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RESEARCH ARTICLE

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Construction of atrial fibrillation-related circRNA/lncRNA-miRNA-mRNA regulatory network and analysis of potential biomarkers

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Abstract

Background: The specific pathogenesis of atrial fibrillation (AF) remains unclear. In this study, we examined the expression of differential messenger RNAs (mRNAs), circular RNAs (circRNAs), and long-stranded noncoding RNAs (lncRNAs) from human peripheral blood mononuclear cells to initially construct a circRNA/lncRNA-miRNA-mRNA ceRNA regulatory network to explore the pathogenesis of AF and to screen for potential biomarkers.

Methods: A total of four pairs of AF cases and healthy subjects were selected to detect differentially expressed mRNAs, circRNAs, and lncRNAs in peripheral blood mononuclear cells by microarray analysis. And 20 pairs of peripheral blood from AF patients and healthy subjects were selected for validation of mRNA, circRNA, and lncRNA by quantitative real-time PCR (qRT-PCR).The relevant ceRNA networks were constructed by GO and KEGG and correlation analysis.

Results: The results showed that compared with healthy subjects, there were 813 differentially expressed mRNAs (DEmRNAs) in peripheral blood monocytes of AF, including 445 upregulated genes and 368 downregulated genes, 120 differentially expressed circRNAs (DEcircRNAs), including 65 upregulated and 55 downregulated, 912 differentially expressed lncRNAs (DElncRNAs), including 531 upregulated and 381 downregulated lncRNAs. GO and KEGG analysis of DERNA revealed the biological processes and pathways involved in AF. Based on microarray data and predicted miRNAs, a ceRNA network containing 34 mRNAs, 212 circRNAs, 108 lncRNAs, and 38 miRNAs was constructed.

Conclusion: We revealed a novel ceRNA network in AF and showed that downregulated XIST, circRNA_2773, and CADM1 were negatively correlated with miR-486-5p expression and had a potential targeting relationship with miR-486-5p.

KEYWORDS
atrial fibrillation, biomarkers, ceRNA network, circRNA, lncRNA, mRNA

1 | INTRODUCTION

Atrial fibrillation (AF) is the most common arrhythmia in clinical practice, which can lead to the complications such as heart failure and stroke, and is closely related to high mortality and disability.[1,2] Meanwhile, its prevalence has increased due to an aging population.[2–4] But so far, the mechanisms of AF are still unclear.

In recent years, next-generation sequencing has provided a more efficient approach to study AF, and there is growing evidence that noncoding RNAs (ncRNAs), including microRNAs (miRNAs), long-stranded noncoding RNAs (lncRNAs), and circular RNAs (circRNAs),[5] play a critical role in cardiovascular diseases including arrhythmia, heart failure, and myocardial infarction.[6] Some studies have reported that ncRNAs are involved in the onset and development of AF.[7] lncRNAs/circRNAs can act as competitive endogenous RNAs (ceRNAs) by binding to miRNAs through their miRNA response elements, thereby regulating the miRNA target mRNAs expression levels.[5,8] Therefore, lncRNA/circRNA-miRNA-mRNA interactions may be a key factor or an important mechanism in the occurrence development of AF. CostaMC et al showed that sponge activity of circRNAs isolating specific miRNAs is important in the evolution from paroxysmal AF to permanent AF.[9] In addition, by microarray analysis and bioinformatics analysis, Jiang SY et al constructed a circRNA-miRNA-mRNA related network and proposed the potential role of has-circRNA-100,612, has-miR-1336 in AF.[10] Another comprehensive analysis by Sun HL et al revealed the role of miRNA, circRNA, and lncRNA in paroxysmal AF and persistent AF, and detected a protective factor against persistent AF.[11] Ke XY et al.[12] identified differentially expressed RNAs and constructed LOC101928304/miR-490-3p/LRRC2 ceRNA network in AF and sinus rhythm subjects. Another study constructed AF-associated lncRNA-miRNA-mRNA network and identified AF-associated sensitive lncRNAs by WGCNA.[13] A recent study focused on postoperative atrial fibrillation (POAF) and constructed POAF-related circRNA-miRNA-mRNA network using a bioinformatics approach and identified a novel circ_0007738.[14] These findings suggested that there was a regulatory role of targeting relevant lncRNAs, circRNAs, miRNAs, and target mRNAs in AF. The circRNA/lncRNA-miRNA-mRNA regulatory network might provide novel insights to further understand the mechanisms of AF and provide new potential targets for clinical treatment of AF.

