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Bioinformatics analysis of the circRNA-miRNA-mRNA network for atrial fibrillation

Xing Liu, MS[a], Yiqian Zeng, MS[b], Zhao Liu, MS[b], Wenbin Li, MS[a], Lei Wang, MD[a], Mingxing Wu, MS[a,*]

Abstract

Atrial fibrillation (AF) is a chronic and progressive disease, with advancing age, the morbidity of which will increase exponentially. Circular ribonucleic acids (RNAs; circRNAs) have gained a growing attention in the development of AF in recent years. The purpose of this study is to explore the mechanism of circRNA regulation in AF, in particular, the intricate interactions among circRNA, microRNA (miRNA), and messenger RNA (mRNA). Three datasets (GSE129409, GSE68475, and GSE79768) were obtained from the Gene Expression Omnibus database to screen differentially expressed (DE) circRNAs, DE miRNAs, and DE mRNAs in AF, respectively. Based on circRNA-miRNA pairs and miRNA-mRNA pairs, a competing endogenous RNAs (ceRNAs) network was built. Then, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analysis of DE mRNAs in the network were performed and protein-protein interaction (PPI) networks were established to identify hub genes. Finally, a circRNA-miRNA-hub gene subnetwork was constructed. A total of 103 DE circRNAs, 16 DE miRNAs, and 110 DE mRNAs were screened in AF. Next, ceRNAs network in AF was constructed with 3 upregulated circRNAs, 2 downregulated circRNAs, 2 upregulated miRNAs, 2 downregulated miRNAs, 17 upregulated mRNAs, and 24 downregulated mRNAs. Thirty GO terms and 6 KEGG pathways were obtained. Besides, 6 hub genes (C-X-C chemokine receptor type 4 [*CXCR4*], C-X-C chemokine receptor type 2 [*CXCR2*], C-X-C motif chemokine 11 [*CXCL11*], neuromedin-U, B1 bradykinin receptor, and complement C3) were screened from constructing a PPI network. Finally, a circRNA-miRNA-hub gene subnetwork with 10 regulatory axes was constructed to describe the interactions among the differential circRNAs, miRNA, and hub genes. We speculated that hsa_circRNA_0056281/hsa_circRNA_0006665 -hsa-miR-613-CXCR4/CXCR2/CXCL11 regulatory axes and hsa_circRNA_0003638-hsa-miR-1207-3p-CXCR4 regulatory axis may be associated with the pathogenesis of AF.

Abbreviations: AF = atrial fibrillation, BDKRB1 = B1 bradykinin receptor, C3 = complement C3, ceRNAs = competing endogenous RNAs, CXCL11 = C-X-C motif chemokine 11, CXCR2 = C-X-C chemokine receptor type 2, CXCR4 = C-X-C chemokine receptor type 4, GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, NMU = neuromedin-U, PPI = protein-protein interaction.

Keywords: atrial fibrillation, circular RNA, microRNA, mRNA, network

1. Introduction

Atrial fibrillation (AF) is a chronic and progressive disease; survey has shown that the prevalence of AF is ≈0.77% in the overall population, with the increase of age, the incidence, and prevalence of which increase exponentially, reaching ≥7.5% in those older than 80 years.[1] In addition, AF is closely associated with poor life quality, a significantly increased risk of heart failure, embolic stroke, and also recognized as a underlying factor for high all-cause mortality, and hospitalization rates, in particular elderly individuals.[1–3] However, current treatment effect of drugs and radiofrequency ablation of AF are still not satisfactory, especially in the radiofrequency ablation treatment of persistent AF.[4] At present, the mechanisms underpinning the pathogenesis of AF are mostly represented by atrial structural remodeling, electrical remodeling, inflammation, and genes,[5] but initial and definite mechanism of how AF occurs is still unclear.

Circular ribonucleic acids (RNAs; circRNAs) are a family of non-coding RNAs formed by a special splicing mechanism, which has a closed loop structure and good stability.[6] It is well known that dysregulated miRNAs can lead to the occurrence of AF by disentangling transcription factors, modulating atrial excitability, and enhancing atrial arrhythmogenicity.[7,8] As a novel kind of molecule with exceptional biological properties, circRNAs can be used as microRNA (miR or miRNA) sponge to bind miRNAs competitively, affect alternative splicing, and control the transcription of parental genes.[9] A growing body of evidence suggests that circRNA-associated competing endogenous RNA (ceRNA) networks are involved in cardiovascular disease's pathogenesis, mainly relating to myocardial infarction,[10,11] dilated cardiomyopathy,[12] heart failure, and cardiac hypertrophy.[13,14] Recent animal experiments[15] confirmed that mmu_circ_0005019 played biological functions by acting as a miR-499-5p sponge to regulate the expression of its target gene

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Kcnn3, inhibiting the fibrosis of cardiac fibroblasts and reversing the electrical remodeling of cardiomyocytes, which is associated with the pathogenesis of AF. Therefore, circRNAs may be a novel kind of latent biomarkers and therapeutic targets for AF, and it is particularly important to further construct and understand circRNA-associated ceRNA networks associated with AF.

The aim of this study was to integrate available microarray data concerning circRNAs, miRNAs, and messenger ribonucleic acids (mRNAs) in AF with bioinformatics tools to construct circRNA-associated ceRNA networks and predict the latent functions of those circRNA-miRNA-mRNA regulatory axes in the pathogenesis of AF. First, differentially expressed (DE) circRNAs, DE miRNAs, and DE mRNAs in AF were identified. Then, a dysregulated circRNA-miRNA-mRNA network was built in AF as well as protein-protein interaction (PPI) networks. Next, the hub genes were subsequently identified. To better understand the molecular genetic mechanism of AF, a subnetwork related to AF was constructed. Our research provides a novel regulatory network underlying the genesis of AF and uncovers molecular linkages among dysregulated transcripts in AF.

2. Methods

2.1. Data acquisition

Three datasets (GSE129409, GSE68475, and GSE79768) were downloaded from gene expression omnibus (http://www.ncbi.nlm.nih.gov/geo/).[16] The circRNA expression profile of GSE129409 contained the heart tissues (left atrial appendages) from 3 AF patients and 3 healthy controls. The miRNA expression profile of GSE68475 contained right atrial appendages from 10 AF patients and 11 sinus rhythm (SR) patients. The mRNA expression profile of GSE79768 contained left atrial appendages from 7 AF patients and 6 control patients. The flowchart of bioinformatics analyses is presented in Figure 1. All datasets originated from a free open-access database on the internet; thus no ethical approval and patient consent are required in this study.

2.2. Identification of DE circRNAs, DE miRNAs, and DE mRNAs in AF

The Limma and pheatmap packages of R (https://www.rproject.org/)/Bioconductor (http://www.bioconductor.org/) were utilized to screen DE circRNAs, DE miRNAs, and DE mRNAs between SR samples and AF samples. Two datasets, GSE68475 (platform GPL15018) and GSE79768 (platform GPL570), were analyzed with a fold change ≥|2| and P value < 0.05 set as the cutoff point for the selection of DE miRNA and DE mRNA separately. To assess DE circRNA in AF, GSE129409 (platform GPL21825) was used, and transcripts with a cutoff point of $\log_2$ (fold change) ≥|3| and P value < .05 were retrieved.

