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Identification of atrial fibrillation-related circular RNAs and constructing the integrative regulatory network of circular RNAs, microRNAs and mRNAs by bioinformatics analysis

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Abstract: Atrial fibrillation (AF) is the most common cardiac arrhythmia with a high incidence of stroke. Many circular RNAs (circRNAs) have been demonstrated they are elated to various heart diseases and may play important roles in diagnostics or many pathophysiological processes. Nevertheless, there is Few studies on circRNAs functions in persistent AF. To identify AF-related circRNAs and construct the integrative regulatory network of circular RNAs, miRNAs, and mRNAs, we collected human right atrial appendage tissues from 5 patients suffering persistent AF (AF group) and 5 patients with normal sinus rhythm (NSR group) and characterized the global changes in circRNA expression with high-throughput sequencing technology. The differential expression of circRNAs and the interactions between circRNAs and microRNAs were analyzed. The microRNA expression file GSE68475 dataset was downloaded from the Gene Expression Omnibus (GEO) database to explore the differentially expressed microRNAs. The target genes of overlapped miRNAs were predicted by using DIANA-TarBase v8. We constructed the circRNA- miRNA-mRNA network using Cytoscape (version 3.4.0) and the network topology was analyzed by utilizing CentiScaPe app. Results showed that all of 600 differentially expressed circRNAs related to AF were screened, including 340 up-regulated and 260 down-regulated circRNAs. An integrative regulatory network was constructed, which included 30 circRNAs, 9 miRNAs and 130 target mRNAs of these miRNAs. It was concluded that that 30 circRNAs, including 8 upregulated circRNAs and 22 downregulated circRNAs, were predicted to highly possibly function as sponges of 9 miRNAs to regulate gene expression by using bioinformatics analysis. Moreover, the interactions of hsa-miR-339-5p with its related circRNAs and target mRNAs constructed the hub regulatory network in persistent AF by utilizing topology analysis. Our proposed regulatory network of circRNAs-miRNAs-mRNAs may provide new insight into the potential mechanism underlying persistent AF. Additionally, these important molecular may become novel biomarkers providing a new strategy in diagnosis and therapy of AF.

Key words: Atrial fibrillation; Circular RNAs; microRNAs; mRNAs; In silico analysis.

Introduction

Atrial fibrillation (AF) is the most prevalent cardiac arrhythmia which influences approximately 1.5% of the total population (1), and more than 50% of paroxysmal AF will progress to persistent AF within 10 years (2). Although radiofrequency ablation of AF is an effective radical strategy that reduces heart failure mortality, the risk of stroke, and significantly improves the quality of life (3, 4), there was higher recurrence rate after catheter ablation for patients suffering persistent AF (5).

AF, sometimes referred to in medicine as AFib, does not have a specific electrical stimulation in the heart. It occurs when there is no electrical stimulation wave in the atria in a specific direction, ie the atrial muscle cells are irregularly stimulated and as a result contract. As a result, there is no regular atrial contraction, so the atria cannot pump blood completely to the ventricles, and the ventricular beats do not follow the atria, and the ventricles contract without normal order and without following and waiting for the atria (6, 7). Cardiac arrhythmia means that the heart rhythm is abnormal. Normally, the heart rhythm starts at the sinus node and spreads to the ventricles after transfer to the atrioventricular node. As a result of this way of conducting electrical stimulation, the atria first contract and the ventricles contract at short intervals, and also the atrial muscles relax and then the ventricles relax (8, 9).

AF usually has no symptoms and the patient does not notice a change in his condition. Patients are often the elderly who have other illnesses. It does not usually endanger the patient's life, but causes other problems. These problems include chronic fatigue, congestive heart failure, and most importantly, stroke (10, 11). The mechanisms behind AF include various pathophysiological processes, and there is obvious heterogeneity among patients. What's more, for the moment the treatment strategies used for AF are not dependent upon the specific mechanism of AF, with infrequent exceptions. Therefore, further investigation of mechanisms underlying AF could alternate current therapy, and clinicians might be better able to tailor therapy in each specific patient (12).

An international research team found a strong link between premature atrial fibrillation and mutations in the TTN gene. The TTN gene encodes a protein called "titin", which acts as a molecular scaffold in heart muscle cells and helps maintain the structure of the heart muscle. Mutations in the TTN gene are associated with a variety of heart diseases, including heart muscle

disease. According to new findings, approximately 2% of the approximately 2,800 patients with premature atrial fibrillation examined in this study had this gene mutation (13).