However, AF-related lncRNA/circRNA-miRNA-mRNA ceRNA regulatory mechanisms are still fewer reported. In our previous study, we detected the differential expression of lncRNAs and circRNAs in peripheral blood of AF patients, and initially explored the role of circRNAs in the diagnosis and development of AF.[15,16] In this study, we constructed a lncRNA/circRNA-miRNA-mRNA regulatory network using microarray and preliminarily identify ceRNA regulatory mechanisms in AF. This study may provide novel targets to analyze the occurrence and development of AF and provide potential diagnostic markers and therapeutic targets for AF.

2 | MATERIALS AND METHODS

2.1 | Study population and specimen collection

Twenty patients with AF(AF group) hospitalized in Taizhou People's Hospital were screened from 1 May to 1 December, 2021, while 20 matched healthy controls (control group) were selected. Three milliliters of peripheral blood was collected, and mononuclear cells were purified from peripheral blood and frozen for analysis. The diagnosis of AF was based mainly on the criteria listed in the 2020 ESC guidelines for the management of atrial fibrillation.[17] Patients with hyperthyroidism, chronic pulmonary heart disease, valvular heart disease, previous coronary atherosclerotic heart disease, infective endocarditis, severe liver and kidney dysfunction, autoimmune disease, and malignancy were excluded. The study was approved by the ethics committee of Taizhou People's Hospital, and all enrolled subjects signed a written informed consent form.

2.2 | RNA extraction and cDNA synthesis

Total RNA was extracted from monocytes of four AF patients and four matched healthy subjects using Trizol reagent (Invitrogen) according to the instructions. The RNA concentration was detected by NanoDrop ND-2000 spectrophotometer (Thermo), and cDNA was synthesized by 5X ALL-In-One RT MasterMix (abm Zhenjiang Abbey Dream Biotechnology Co., Ltd.) to further synthesize cDNA from the qualified total RNA.

2.3 | Microarray analysis and data acquisition

Significant differentially expressed lncRNAs, circRNAs, and mRNAs were analyzed by high-throughput sequencing, a value of $|\log_2 FC| \geq 1$ and $p < 0.05$ was considered as statistically significance. Image scans were entered into the Aglient Feature Extraction 11.0 Software using an Agilent scanner for image data analysis (selection of mRNAs, circRNAs, lncRNAs chips, probe design, image acquisition, data analysis, etc. were provided by Shanghai Core Ultra Bio).

2.4 | GO and KEGG pathway analysis

The differentially expressed RNAs filtered by Volcano Plot were further subjected to GO enrichment analysis using GO (http://www.geneontology.org) to characterize the function of the differentially expressed genes (combined with GO annotation results). CC, BP, and MF enrichment analyses were performed separately for differentially expressed genes between samples using the fisher algorithm. Metabolic signaling pathway analysis of their differential genes was performed using the Kyoto Gene and Gene Encyclopedia KEGG database (http://www.genome.jp/kegg).

2.5 | ceRNA network construction

miRanda database (version 1.0) was used to predict miRNAs interacted with DEcircRNAs. startbase database (version 1.0) was used to predict miRNAs interacted with DElnRNAs. miRWalk database (version 2.0) was used to predict miRNAs interacted with DEmRNAs. Then, the obtained miRNAs were intersected with circRNA or lncRNA predicted miRNAs to construct circRNA-miRNA-mRNA, lncRNA-miRNA-mRNA networks, respectively. DEcircRNAs- and DElnRNAs-related ceRNA networks were constructed based on miRNAs that interacted with DEmRNAs, DEcircRNAs, and DElnRNAs together. The circRNA/lncRNA-miRNA-mRNA ceRNA networks were visualized using the Cytoscape software (version 3.6.1).

2.6 | Protein–protein interaction (PPI)

Set up STRING database with high confidence (≥0.900) and CytoScape for constructing PPI interaction network. The MCODE plug-in in Cytoscape is used to identify functional-related and highly interconnected key modules in PPI networks.