2.3. Construction of a circRNA-miRNA-mRNA regulatory network

CircBase (http://www.circbase.org/)[17] is a repository for information about circRNAs. Target miRNAs were predicted by the cancer-specific circRNA database (CSCD, https://gb.whu.edu.cn/CSCD/).[18] Each DE circRNA's predicted miRNAs were obtained. Compilation of miRNAs that overlapped with anticipated and DE miRNAs followed. Then, the TargetScan database (http://www.targetscan.org/vert_72/) was used to predict the miRNA-targeted mRNAs.[19] To this end, mRNAs identified by TargetScan database were regarded as candidate targets and intersected with the identified DE mRNAs. The ggpubr and reshape2 packages of R/Bioconductor were used to screen the DE circRNAs, DE miRNAs, and DE mRNAs in the ceRNA network in the microarray datasets. Finally, the ceRNAs regulatory network in AF was established by Cytoscape version 3.8.0 (https://cytoscape.org/).

2.4. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes functional enrichment analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analysis of DE mRNAs in the ceRNA network were performed using the clusterprofiler, org.Hs.eg.db, enrichplot, ggplot2 Goplot, and digest packages of R/Bioconductor. P value < .05 was regarded as a statistically significant difference.

2.5. Construction of PPI regulatory network and screening of hub genes

A PPI network was established by using the STRING database (http://string-db.org)[20] and visualized using the CytoCope 3.8.0 software. MCODE, a Cytoscape app, was run to find the most significant protein module. In addition, hub genes in the PPI network were filtered by the Maximal Clique Centrality arithmetic of Cytoscape plug-in cytoHubba. Finally, we constructed a circRNA-miRNA-hub genes subnetwork.

3. Results

3.1. Identification of DE circRNAs, DE miRNAs, and DE mRNAs in AF

Table 1 lists the basic information on the expression of RNAs in AF and SR tissues from 3 microarray datasets (GSE129409, GSE68475, and GSE79768). In total, 103 DE circRNAs (59 upregulated and 44 downregulated circRNAs) were screened in the circRNA expression profile data (Fig. 2A and D). There was no search for fifteen circRNAs in the CSCD database. We predicted, using the CSCD database, that the remaining 88 circRNAs could target 1955 miRNAs. 5 upregulated and 11 downregulated miRNAs were screened in the miRNA expression profile data (Fig. 2B and E). Then, 5 intersecting miRNAs were acquired (Fig. 2G). Via the TargetScan database, 11403 potential target genes for the 5 miRNAs were predicted. In the mRNA dataset, a total of 110 DE mRNAs (44 upregulated and 66 downregulated mRNAs) were identified in AF (Fig. 2C and F). Sixty-two intersecting mRNAs were obtained (Fig. 2H).

3.2. Construction of ceRNAs regulatory networks in AF

A circRNA-miRNA-mRNA network in AF was constructed with 3 upregulated circRNAs, 2 downregulated circRNAs, 2 upregulated miRNAs, 2 downregulated miRNAs, 17 upregulated mRNAs, and 24 downregulated mRNAs (Fig. 3). The DE circRNAs, DE miRNAs, and DE mRNAs in the ceRNA network in the microarray datasets are shown in Figure 4. The basic information and basic structural pattern of the 5 circRNAs in the ceRNA network are listed in Table 2 and Figure 5, respectively.

3.3. Functional enrichment analyses for mRNAs in the ceRNAs network

The results showed that the identified mRNAs in the network were predominantly enriched in "response to metal ion," "positive regulation of cytosolic calcium ion concentration," "regulation of cytosolic calcium ion concentration" and "regulation of cell junction assembly" (biological processes) (Fig. 6A); "focal adhesion," "cell-substrate junction" and "A band" (cellular components) (Fig. 6A); and "G protein–coupled peptide receptor activity," "peptide receptor activity" and "C-C chemokine receptor activity" (molecular functions) (Fig. 6A). KEGG pathway