To investigate the genetic effects of premature atrial fibrillation, the researchers collected all genomic sequence data from 2781 American patients under the age of 66. Then, by performing the GWAS process, they sought to determine which common genetic variable increases the risk of premature atrial fibrillation in individuals. Researchers have developed a list of more than 8.2 million variables into 26 genomic regions with previous studies, and of the 84 genes in these 26 regions, only TTN was highly associated with premature atrial fibrillation (13). Today, the standard approach to treating premature atrial fibrillation is to use drugs that control heart rhythm, or to use an invasive procedure called catheter ablation. However, the researchers in this study acknowledge that using a more dynamic approach to treating premature atrial fibrillation should be on the agenda (14).

At present, non-coding RNAs (ncRNAs), counting long non-coding RNAs (lncRNAs), micro-RNAs (miRNAs), and circular RNAs (circRNAs), have been shown to interact with the transcriptome in several ways that alter gene expression, regulate cell function, and influence pathogenesis of heart disease (15, 16). As a new class of ncRNAs, circRNAs, with covalently closed loop structures which are seemingly more stable and conserved, have been demonstrated that are associated with numerous heart diseases and may play important roles in diagnostics or many pathophysiological processes (17, 18). Recently, the role of circRNAs in cardiac diseases showed their crucial functions as modulators of miRNA levels (19). Sponging activity of circRNAs sequestering specific miRNAs is an important factor to be considered for the determination of the molecular mechanisms involved in AF progression (20). Studies have demonstrated that circRNA-miRNA-mRNA networks are associated with certain diseases (18, 21). circRNAs may be a novel kind of potential biomarkers and therapeutic targets. However, there is little research regarding the functions of circRNAs in persistent AF.

To identify of atrial fibrillation-related circRNAs and construct the integrative regulatory network of circular RNAs, miRNAs, and mRNAs, we collected human right atrial appendage tissues from 5 patients suffering persistent AF (AF group) and 5 patients with normal sinus rhythm (NSR group) undergoing open-heart surgery at the Second Affiliated Hospital of Nanchang University and characterized the global changes in circRNA expression in human atrial appendages with high-throughput sequencing technology. The differentially expressed circRNAs were identified and the biological functions of their interaction miRNAs and mRNAs were analyzed. Our study provided new insights of the circRNA roles in the pathogenesis behind persistent AF and proposed highly possible interaction mechanisms among circRNAs, miRNAs, and mRNAs. We screened overlapped miRNAs based on differentially expressed miRNAs in microarray and target miRNAs of circRNAs using Draw Venn Diagram.

Materials and Methods

Clinical specimen acquisition

Specimens of heart tissues were obtained from 10 patients undergoing surgery at the Second Affiliated Hospital of Nanchang University (Nanchang, China). The samples were taken within 10 minutes after tissue excision, immediately immersed in RNAlatar@ stabilization, and then stored at -80 degrees Centigrade. This research was performed in accordance with the 1964 Helsinki Declaration as amended or similar moral criteria and was approved by the Ethics Committee of the Second Affiliated Hospital of Nanchang University. Written informed consent was obtained from all patients. Supplementary Table S1 shows the clinical specimen information of the 10 patients.

Library construction and sequencing circRNA expression profiles of clinical specimens

Total RNAs were isolated from cells/tissues using the Trizol (invitrogen) and purity was assessed by ND-1000 Nanodrop required A260/280≥1.8, A260/A230≥2.0. RNA integrity (RIN) was evaluated by Agilent 2200 TapeStation (Agilent Technologies, USA) requiring RIN≥7.0. Epicentre Ribo-Zero rRNA Removal Kit (illumina, USA) was utilized to remove rRNAs from Total RNAs, and then RNase R (Epicentre, USA) were utilized to randomly break the circRNA into small 200 bp fragments. Subsequently, the purified RNA fragments were subjected to first strand and second strand cDNA synthesis followed by adaptor ligation and enrichment with a low-cycle according to instructions of NEBNext® UltraTM RNA Library Prep Kit for Illumina (NEB, USA). The purified library products were evaluated using the Agilent 2200 TapeStation and Qubit®2.0(Life Technologies, USA) and then sequenced on HiSeq 3000 with 2 X 150 bp mode.

To ensure the accuracy of subsequent bioinformatics analysis, the quality of raw sequencing data was inspected using the FastQC software. Two algorithms, CIRI2 and CIRCexplorer2, were used to detect circRNAs. Reads were mapped to human reference genome GRCh37/ hg19 (http://genome.ucsc.edu/) by BWA-MEM or Tophat, respectively. Candidate circRNAs with junction read counts >2 were screened as the final identified circRNAs. Built on the location of the circRNA in the genome and the relationship with the gene, the selected circRNA candidate was annotated with the Ensembl database and RefSeq database.