2.7 | QRT-PCR validation of differentially expressed circRNAs, lncRNAs, and mRNAs

One microlitercDNA was added to 10 μl SYBR-Green Gene Expression Master Mix (Applied Biosystems, Inc.), 8 μl DEPC enzyme-free water and 0.5 μl reverse and forward primers according to the kit instructions. u6 and GAPDH expression were used as controls. The expression results of FTX, XIST; circRNA_7571, circRNA_2773; hsa-miR-149-5, hsa-miR-486-5p; MMP9, CADM1 were determined and quantified by the $2(-\Delta\Delta C_t)$ method. The primers are listed in Table 1.

TABLE 1 Primer sequences for reverse transcription polymerase chain reaction

Primer name	Primer sequences (5′-3′)
MMP9-Forward primer	GACAAGCTCTTCGGCTTCTG
MMP9-Reverse primer	CAAAGTTCGAGGTGGTAGCG
CADM1-Forward primer	GTCAGCTGATGCTGAAGGTG
CADM1-Reverse primer	CCCGGGTTAAGCCTTGTAGA
XIST-Forward primer	GCCACTAGTGTACAGGGTGT
XIST-Reverse primer	CGAGGAGCTAGTAGGGCAAA
FTX-Forward primer	GCCCAGCAAGTTCATCAGAG
FTX-Reverse primer	CTGCATGGTCACTCACATGG
circRNA-7571-Forward primer	GGTCCAGAGGGCCGTCGT
circRNA-7571-Reverse primer	ATCCCTGTCCATCTCTGGACC
circRNA-2773-Forward primer	GGGGTTCCTGGGGGATG GGATTTT
circRNA-2773-Reverse primer	TCAAAAAGAACCCTAGG AACCCC
has-miR-149-5p-Forwardprimer	TCTGGCTCCGTGTCTTCACTC
has-miR-149-5p-Reverse primer	ATCCAGTGCAGGGTCCGAGG
GAPDH-Forward primer	GAGAAGTATGACAACAG CCTCAA
GAPDH-Reverse primer	GCCATCACGCCACAGTTT
U6-Forward primer	CTCGCTTCGGCAGCACA
U6-Reverse primer	AACGCTTCACGAATTTGCGT
has-miR-149-5p RT	GCCGTATCCAGTGCAGGGTC CGAGGTAT
	TCGCACTGGATACGACGGGAGT
has-miR-486-5p RT	GTCGTATCCAGTGCAGGGTC CGAGGTA
	TTCGCACTGGATACGAC CTCGGG

2.8 | Statistical analysis

The data were analyzed using the GraphPad Prism 9.0.2 software. Data were expressed as mean ± standard error of the mean (SEM). Significant differences between the two groups were analyzed by Student's *t*-test; differences between more than two groups were analyzed by one-way analysis of variance (ANOVA) followed by a test. Pearson or Spearman correlations were chosen depending on whether the variables followed a normal distribution. $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Identification and analysis of DEmRNA

Based on the preset threshold ($p < 0.05$ and $|\log_2 FC| \geq 1$), 813 DEmRNAs were obtained from the AF group compared with healthy controls, of which 445 genes were upregulated and 368 ($p < 0.05$) were downregulated (Figure 1A). The top 10 upregulated mRNAs: ADGRE3, MME, TRPM6, TCTEX1D4, ALOX15, CCNJL, CSF1, DDIT3, JUN, CYP4F3. The top 10 downregulated mRNAs: ZCWPW2, LTF, AKAP5, ABCA13, OLFM4, KIR2DS4, PCDH1, C1orf21, KRT73, KIR3DL1. Based on hierarchical clustering analysis, some of the 813 DEGs may be involved in the regulation of the same pathway or have similar functions (Figure 1B). As shown in Figure 1C, differentially expressed DEmRNAs in BP were mainly involved in neutrophil degranulation, antimicrobial humoral response, and inflammatory response. Differentially expressed DEmRNAs in CC were mainly involved in plasma membrane, components of plasma membrane. Differentially expressed DEmRNAs in MF were mainly involved in antigen binding, G protein-coupled receptor activity, sphingosine-1-phosphate receptor activity. KEGG pathway enrichment indicated that the significant pathways were involved in PI3K-Akt signal pathway, MAPK signal pathway, Rap1 signal pathway, and TNF signal pathway ($p < 0.05$) (Figure 1D) .