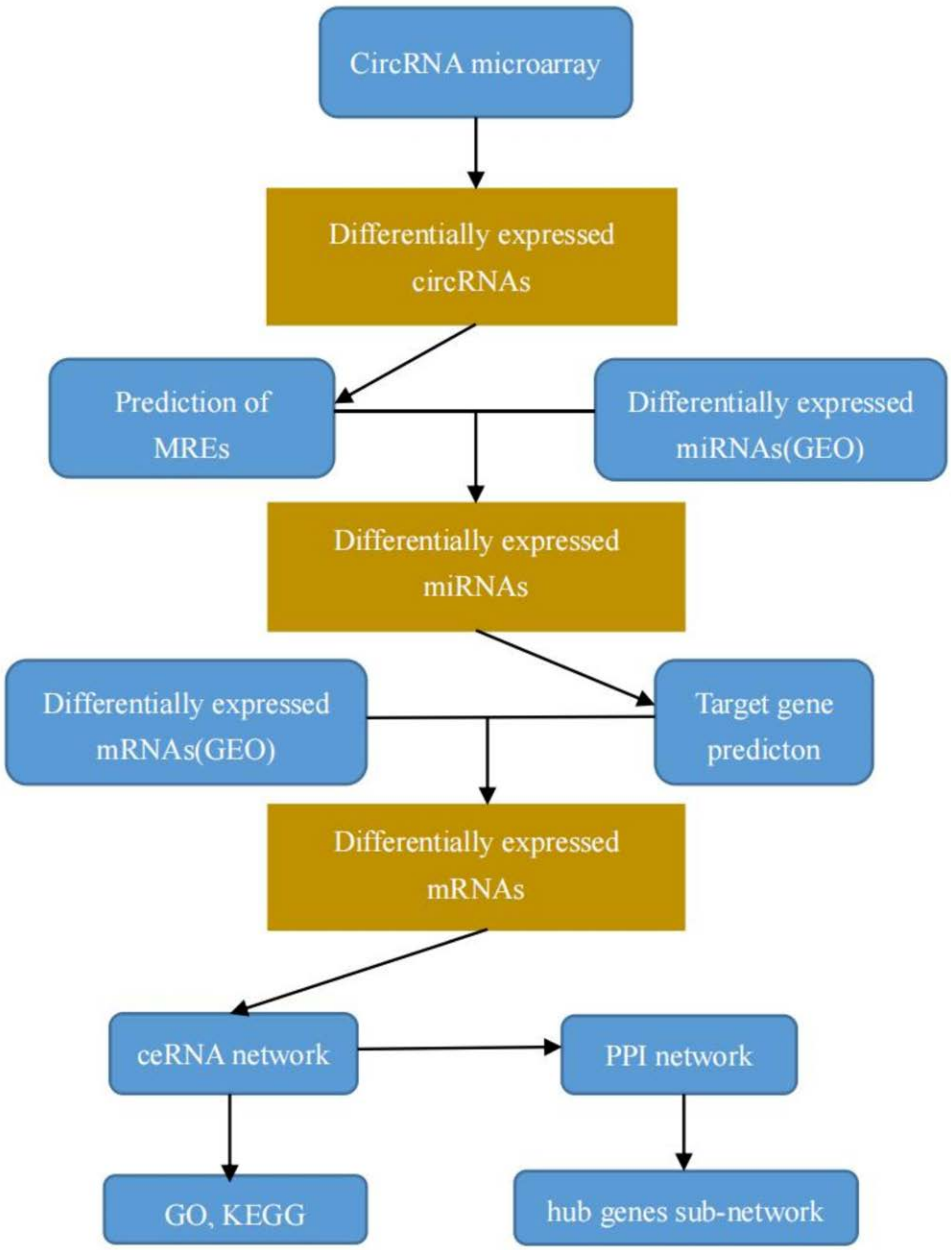

Figure 1. Flow diagram of study. ceRNA = competing endogenous RNAs, CicRNA = circular RNA, GEO = Gene Expression Omnibus, GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, MiRNA = microRNA, MREs = microRNA response elements, PPI = protein-protein interaction, RNA = ribonucleic acid.

Table 1

Basic information of the 3 microarray datasets from GEO.

Data source	Platform	Series	Sample size (AF/SR)
CircRNA	GPL21825	GSE129409	3/3
miRNA	GPL15018	GSE68475	10/11
mRNA	GPL570	GSE79768	7/6

AF = atrial fibrillation, CicRNA = circular RNA, GEO = Gene Expression Omnibus, MiRNA = microRNA, mRNA = messenger RNA, RNA = ribonucleic acid, SR = sinus rhythm.

analysis showed significant enrichment in the "Complement and coagulation cascades," "Viral protein interaction with cytokine and cytokine receptor," "Chemokine signaling pathway" (Fig. 6C). The main results of GO enrichment and KEGG pathway analysis and related genes are shown in Figure 6B and D.

3.4. Construction of PPI network

A PPI network including 14 nodes and 20 edges was built for the 41 mRNAs of the ceRNAs network by using the STRING database after removing unconnected nodes (Fig. 7A). To explore and construct the crucial circRNA-miRNA-hub genes regulatory axis in the progression of AF, hub genes in the PPI network were identified by using MCODE software. The vital module consisting of 6 nodes and 15 edges was selected (Fig. 7A). These 6 hub genes (Table 3) were C-X-C chemokine receptor type 4 (*CXCR4*), C-X-C chemokine receptor type 2 (*CXCR2*), C-X-C motif chemokine 11 (*CXCL11*), neuromedin-U (*NMU*), B1 bradykinin receptor (*BDKRB1*), complement

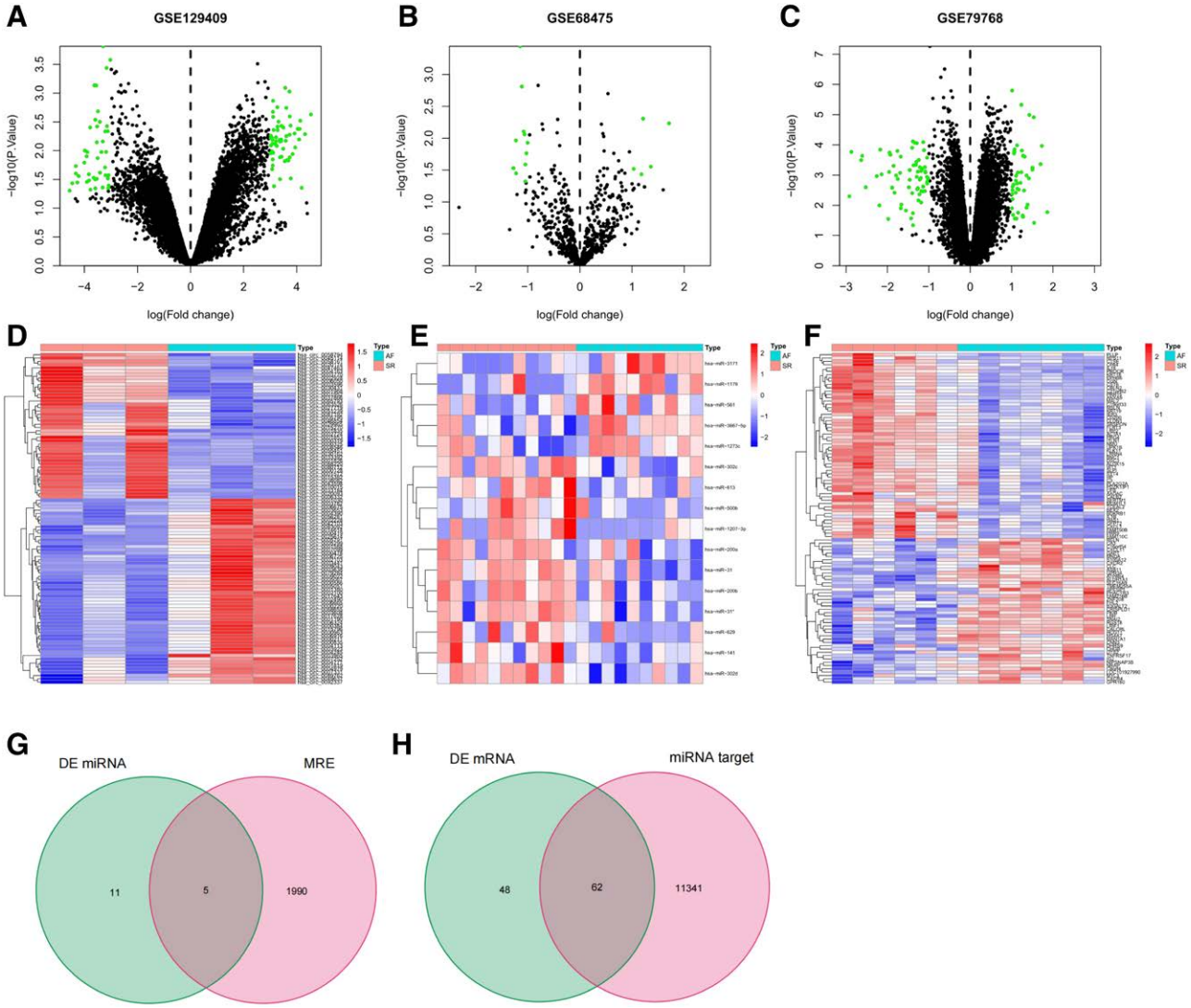

Figure 2. Identification of DE circRNAs, DE miRNAs, and DE mRNAs in AF. Volcano plots (A) and heat map (D) of DE circRNAs in the GSE129409 dataset. Volcano plots (B) and heat map (E) of DE miRNAs in the GSE68475 dataset. Volcano plots (C) and heat map (F) of DE mRNAs in the GSE79768 dataset. Green dots show DE RNAs. (G) Venn diagram of overlapping DE miRNAs from predicted MRE and DE miRNAs in the GSE68475 dataset. (H) Venn diagram of overlapping DE mRNAs from DE mRNAs in the GSE79768 dataset and targeted mRNAs. AF = atrial fibrillation, DE = differentially expressed, MRE = microRNA response elements, SR = sinus rhythm.