Identification of the differentially expressed circRNAs and miRNAs between AF group and NSR group

Based on circRNA expression, the t-test method of the R software limma package was used for differential expression analysis. Significantly differentially expressed circRNAs between AF group and NSR group were filtered with defined threshold values >1.0 (|logFC| > 1) and *P* values <0.05.

The dataset of miRNA microarray GSE68475 (GPL15018, Agilent-031181 Unrestricted_Human_miRNA_V16.0_ Microarray 030840; Agilent Technologies) was downloaded from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/), using the keywords atrial fibrillation and

microRNA. Human atrial right appendages were collected from patients who had received open-heart surgery at Oita University Hospital; 10 samples were from patients with persistent AF (AF group) and 11 samples from patients with normal sinus rhythm (NSR group). The AF group included patients with a documented record of sustained AF for 6 months or longer. The NSR group included patients with no documented history of AF. The statistical significance of differential expression between two groups was also estimated with t-test using the R software limma package and further filtered with fold change. microRNAs with *P* values <0.05 and |logFC| > 1 were identified as significantly differentially expressed miRNAs.

Prediction the target miRNAs of circRNAs and screening overlapped miRNAs

Differentially expressed circRNAs contain corresponding miRNA binding sites. To facilitate the investigation, the interactions between circRNAs and miRNAs were predicted by Targetscan, miRanda, and RNAhybrid. circRNAs of which target miRNAs were simultaneously predicted by three tools mentioned above were identified as candidate circRNAs. The candidate circRNAs, which were annotated in circBase database, with an absolute value of fold change >2.0 were selected for further analysis. We screened overlapped miRNAs based on differentially expressed miRNAs in microarray and target miRNAs using Draw Venn Diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/).

Prediction of the target genes of overlapped miRNAs and performing the GO and KEGG enrichment analysis of target genes

The target genes of overlapped miRNAs were predicted by using DIANA-TarBase v8, which is a decade-long collection of experimentally supported miRNA–gene interactions database, and facilitates the extraction of miRNA interactions derived from >33 experimental methodologies, applied to about 600 distinct cell types/tissues under approximately 451 experimental conditions (22). We utilized DAVID (http://david.abcc.ncifcrf.gov/) tool to perform Gene ontology (GO) enrichment analysis of miRNA target genes. GO terms, including molecular function (MF), biological processes (BP), and cellular components (CC), with *P* value < 0.05 were considered significantly enriched by target genes. KEGG enrichment analysis of target genes was conducted with Kyoto Encyclopedia of Genes and Genomes (KEGG), which is a database resource for further understanding high-level functions and effects of the biological system (http://www.genome.jp/kegg/)(23).

Construction of the circRNA-miRNA-mRNA integrative regulation network

Based on the hypothesis called competing endogenous RNA (ceRNA) mechanism, which proposed that transcripts such as mRNAs and circRNAs can serve as natural miRNA sponges by competitive binding to miRNA response elements (MREs) to suppress their expression and functions, we constructed the genome wide integrative regulatory network of circRNAs, microRNAs and mRNAs (24). Significantly expressed circRNAs with an absolute value of fold change >2.0, overlapped miRNAs, and target genes related to overlapped miRNAs were superimposed onto the circRNA-miRNA-mRNA network by by using Cytoscape (version 3.4.0) and the network topology was analyzed by utilizing CentiScaPe app (25).

Results

Identification of differentially expressed circRNAs and miRNAs between AF group and NSR group

Expression profiling data of 41541 circRNAs were obtained by using high-throughput sequencing. The scatter plot was used to visualize the correlation analysis which can measure the reliability of sequencing results to a certain extent and reflect the degree of the differences between samples as a whole (Figure S1). The Pearson coefficient of correlation of samples was 0.847-a positive correlation, indicating a high degree of similarity in expression patterns between samples and a great degree of standardization. Based on the threshold of *P* <0.05 and absolute value of fold change >1.0, a total of 600 differentially expressed circRNAs were screened in the AF group compared with the NSR group, including 340 up-regulated and 260 down-regulated circRNAs. Volcano plot was utilized to visualize differential expression between two groups (Figure 1). Hierarchical clustering was implemented based on differential expression of circRNAs and the result of hierarchical clustering showed a distinguishable circRNA expression profiling among samples (Figure 2). The box plot showed the median of different samples was almost on the same line after Background Correction and Quantile Normalization of the miRNA expression profile (Figure 3). The differentially expressed miRNAs of the miRNA expression profile of GSE68475 was generated by utilizing the R software limma package. For the same criteria, 60 differentially expressed miRNAs were identified from the miRNA expression profile, including 31 up-regulated and 29 down-regulated miRNAs. Based on the differential expression of miRNAs, we mapped the volcano plot to visualize the differential expression (Figure 4).