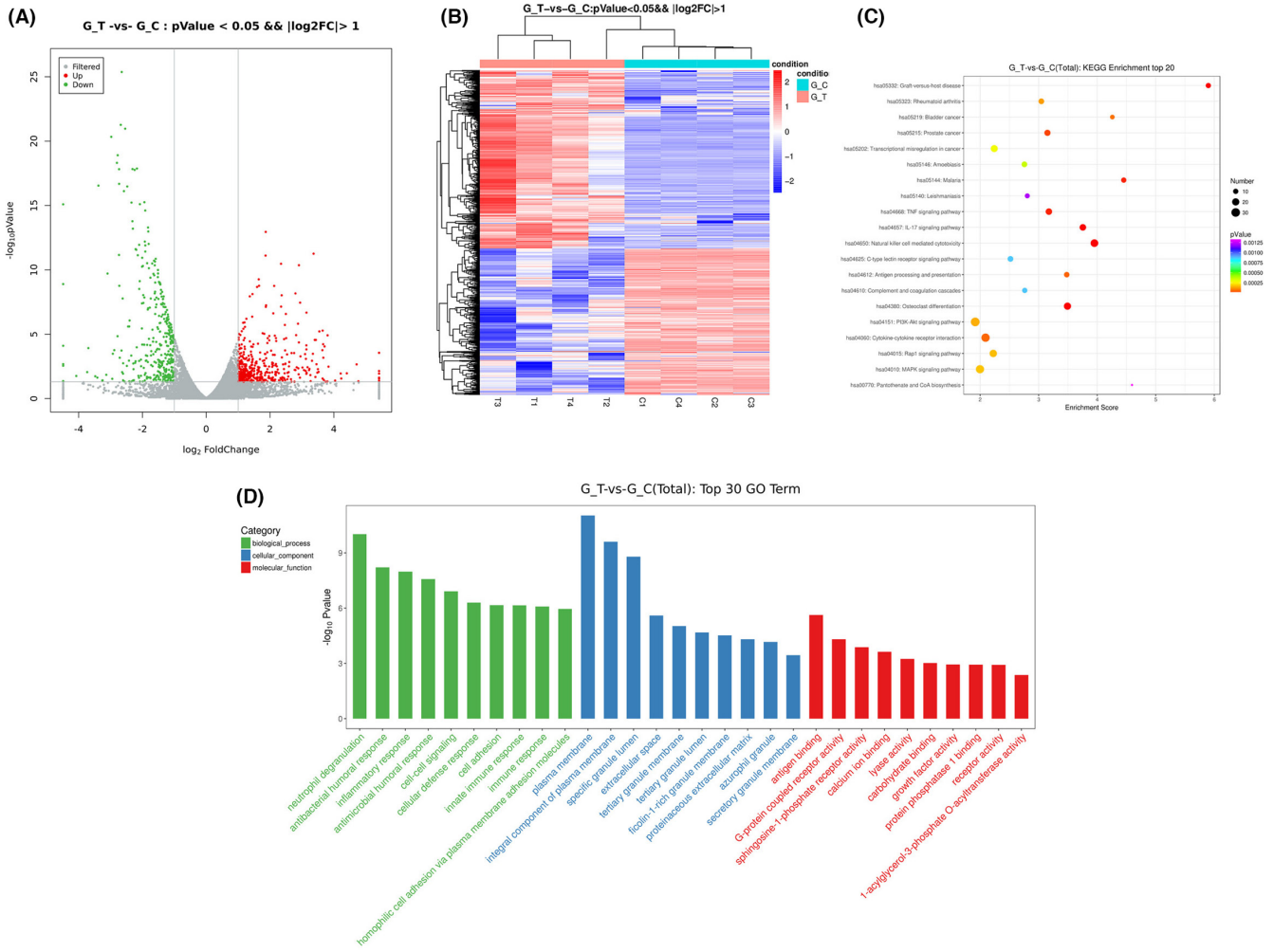

FIGURE 1 mRNA expression profiles. (A) Volcano map of DEmRNA, red and green indicate up- and downregulation, respectively. (B) Hierarchical clustering analysis heat map analysis of DEmRNA. Blue: low expression; red: high expression. "T" represents AF samples and "C" represents healthy controls. (C) GO analysis of DEmRNA. (D) KEGG analysis of DEmRNA.