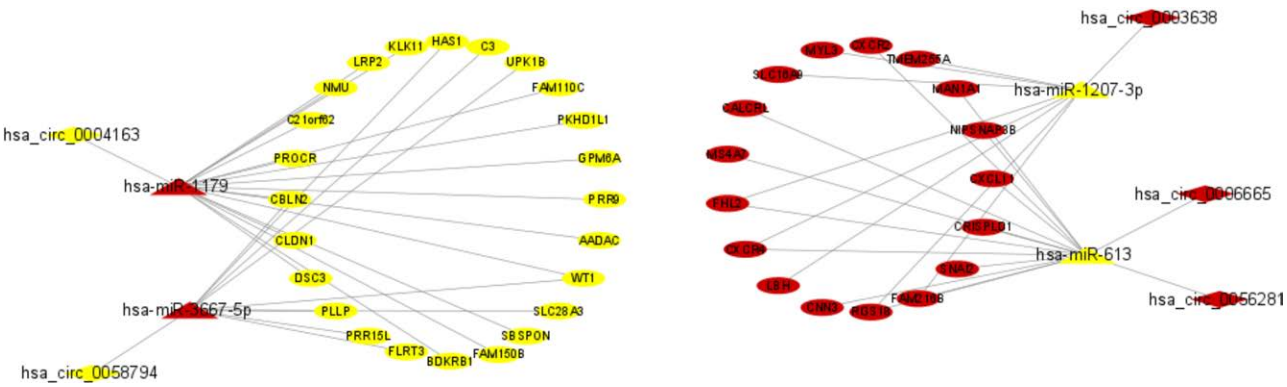

Figure 3. The circRNA-miRNA-mRNA network in AF. Red indicates upregulated circRNAs, miRNAs, or mRNAs; Yellow indicates downregulated circRNAs, miRNAs or mRNAs. AF = atrial fibrillation, circ = circular RNA, CicRNA = circular RNA, MiRNA = microRNA, hsa = homo sapiens, miR = microRNA, mRNA = messenger RNA, RNA = ribonucleic acid.

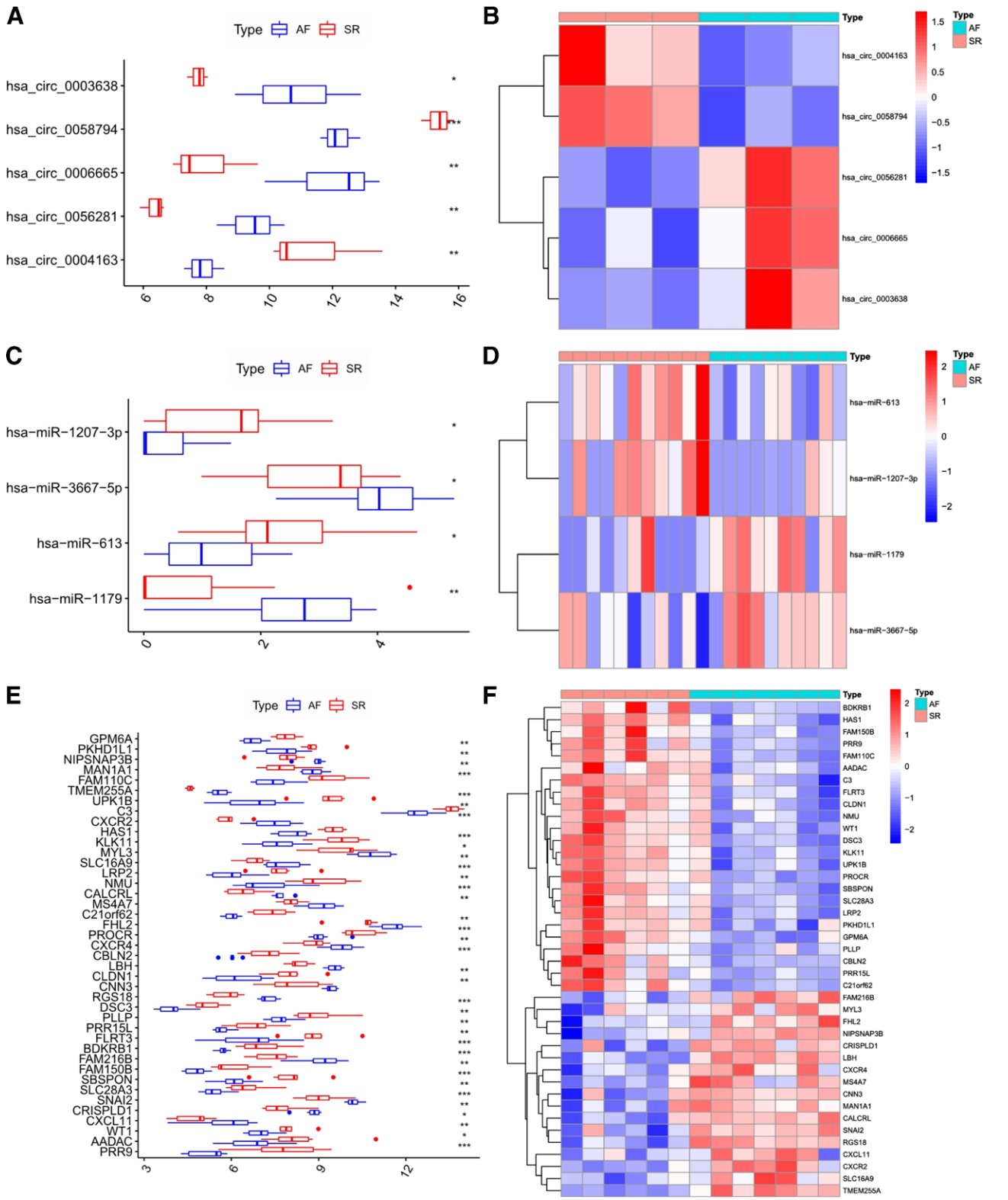

Figure 4. The DE circRNAs, DE miRNAs, and DE mRNAs in the ceRNA network. Boxplot (A) and heat map (B) of DE cicrRNA. Boxplot (C) and heat map (D) of DE miRNA. Boxplot (E) and heat map (F) of DE mRNA. AF = atrial fibrillation, ceRNA = competing endogenous RNAs, CicRNA = circular RNA, DE = differentially expressed, mRNA = messenger RNA, RNA = ribonucleic acid, SR = sinus rhythm. *$P < .05$, **$P < .01$, ***$P < .001$.