Prediction of circRNA-miRNA and miRNA-mRNA interaction

We utilized three online tools, including Targetscan,

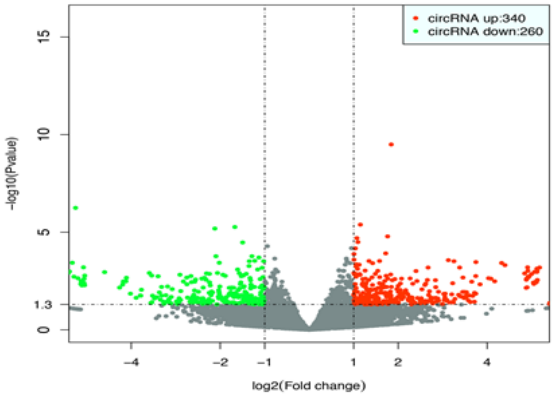

Figure 1. The volcano plot was utilized to visualize differential expression of circRNAs between AF group and NSR group. Red plots indicated significantly upregulated circRNAs and green plots indicated significantly downregulated circRNAs.

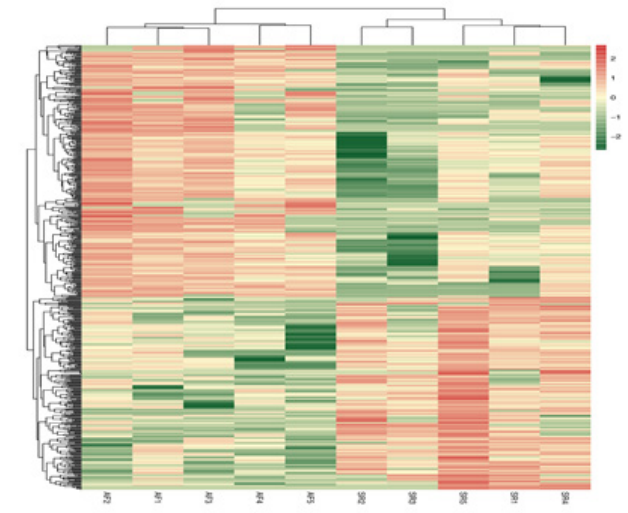

Figure 2. The heat map was to illustrated the result of the hierarchical clustering analysis based on differential expression of circRNAs and the result of hierarchical clustering showed a distinguishable circRNA expression profiling among samples. Each group consisted of five samples. circRNA expression profiles were shown in rows. "Red" indicated high relstively expression and "Green" indicated low relatively expression. AF: atrial fibrillation group; SR: normal sinus rhythm group.

miRanda, and RNAhybrid, to predict the interactions between circRNAs and miRNAs. The candidate circRNAs annotated in circBase database with *P* values <0.05 and |logFC| > 2 were selected for further analysis. A toltal of 15482 interactions between 133 circRNAs and 2403miRNAs were identified. Based on differentially expressed miRNAs in microarray and target miRNAs of 133 circRNAs, 31 overlapped miRNAs were screened by using Draw Venn Diagram (Figure 5). The target miRNAs of the 31 overlapped miRNAs were predicted by using Tarbase V8.0 and 293 interactions between 278 genes and 31 overlapped miRNAs. What's more, 9 miRNAs, all of 130 target mRNAs of the 9 miRNAs with a prediction score >0.4, and 30 circRNAs which function as the sponge of the 9 miRNAs were utilized to construct the circRNA-miRNA-mRNA integrative regulation network.