3.2 | Identification and analysis of DElncRNAs

912 DElncRNAs in the AF group compared with the control group, including 531 upregulated and 381 downregulated lncRNAs (Figure 2A); the top 10 upregulated lncRNAs: ENST00000648905, TCONS_00001709, ENST00000609281, XR_001754622.1, TCONS_00038119, ENST00000649467, XR_947046.2, TCONS_00019391, XR_001739616.1, TCONS_00019687. The top10 down-regulated lncRNAs:TCONS_00026213, XR_942786.2, TCONS_00015252, XR_001738325.1, XR_001740454.1, TCONS_00026209, NR_037650.1, TCONS_00026208, XR_944963.2, TCONS_00026211. Hierarchical clustering analysis is shown in Figure 2B. Size distribution analysis showed that most lncRNAs were longer than 2000bp (Figure 2E). There are 7813 lncRNAs (Figure 2F) with two exons, and GO analysis was performed based on 912 differentially expressed lncRNAs screened by ($|\log_2 FC| \geq 1$ vs. p-value <0.05), yielding a total of 1111 GO entries ($p < 0.05$). As shown in Figure 2C, differentially expressed DElncRNAs in BP were mainly involved in negative regulation, negative of multicellular organismal metabolic process; Differentially expressed DElncRNAs in CC were mainly involved in lysosome, ficolin-1-rich granule lumen; differentially expressed DElncRNAs in MF were mainly involved in beta-3 adrenaline, ficolin-1 rich granule lumen; KEGG pathway enrichment indicated that the significant pathways were involved in Fat digestion and absorption, Epstein–Barr virus, Ether lipid metabolism, and human immunodeficiency virus 1 infection ($p < 0.05$) (Figure 2D).

3.3 | Identification and analysis of DEcircRNAs

In our previous research,[12] by microarray analysis, a total of 120 DEcircRNAs were identified in AF and control samples based on predefined thresholds (p-value <0.05 and $|\log_2 FC| \geq 1$), of which 65 were upregulated and 55 were downregulated; The top 10 upregulated circRNAs: hsa_circ_0039161, circRNA_0161, hsa_circ_0001947, hsa_circ_0003916, circRNA_6991, hsa_circ_0008021, hsa_circ_0001394, circRNA_7571, hsa_circ_0008699, hsa_circ_0072697. The top 10 down-regulated circRNAs: circRNA_1834,