C3 (*C3*) (Fig. 7B). Subsequently, a circRNA-miRNA-hub gene subnetwork with 10 regulatory axis was constructed (Fig. 8), including hsa_circ_0003638-hsa-miR-1207-3p-CXCR4 axis, hsa_circ_0006665-hsa-miR-613-CXCR4 axis, hsa_circ_0006665-hsa-miR-613-CXCR2 axis, hsa_circ_0006665-hsa-miR-613-CXCL11 axis, hsa_circ_0056281-hsa-miR-613-CXCR4 axis, hsa_circ_0056281-hsa-miR-613-CXCR2 axis, hsa_circ_0056281-hsa-miR-613-CXCL11 axis, hsa_circ_0058794-hsa-miR-3667-5p-C3 axis, hsa_circ_0004163-hsa-miR-1179-NMU axis, and hsa_circ_0004163-hsa-miR-1179-BDKRB1 regulatory axis.

Table 2

Basic characteristics of the 5 differentially expressed circRNAs in GSE129409 which are involved in ceRNA network.

CircRNA ID	logFC	Position	Genomic length	Strand	Best transcript	Gene symbol
hsa_circ_0056281	3.1	chr2:120885263-120922486	37223	+	NM_020909	EPB41L5
hsa_circ_0058794	–3.2	chr2:236626200-236659132	32932	+	NM_001037131	AGAP1
hsa_circ_0006665	3.9	chr10:15875628-15889942	14314	–	NM_024948	FAM188A
hsa_circ_0003638	3.1	chr17:26490568-26499644	9076	+	NM_016231	NLK
hsa_circ_0004163	–3.5	chr18:662145-671451	9306	+	NM_001071	TYMS

AGAP1 = ANK repeat and PH domain-containing protein 1, ceRNA = competing endogenous RNA, chr = chromosome, CicRNA = circular RNA, EPB41L5 = Band 4.1-like protein 5, FC = fold change, NLK = Nemo-like kinase, RNA = ribonucleic acid, TYMS = thymidylate synthase.

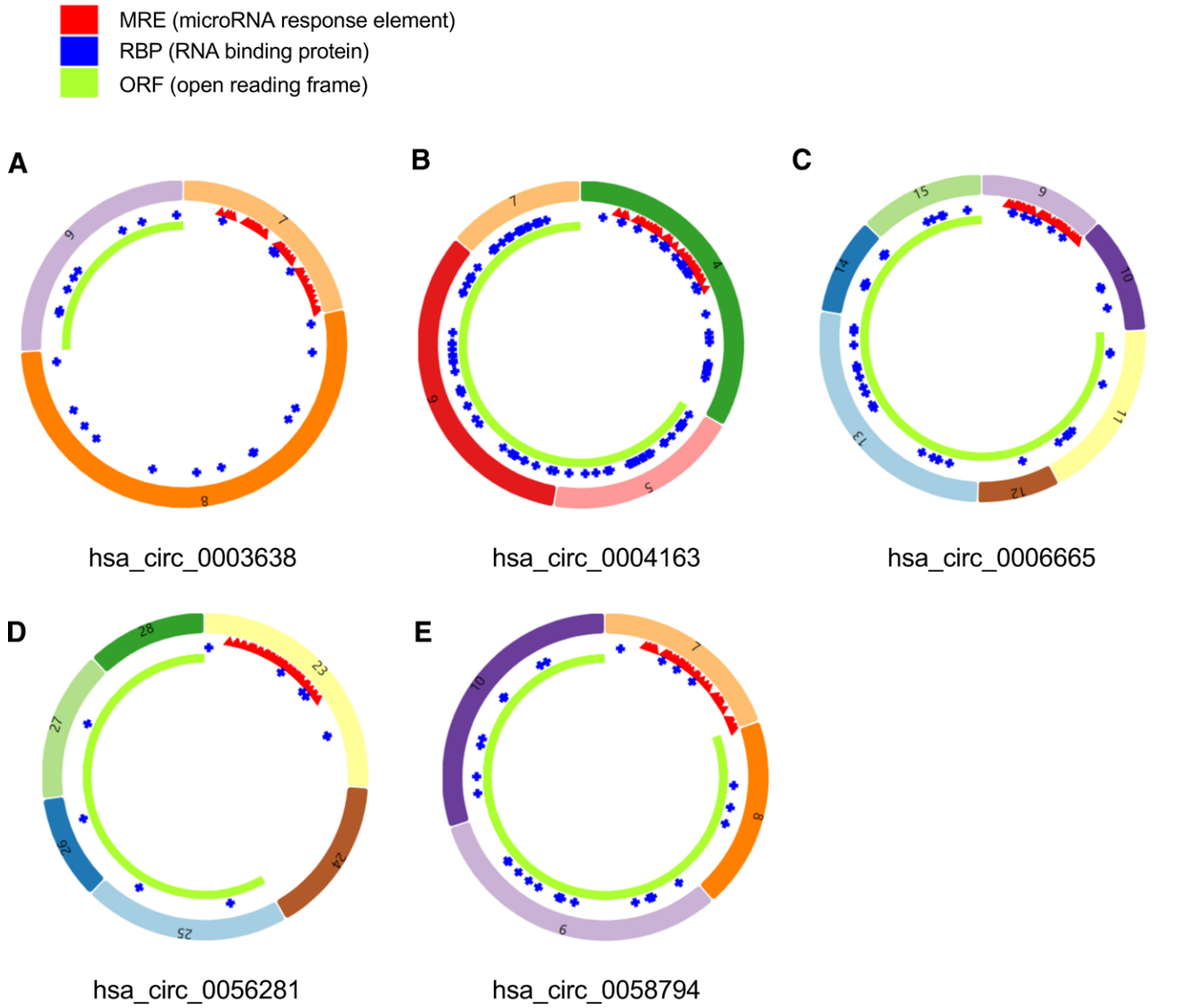

Figure 5. Structural patterns of the 5 circRNAs (A–E) in the ceRNA network. ceRNA = competing endogenous RNAs, circ = circular RNA, CicRNA = circular RNA, hsa = homo sapiens, MREs = microRNA response elements, ORF = open reading frame, RBP = RNA binding protein, RNA = ribonucleic acid.

4. Discussion

AF is by far the most prevalent form of arrhythmia, and its incidence increases with age, which is closely connected with a decline in quality of life, stroke, heart failure, and an elevated risk of mortality. Although treatment strategies have advanced dramatically in recent years, their efficacy is not ideal. The reason is the incomplete knowledge of the AF mechanisms. Consequently, it is essential to get a deeper knowledge of the molecular and cellular mechanisms of AF and to develop more effective treatments for AF.