The GO and KEGG enrichment analysis of the target mRNAs of overlapped miRNAs

GO functional enrichment analysis, which involved BP, MF and CC categories, was conducted to investigate the biological function of the target mRNAs of overlapped miRNAs. All of the results of GO analysis were ranked by enrichment score (-log(*P* value)) and top ten of every category were showed in Figure 6. In BP analysis, regulation of cellular metabolic process, cellular macromolecule metabolic process, regulation of macromolecule metabolic process, and regulation of metabolic process were the top 4 enriched terms. In MF analysis, enzyme binding, macromolecular complex binding, protein binding, and kinase binding were top 4 enriched terms. In CC analysis, intracellular organelle, intracellular, organelle, and intracellular membrane-bounded organelle were top 4 enriched terms. KEGG pathway enrichment analysis was performed for a further understanding of the target genes. In KEGG pathway analysis, the results were also ranked by enrichment score and 19

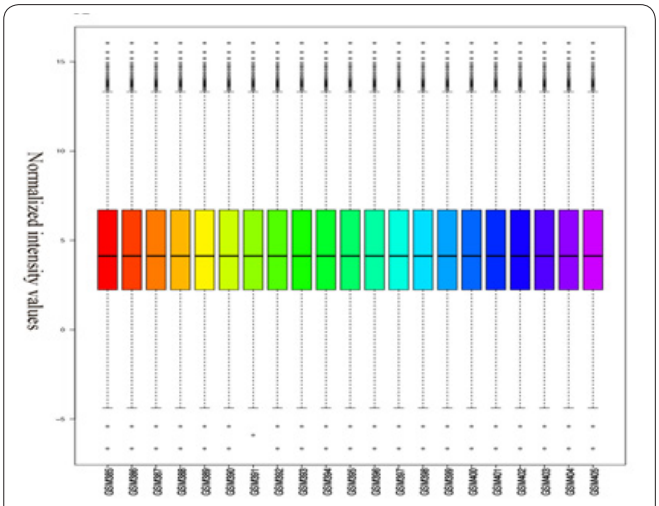

Figure 3. The box plot showed the median of different samples was almost on the same line after Background Correction and Quantile Normalization of the miRNA expression profile.

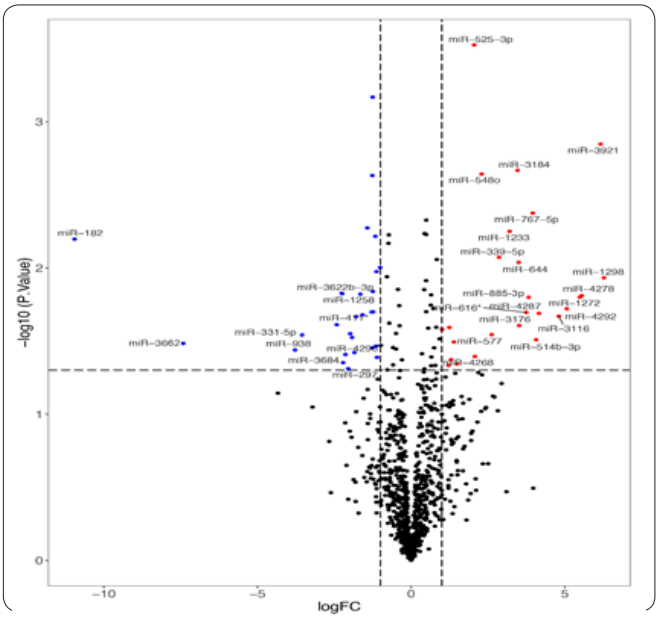

Figure 4. The volcano plot was utilized to visualize differential expression of miRNAs between AF group and NSR group. Red plots indicated significantly upregulated miRNAs and blue plots indicated significantly downregulated miRNAs. The plots with the label represented the miRNAs with with *P* values <0.05 and |logFC| > 2.

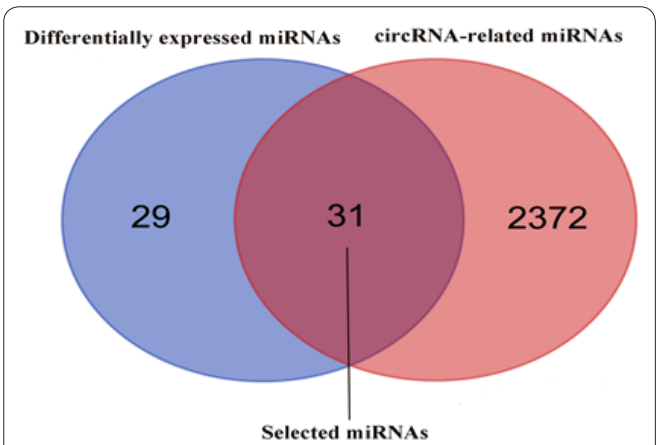

Figure 5. Based on differentially expressed miRNAs in microarray and target miRNAs of 133 candidate circRNAs, 31 overlapping miRNAs were screened by using Draw Venn Diagram.

pathways associated with the target mRNAs were displayed in Figure 7.