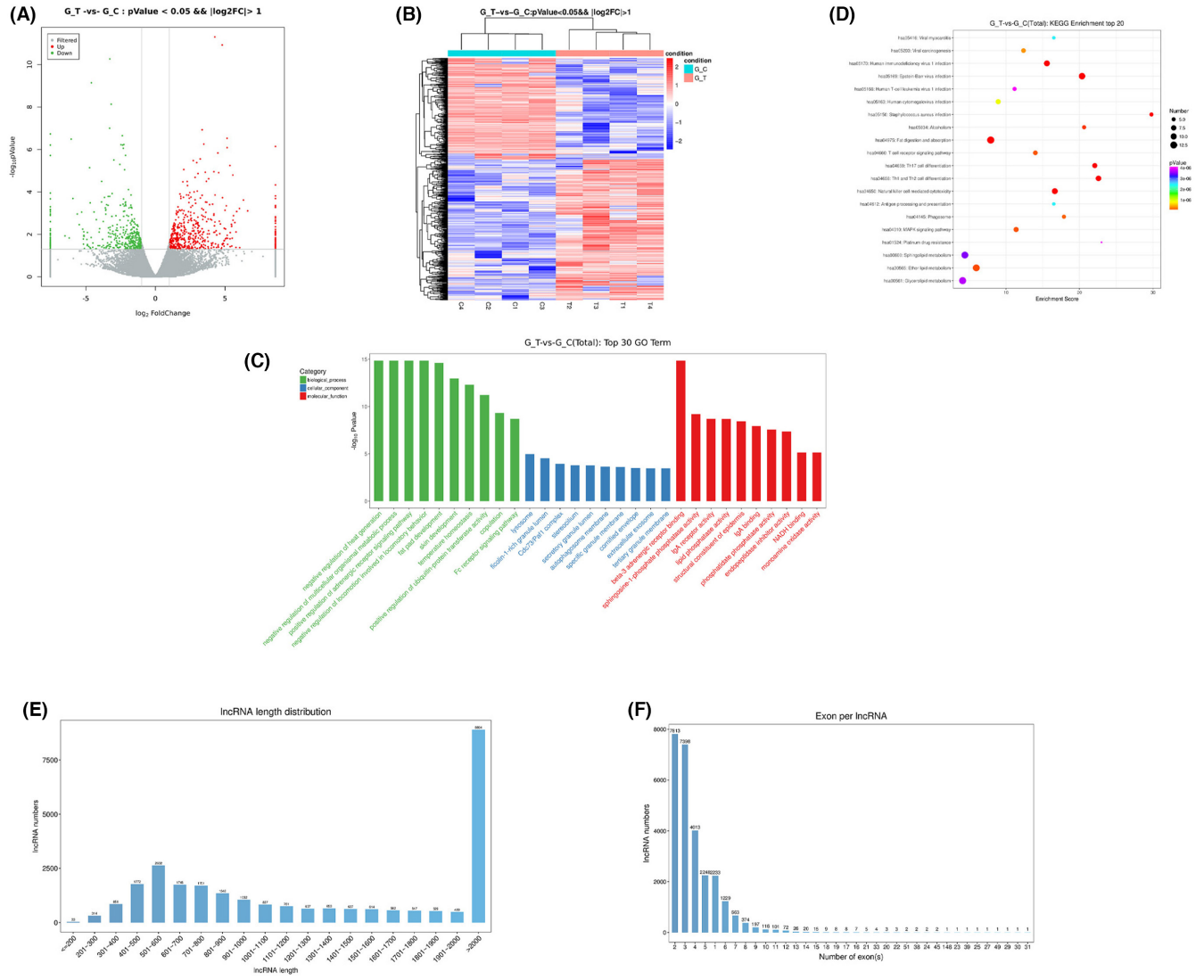

FIGURE 2 lncRNA expression profiles. (A) Volcano plot of DElncRNA, red and green indicate up- and downregulation, respectively. (B) Hierarchical clustering analysis heat map analysis of DElncRNA. Blue: low expression; red: high expression. "T" represents AF samples and "C" represents healthy controls. (C) GO analysis of DElncRNA. (D) KEGG analysis of DElncRNA. (E) Gene length. (F) lncRNA number of exons.

circRNA_4184, circRNA_9064, hsa_circ_0004096, circRNA_8108, circRNA_4624, hsa_circ_0085438, hsa_circ_0006208, circRNA_3830, circRNA_1800.

In addition, GO analysis was performed on the 120 differentially expressed circRNAs screened, and a total of 481 GO entries were obtained ($p < 0.05$). And differentially expressed DEcircRNAs in BP were mainly involved in phosphatidylethanolamine acyi-chain remodeling, phosphatidylcholine acyi-chain remodeling; differentially expressed DEcircRNAs in CC were mainly involved in specific granule lumen, cell surface. Differentially expressed DEcircRNAs in MF were mainly involved in 2-cylglycerol-3-phosphate O-acyltransferase activity and transmembrane signaling receptor activity. KEGG pathway enrichment indicated that the significant pathways were involved in Endocytosis, Natural killer cell-mediated cytotoxicity, tuberculosis, and influenza ($p < 0.05$).

3.4 | ceRNA network construction

Based on the relationship between the microarray data and the predicted results, the ceRNA networks of related circRNA-miRNA-mRNA (Figure 3A), lncRNA-miRNA-mRNA (Figure 3B) were constructed. ceRNA networks were visualized using the Cytoscape software (version 3.6.1). The above circRNA-miRNA, lncRNA-miRNA, and mRNA-miRNA were crossed to obtain six common miRNAs (Figure 3D), and the lncRNA/circRNA-miRNA-mRNA was then constructed according to the interaction between miRNA and mRNA, lncRNA, and circRNA (Figure 3C). As shown below, in the circRNA/lncRNA-miRNA-mRNA network, CCNL1, TNFAIP3, BTG2, CADM1, MYBL1, ENPP5, PLEKHO2 were identified as key genes. BTG2 and CADM1 were found to be involved in PI3K/AKT pathway, while ENPP5 was associated with phosphorylation