In this study, to explore the role of circRNAs in AF, a circRNA-miRNA-mRNA regulatory network with 3 upregulated circRNAs, 2 downregulated circRNAs, 2 upregulated miRNAs, 2 downregulated miRNAs, 17 upregulated mRNAs, and 24 downregulated mRNAs was established based on bioinformatics analysis and transcriptome data, indicating that these DE circRNAs, DE miRNA, and DE mRNA in the network may play an significant

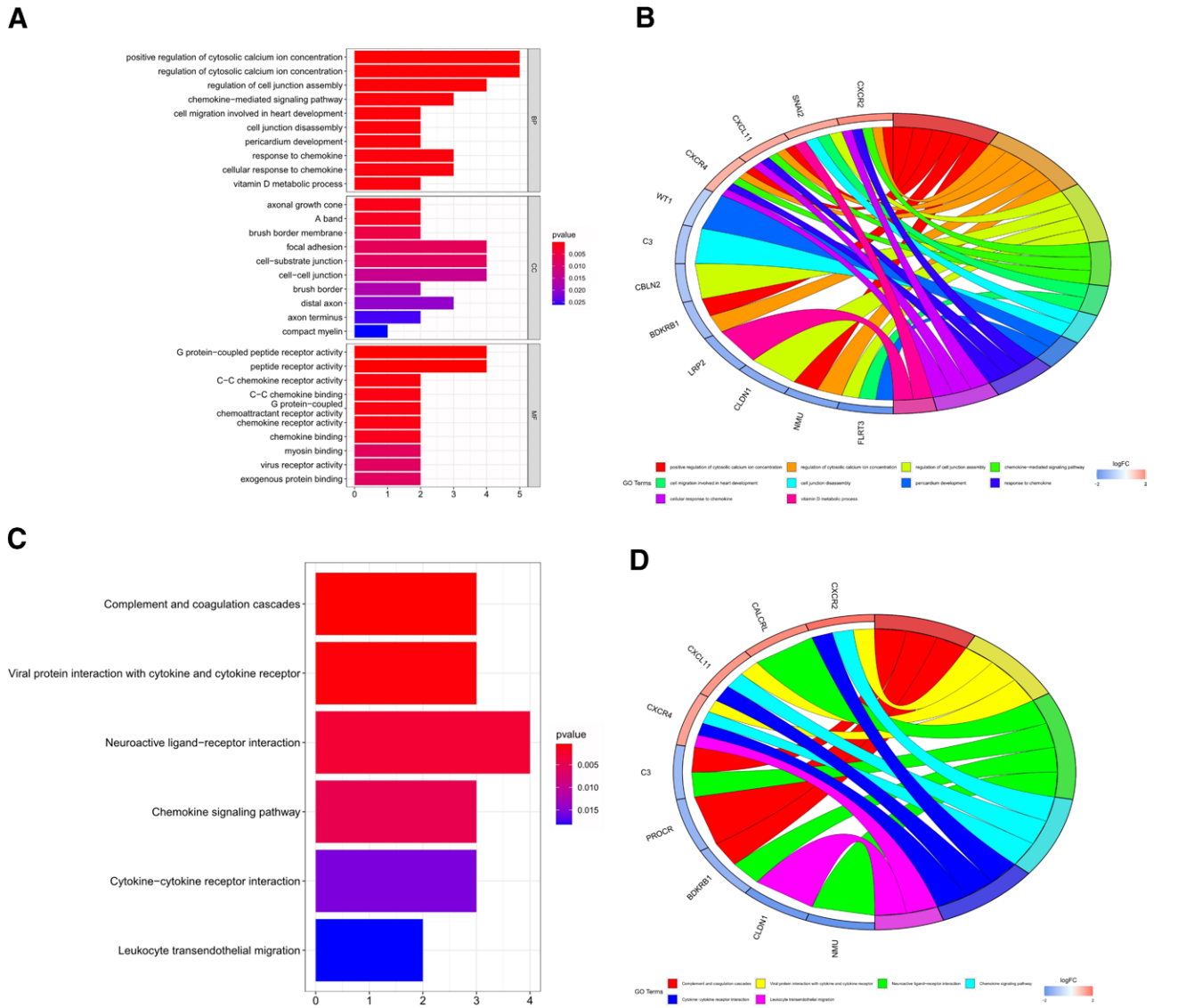

Figure 6. GO and KEGG pathway enrichment analysis for mRNAs in the ceRNA network. **A**, GO analyses of BP, CC, and MF. **C**, The enrichment analysis of the KEGG pathways. **B** and **D**, The main results of GO enrichment and KEGG pathway analysis, and related genes. BDKRB1 = B1 bradykinin receptor, BP = biological process, C3 = complement C3, CALCRL = Calcitonin gene-related peptide type 1 receptor, CBLN2 = common variants in cerebellin 2, CC = cellular component, ceRNA = competing endogenous RNAs, CLDN1 = claudin 1, CXCL11 = C-X-C motif chemokine 11, CXCR2 = C-X-C chemokine receptor type 2, CXCR4 = C-X-C chemokine receptor type 4, FC = fold change, FLRT3 = fibronectin-leucine-rich transmembrane protein 3, GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, LRP2 = Low-density lipoprotein receptor-related protein 2, MF = molecular function, NMU = neuromedin-U, PROCR = endothelial protein C receptor, RNA = ribonucleic acid, SNAI2 = snail family transcription repressor 2, WT1 = Wilms tumour 1.

role in AF pathogenesis. GO analysis results showed that identified mRNAs in ceRNA network were significantly implicated in the positive regulation of cytosolic calcium ion concentration, chemokine-mediated signaling pathway, G protein–coupled peptide receptor activity, and C-C chemokine receptor activity, including 6 genes (*CXCL11*, *BDKRB1*, *CXCR4*, *NMU*, *CXCR2*, *calcitonin gene-related peptide type 1 receptor*). KEGG pathway analysis results were predominantly enriched in the chemokine signaling pathway, complement and coagulation cascades, and cytokine-cytokine receptor interaction, including 6 genes (*CXCL11*, *CXCR4*, *CXCR2*, *BDKRB1*, *C3*, *endothelial protein C receptor*). The chemokine signaling pathway plays a key role in cardiovascular disease. For example, chemokines and their receptors are critical for the recruitment and activation of immune cells and the sustaining of the local inflammatory response in atherosclerosis.[21] Chemokines and their receptors have also been shown to be involved in the pathophysiology of cardiac remodeling and heart failure resulting from excessive pressure load.[22] According to GO and KEGG results, ceRNA network about AF in this study was mainly implicated in the regulation of cytosolic calcium ion, inflammation, and the immune system. On the one hand, studies have found that large influx of calcium ions can lead to calcium overload in atrial myocytes, which can decrease atrial muscle contractility, enlarge atrial volume, and increase atrial pressure. Fibroblasts play an important role in heart structural remodeling, and the increase of intracytoplasmic Ca^{2+} in fibroblasts can promote their proliferation and differentiation into myofibroblasts, leading to atrial fibrosis, the basis of electroanatomical remodeling, and AF maintenance and progression.[23] Intracellular Ca^{2+} aggregation not only causes delayed afterdepolarizations and maintains arrhythmias but also leads to atrial fibrosis.[23–25] On the other hand, numerous studies have shown a relationship between inflammation and atrial fibrosis, and inflammation and its associated immune response play a vital role in the etiology and progression of AF.[26,27] As an acute phase reactive protein, C-reactive protein (CRP) is a marker of the inflammatory system. Study found that CRP was independently associated with the occurrence of AF, and the incidence of AF increased with the increase in CRP expression.[28,29] Thus, further exploration of the