Construction of the circRNA-miRNA-mRNA integrative regulation network

An integrative regulation network revealing the interactions of circRNA-miRNA-mRNA was displayed in Figure 8. In the network of 30 circRNAs, including 8 upregulated circRNAs and 22 downregulated circRNAs respectively, 9 miRNAs ranked relatively higher, of which 6 are upregulated miRNAs and 3 are downregulated miRNAs, and 130 target mRNAs of these miRNAs were collected. Moreover, the target genes of these miRNAs were predicted by using TarBase v8.0 with a prediction score > 0.4 and all the interactions of miRNA-mRNA were experimentally validated. Dramatically, the number of the target genes of hsa-miR-3662, hsa-miR-3176, or hsa-miR-331-5p are almost the same. In graph theory, betweenness centrality is a measure of centrality in a graph based on shortest paths and devised as a general measure of centrality. A node with higher betweenness centrality would have more control over the network, because more information will pass through that node. As shown in Figure 8, hsa-miR-339-5p had the most interactions with circRNAs and mRNAs; SOS1 and TOR1AIP2 that are two target mRNAs of hsa-miR-339-5p are also the target genes of hsa-miR-331-5p which regulate the expressions of other 22 mRNAs; hsa_circ_0091017, hsa_circ_0053083, and hsa_circ_0011293 that function as sponge of hsa-miR-339-5p also regulate the expression of hsa-miR-3667-5p, hsa-miR-3176, or hsa-miR-525-3p respectively, which indicated that hsa-miR-339-5p was the hub miRNAs in the regulation network.

Discussion

Over the past few decades, although many researches have demonstrated that large numbers of mRNAs were associated with the development and progression of atrial fibrillation (AF), the molecular mechanism underlying AF remains unclear owing to the fact that more focus has been put on mRNAs or miRNAs (26, 27). Acting as microRNA target decoys, RNA binding protein sponges, and transcriptional regulators, more recently circRNAs have been reported to be involved in many complicated biological processes and the dysregulations of circRNAs contribute to a wide variety of diseases (28). The more important fact is that circRNAs may be novel biomarkers for the diagnosis, prediction of therapeutic response, and prognosis (29, 30). In addition, identification new circRNAs relating to atrial fibrillation could provide novel insight into the potential mechanisms of AF.

In our present study, high throughput sequencing of 5 persistent AF samples and 5 NSR samples was conducted to obtain the expression profiles of circRNA. The expression profiles of miRNAs including 10 persistent AF samples and 11 NSR samples were downloaded from GEO database and analyzed by utilizing the R software limma package. With the cut off criterion of *P* value <0.05 and absolute FC >1.0, differen-

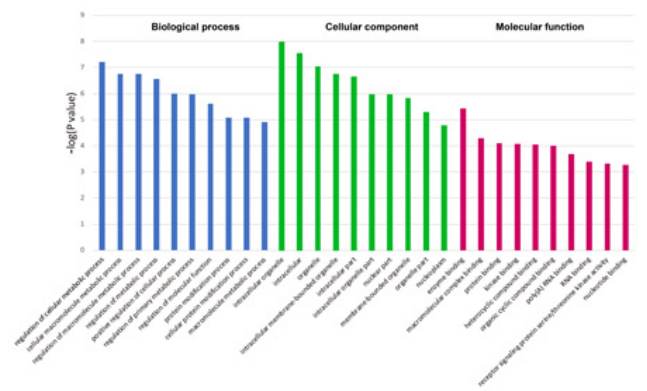

Figure 6. The GO enrichment analysis of the target mRNAs of 31 overlapping miRNAs. The plot showed the top 10 enriched terms of molecular function (MF), biological processes (BP), and cellular components (CC) analyses.

