	5	.01191331	*TYMS, CKS2, CENPF, MCM10, MELK*
hsa04020	Calcium signaling pathway	3	.049507704	*SLC25A4, CAMK2G, RYR2*
GO:0045892	negative regulation of transcription, DNA-templated	5	.032806081	*TIMELESS, EZH2, PER2, CENPF, HMGA1*

DEGs = differentially expressed genes.

enriched in cell proliferation biological process and *L3MBTL3*, *CDCA7*, and *BAMBI* were enriched in apoptotic biological process. Furthermore, the hub genes MCM4 and MCM10, which had been confirmed to regulate cell cycle, were target genes of hsa-miR-93 and hsa-miR-485-3p, respectively. Meanwhile, it was reported that miR-93 and miR-485 were all involved in cardiac hypertrophy in mouse model.[35–37] Furthermore, miR-93 was also confirmed to regulate rat cardiac fibroblasts proliferation and mouse endothelial and skeletal muscle cells apoptosis and proliferation through controlling multiple genes involving cell cycle.[38] Taken together, we speculate hsa-miR-93 and hsa-miR-485-5p are closely related to development and progression of AD through affecting vascular stability by regulating the biological activity of cell vascular walls, such as proliferation and apoptosis.

There are some limitations to our study that should be noted. Firstly, these differential expression RNAs between aortic dissection group and control group, especially for the hub DEMs and DEMIs, need to be further verified using cell experimentation and clinical samples. Secondly, these DEMs and DEMIs were screened from different tissue samples. Therefore, the results, especially for miRNA-mRNA network, could be affected by samples derived from different patients.

Taken together, we identified core genes and crucial pathways, especially vascular wall cell biological function alteration, involved in the pathogenesis of AD. These findings build upon the understanding of potential molecular mechanism in AD and provided some new insights for use in further identification and development of new AD therapeutic targets.

5. Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Author contributions

Data curation: Qiyi Li, peiyong Hou.
Formal analysis: zhiyong zheng, xiaomin Wei.
Funding acquisition: peiyong Hou.
Investigation: Qiyi Li.
Project administration: peiyong Hou.
Software: xiaomin Wei.
Visualization: zhiyong zheng.
Writing – original draft: Yiming Su.
Writing – review & editing: Yiming Su.

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