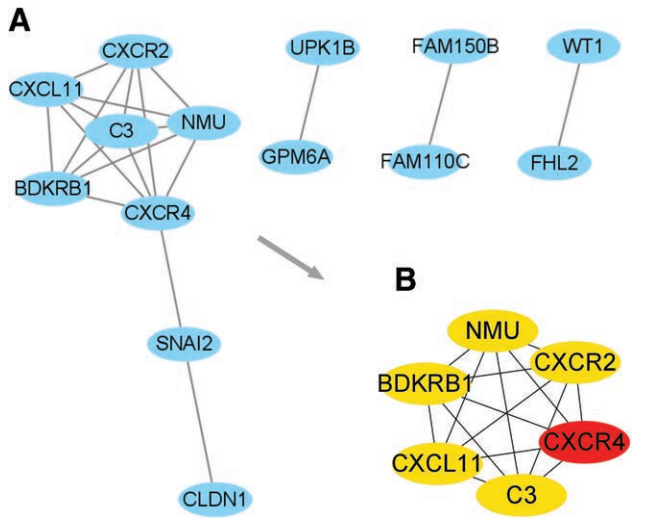

Figure 7. PPI network (A) construction and hub gene (B) selection for mRNAs in the ceRNA network. BDKRB1 = B1 bradykinin receptor, C3 = complement C3, ceRNA = competing endogenous RNAs, CLDN1 = claudin 1, CXCL11 = C-X-C motif chemokine 11, CXCR2 = C-X-C chemokine receptor type 2, CXCR4 = C-X-C chemokine receptor type 4, FHL2 = four and a half LIM domains 2, GPM6A = neuronal membrane glycoprotein M6-a, mRNA = messenger RNA, NMU = neuromedin-U, PPI = protein-protein interaction, RNA = ribonucleic acid, UPK1B = Uroplakin-1b, WT1 = WT1 = Wilms tumour 1 , SNAI2 = snail family transcription repressor 2.

Table 3

Hub genes in protein-protein interaction network.

Gene symbol	MCC score	logFC	*P* value	Gene title
CXCR4	121	1.020	.0031	C-X-C chemokine receptor type 4
CXCR2	120	1.505	.0006	C-X-C chemokine receptor type 2
CXCL11	120	1.119	.0278	C-X-C motif chemokine 11
NMU	120	−1.888	.0026	Neuromedin-U
BDKRB1	120	−1.369	.0005	B1 bradykinin receptor
C3	120	−1.194	.0012	Complement C3

FC = fold change, MCC = maximal clique centrality.

molecular mechanism of atrial fibrosis might be a potential means of preventing and treating AF.

Next, we established a PPI network using genes in this ceRNA network and identified six hub genes (*CXCR4*, *CXCR2*, *CXCL11*, *NMU*, *BDKRB1*, *C3*). Among them, 5 hub genes (*CXCR4*, *CXCR2*, *CXCL11*, *NMU*, *BDKRB1*) were enriched in the positive regulation of cytosolic calcium ion concentration, 3 hub genes (*CXCR4*, *CXCR2*, *CXCL11*) were enriched in chemokine signaling pathway, and 2 hub genes (*BDKRB1*, *C3*) were enriched in complement and coagulation cascades according to GO and KEGG enrichment analyses. Finally, a circRNA-miRNA-hub gene ceRNA subnetwork with 10 regulatory axes was built.

4.1. The potential roles of hsa_circRNA_0056281/ hsa_circRNA_0006665, hsa-miR-613, and CXCR4/CXCR2/CXCL11 in AF

EPB41L5 and *FAM188A* are the host gene of hsa_circRNA_0056281 and hsa_circRNA_0006665, respectively. Lv et al[30] found that circ-EPB41L5 regulated the expression of its host gene *EPB41L5* via sponging miR-19a; consecutively, they showed that the expression of *EPB41l5* mRNA was obviously increased in circ-EPB41L5 glioma cells with overexpressing. *EPB41L5* is a member of the 4.1 protein family, which is implicated in several cellular processes, such as the cell adhesion, organization of cell polarity, response to growth factors, and motility.[31,32] It is known to all that the transforming growth factor-β1 (TGF-β1)/Smad2/3 signaling pathway is vital for atrial fibrosis, which is a hallmark of AF and is also mediated by the inflammatory response.[33,34] Study demonstrated that phosphorylated Smad3 binds to and activates *EPB41L5* gene transcription in response to TGF-β signaling.[35] It suggests that hsa_circRNA_0056281 may be related to atrial fibrosis in AF. Recently, no AF-related hsa_circRNA_ 0006665 was ever reported. In this study, we found that hsa_circRNA_0056281 and hsa_circRNA_0006665 were upregulated in the heart tissue of AF patients. Moreover, downregulated hsa-miR-613 and target upregulated CXCR4/CXCR2/CXCL11 were under the regulation of hsa_circRNA_0056281 and hsa_circRNA_0006665. Study has demonstrated that gap junction alpha-1 is a direct downstream target of miR-613[36] and large-scale genotyping reported novel AF risk loci at or near gap junction alpha-1.[37] Luciferase reporter assay indicated that *CXCR4* was a target of miR-613.[38] CXCR4 is an α-chemokine receptor specific for stromal cell–derived factor 1 (also called CXCL12) that transduces signals by increasing intracellular calcium ion levels and promoting mitogen-activated protein kinase 1/mitogen-activated protein kinase 3 activation,[39] which played a wide role in cardiac hypertrophy and myocardial remodeling.[40] Soppert et al[41] found that CXCR4 was related to myofibroblast necrosis, which may regulate cardiac remodeling in heart failure. In addition, Wang et al[42] also showed that the overexpression of CXCR4 was observed in patients with chronic AF and might lead to the process of AF through modulating atrial fibrosis and structural remodeling. Another vital downstream target gene of miR-613 is CXCR2 which is the major chemokine receptor of neutrophils. Study demonstrated that CXCR2 is associated with the pathogenesis of angiotensin II–induced cardiac remodeling and atrial remodeling, such as atrial fibrosis.[43] And selective blockade of CXCR2 prevents and reverses AF in spontaneously hypertensive rats, which was related to the inhibition of macrophage infiltration, oxidative stress, and multiple signaling pathways (TGF-β1/Smad2/3, NADPH oxidases, and nuclear factor kappa B p65).[44] *CXCL11* is also one of the target genes of miR-613, CXCL11 is a selective ligand for CXCR3, and elevated by cytokine stimulation and plays a vital role in the migration, differentiation, and activation of immune cells, which may be implicated in the structural remodeling of AF.[45] These reports suggested that hsa_circRNA_0056281, hsa-miR-613, and CXCR4/ CXCR2/ CXCL11 play an important role in AF. Our study indicated that the interaction of hsa_circRNA_0056281/ hsa_circRNA_0006665-hsa-miR-613-CXCR4/CXCR2/CXCL11 may be involved in the process of AF.