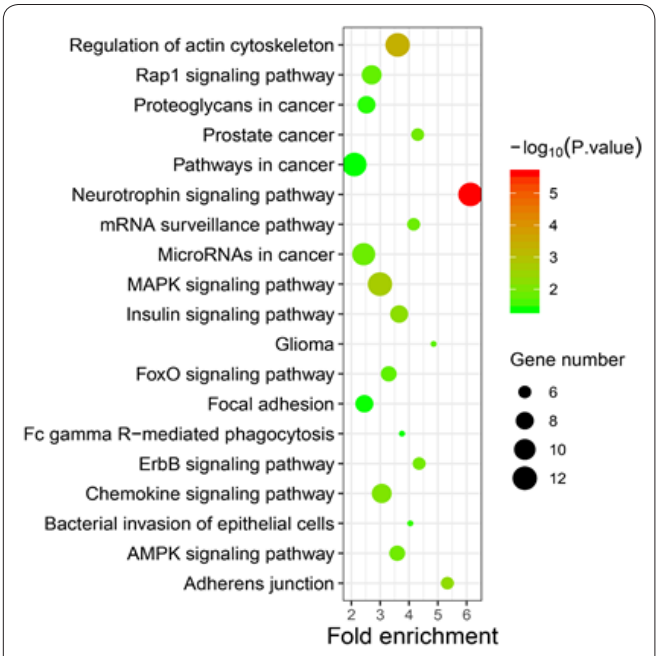

Figure 7. The bubble plot was used to visualize the results of KEGG pathway enrichement analysis and 19 pathways associated with the target mRNAs were displayed.

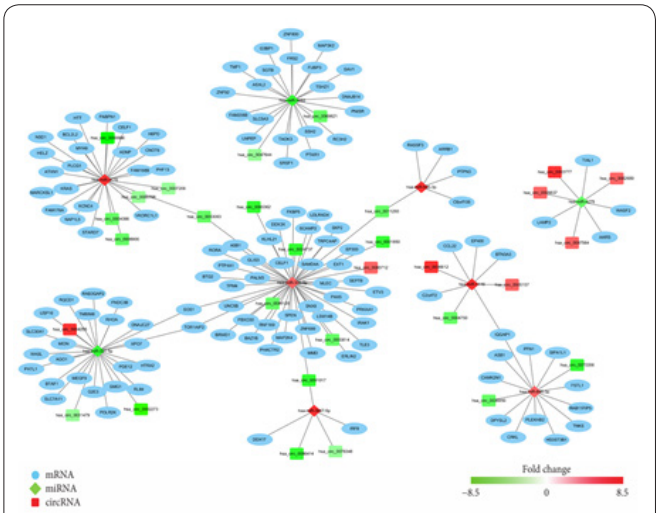

Figure 8. The network consisted 30 circRNAs, 9 miRNAs and 130 mRNAs. In this integrative regulatory network of circRNAs-miRNAs-mRNAs, the circRNAs were marked by the rectangle, the shape of rhombus represented miRNAs, and the shape of circle represented circRNAs. Solid lines represented interactions between two nodes.

tially expressed circRNAs and miRNAs were screening separately. After the target miRNAs of circRNAs with absolute FC >2.0 and dysregulated miRNAs were intersected using using Draw Venn Diagram tool, 31 overlapped miRNAs were used for further study. The target mRNAs of the overlapped miRNAs were used to perform the GO and KEGG pathway analyses. Furthermore, 9 miRNAs with a prediction score >0.4, 130 target mRNAs of the selected miRNAs, and 30 circRNAs which function as the sponge of the 9 miRNAs were utilized to construct the circRNA-miRNA-mRNA integrative regulation network and perform the network toplogy analysis utilizing CentiScaPe app. As shown in Figure 8, hsa-miR-339-5p had the most interactions with circRNAs and mRNAs; SOS1 and TOR1AIP2 that are two target mRNAs of hsa-miR-339-5p are also the target genes of hsa-miR-331-5p which regulate the expressions of other 22 mRNAs; hsa_circ_0091017, hsa_circ_0053083, and hsa_circ_0011293 that function as sponge of hsa-miR-339-5p also regulate the expression of hsa-miR-3667-5p, hsa-miR-3176, or hsa-miR-525-3p respectively, which indicated that hsa-miR-339-5p was the hub miRNAs in the regulation network.