4.2. The potential roles of hsa_circRNA_0003638, hsa-miR-1207-3p, and CXCR4 in AF

Nemo-like kinase (*NLK*) is the host gene of hsa_circRNA_0003638, which is an atypical proline-directed serine/threonine mitogen-activated protein kinase. Study has shown that an inducible transgenic mouse with cardiac-specific NLK expression was more susceptible to left ventricular damage and heart failure. Furthermore, the myocardial tissue–specific transgenic knockout model of NLK protected it from pathology related to pressure overload and infarction injury.[46] Interestingly, we found that hsa_circRNA_0003638 was upregulated in the heart tissue of patients with AF. Furthermore, downregulated hsa-miR-1207-3p and target upregulated CXCR4 were regulated by hsa_circRNA_0003638. Das et al[47] demonstrated that miR-1207-3p regulates proliferation, apoptosis, and migration in prostate cancer by regulating fibronectin-1 via directly targeting fibronectin type III domain containing 1 performed by a dual-luciferase reporter assay. Report about fibronectin type III domain containing 1 has shown that it plays some role in hypoxia-induced apoptosis of cardiomyocytes.[48] Meanwhile, levels of circulating fibronectin-1 were found to be related to atrial remodeling in AF.[49] It suggests that miR-1207-3p may be associated with AF.

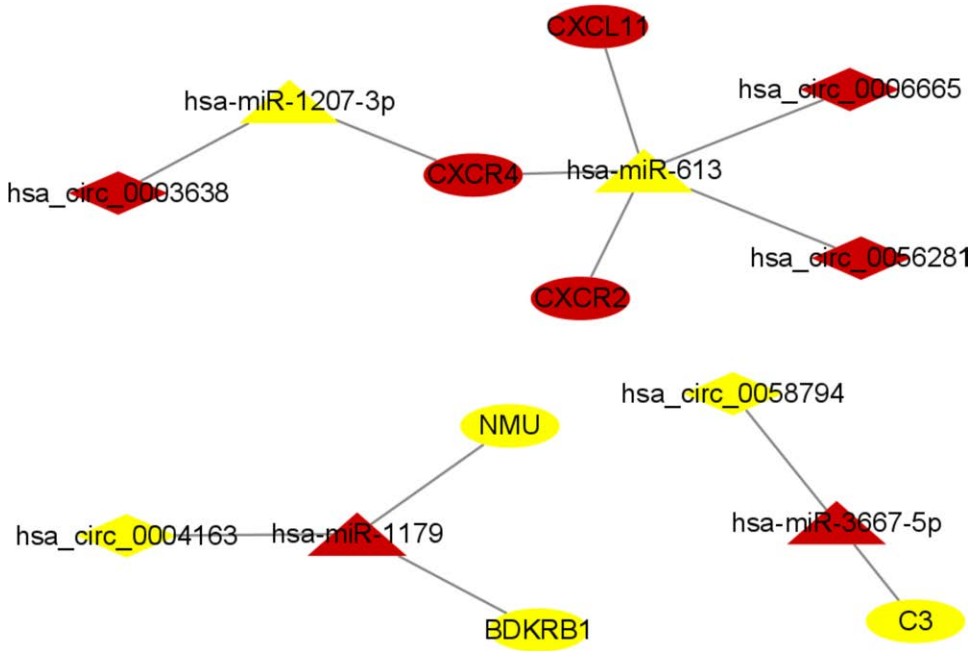

Figure 8. CircRNA-miRNA-hub genes subnetwork construction. Red indicates upregulated circRNAs, miRNAs, or mRNAs; Yellow indicates downregulated circRNAs, miRNAs, or mRNAs. BDKRB1 = B1 bradykinin receptor, C3 = complement C3, circ = circular RNA, CicRNA = circular RNA, CXCL11 = C-X-C motif chemokine 11, CXCR2 = C-X-C chemokine receptor type 2, CXCR4 = C-X-C chemokine receptor type 4, hsa = homo sapiens, miRNA = microRNA, mRNA = messenger RNA, NMU = neuromedin-U, RNA = ribonucleic acid.

The role of CXCR4 in AF hsa been discussed above. Those indicated that hsa_circRNA_0003638, hsa-miR-1207-3p, and CXCR4 may play roles in cardical damage and atrial remodeling. Our result suggested that the interaction between hsa_circRNA_0003638-hsa-miR-1207-3p-CXCR4 could be associated with AF.

NMU and C3 play an important role in the immune and inflammatory diseases; most of the studies have not only shown that NMU and C3 have a proinflammatory role[50,51] but also found that NMU in cutaneous inflammation may be dual function as a proinflammatory mediator at early stage and as an anti-inflammatory regulator at a later phase.[52] In this study, we also found that the expressions of NMU and C3 were relatively low in persistent AF, and long-term experiments are needed to confirm whether they are related to the anti-inflammatory regulation at the late stage of inflammation. In cardiac fibroblasts, the reduction of collagen I–induced by the activation of BDKRB1 plays an antifibrosis role.[53] On the contrary, the inhibition of BDKRB1 expression may be related to promoting fibrosis. The results of this study suggest that the expression of BDKRB1 decreases in AF, suggesting that low expression of BDKRB1 may play a role in AF fibrosis. As for the circRNA-miRNA-hub gene ceRNA subnetwork constructed by us, it was found that the downregulated circRNAs (hsa_circ_0004163, hsa_circ_0058794), upregulated miRNAs (hsa-miR-1179, hsa-miR-3667-5p) and downregulated mRNAs (NMU, C3, and BDKRB1) may be related to AF.

5. Limitations

First, a sample size was small in the present study, which may lead to unrepresentative results. Therefore, larger sample sizes used for microarray analysis can give more reliable statistical values. Second, it is primarily based on sequencing data analysis. Moreover, further experimental validation would be required for future verification in vivo animal models or in vitro cell experiments.

6. Conclusions

The circRNA-miRNA-mRNA network view may provide a new research approach to explore the mechanisms of AF. We speculated that hsa_circRNA_0056281/hsa_circRNA_0006665-hsa-miR-613-CXCR4/ CXCR2/ CXCL11 regulatory axis and hsa_circRNA_0003638-hsa-miR-1207-3p-CXCR4 regulatory axis may be associated with the pathogenesis of AF.

Author contributions

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