In the integrative network of circRNA-miRNA-mRNA in atrial fibrillation, upregulated hsa-miR-339-5p interact with upregulated hsa_circ_0080712, and downregulated hsa_circ_0053083, hsa_circ_0085362, hsa_circ_0011293, hsa_circ_0024737, hsa_circ_0001850, hsa_circ_0003814, hsa_circ_0091017, hsa_circ_0084129 and interact with 40 downregulated mRNAs. Recent studies have demonstrated that hsa-miR-339-5P can serve as a novel biomarker and may provide diagnostic and prognostic value in carcinoma. Jia et al. (31) found that LncRNA MAFG-AS1 facilitated the migration and invasion of NSCLC cell via sponging miR-339-5p from MMP15 and MAFG-AS1-miR-339-5p-MMP15 axis might be a promising therapeutic target for the treatment of patients with NSCLC. Meanwhile, another study have reported that miR-339-5p increases radiosensitivity of lung cancer cells by targeting phosphatases of regenerating Liver-1 (PRL-1) (31, 32). We found for the first time that hsa-miR-339-5p significantly upregulated in right atrial appendage of persistent AF. In our research, we showed hsa_circ_0080712, hsa_circ_0053083, hsa_circ_0085362, hsa_circ_0011293, hsa_circ_0024737, hsa_circ_0001850, hsa_circ_0003814, hsa_circ_0091017, and hsa_circ_0084129, wich located on chromosome 7, 2, 8, 1, 11, 9, 6, X, or 8 respectively, could function as sponge of hsa-miR-339-5p to regulate the expression of genes. Zhang et al. found that cyclic RNA hsa_circ_0091017 inhibits proliferation, migration and invasiveness of bladder cancer cells by binding to microRNA-589-5p (33). Our results provided new insight into highly possible interaction mechanisms between circRNAs and miRNAs in AF. For a further understanding of biological functions of the 40 target mRNAs of hsa-miR-339-5p, we performed KEGG pathway analysis. The top 3 significantly enriched pathways of these mRNAs were Neurotrophin signaling pathway, Regulation of actin cytoskeleton pathway, and MAPK signaling pathway. Recent research has shown that MAPK pathway activation was associated with cardiac fibroblasts in a rat model of atrial fibrillation (34). Moreover, GO enrichment analysis was performed for the target mRNAs to investigate the biological function of the circRNAs. In the molecular function analysis, enzyme binding, macromolecular complex binding, and protein binding were the top 3 enriched terms. In the biological process analysis, regulation of cellular metabolic process, cellular macromolecule metabolic process, and regulation of macromolecule metabolic process were the top 3 enriched terms. In the cellular component analysis, intracellular organelle, intracellular, and organelle were the top 3 enriched terms.

Interestingly, the number of target genes of hsa-miR-3662, hsa-miR-3176, or hsa-miR-331-5p was almost the same. 21 upregulated genes were as competing endogenous mRNAs of downregulated hsa_circ_0069621 and hsa_circ_0047644 by competitively binding to downregulated hsa-miR-3662 (Figure 8). These circRNAs have not been reported to be associated with AF. Previously studies have identified hsa-miR-3662, which is a new actor in the prominent hematopoietic quantitative trait locus in chromosome 6q23.3 played important roles in malignant hematopoietic cell proliferation and suppressed hepatocellular carcinoma growth through inhibition of HIF-1 alpha-mediated Warburg effect (35, 36). The 6 downregulated hsa_circ_0000666, hsa_circ_0007209, hsa_circ_0053083, hsa_circ_0000798, hsa_circ_0004066, and hsa_circ_0008900 were predicted to regulate the expression of 21 mRNAs by functioning as sponge of upregulated hsa-miR-3176. In the KEGG pathway analysis, the top 3 significantly enriched pathways of these mRNAs were Neurotrophin signaling pathway, Regulation of actin cytoskeleton pathway, and MAPK signaling pathway. Previous research has shown that MAPK pathway activation was associated with cardiac fibroblasts in a rat model of atrial fibrillation(Guo et al., 2020). In addition, 22 upregulated genes were as competing endogenous mRNAs of downregulated hsa_circ_0002273, hsa_circ_0031479 and upregulated hsa_circ_0004255 by competitively binding to downregulated hsa-miR-331-5p (Figure 8). Feng DD et al. (37) have reported that down-regulated miR-331-5p and miR-27a are associated with chemotherapy resistance and relapse in leukaemia. In the KEGG pathway analysis, the top 3 significantly enriched pathways of these mRNAs were Neurotrophin signaling pathway, Regulation of actin cytoskeleton pathway, and MAPK signaling pathway. Previous research has shown that MAPK pathway activation was associated with cardiac fibroblasts in a rat model of atrial fibrillation (34).

In summary, our study showed that 30 circRNAs, including 8 upregulated circRNAs and 22 downregulated circRNAs, were predicted to highly possibly function as sponges of 9 miRNAs to regulate gene expression by using bioinformatics analysis. Moreover, the interactions of hsa-miR-339-5p with its related circRNAs and target mRNAs constructed the hub regulatory network in persistent AF by utilizing topology analysis. Our proposed regulatory network of circRNAs-miRNAs-mRNAs may provide new insight into the potential mechanism underlying persistent AF. Additionally, these important molecular may become novel biomarkers providing a new strategy in diagnosis and therapy of AF.

Acknowledgments

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