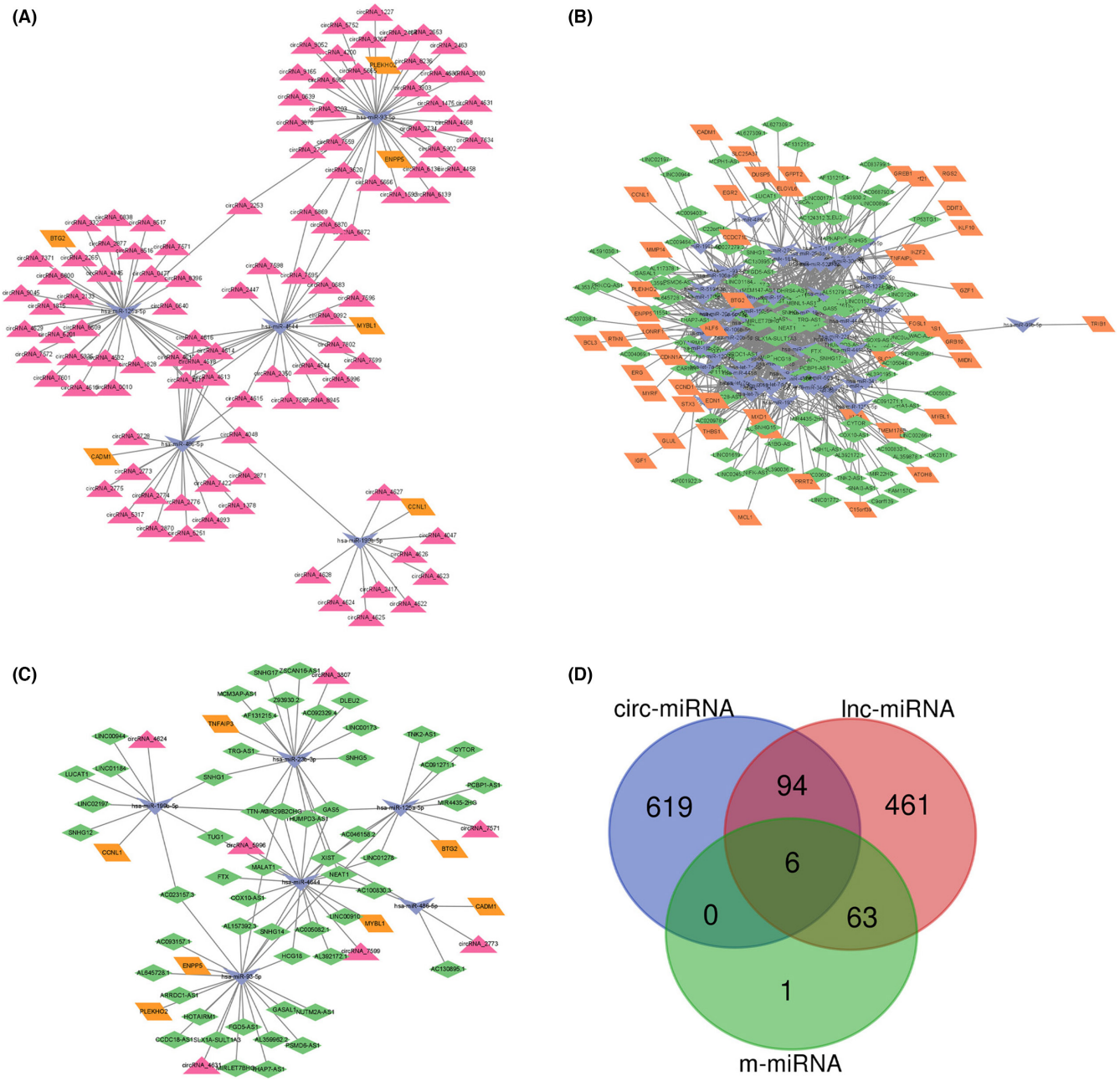

FIGURE 3 Establishment of circRNA/lncRNA-miRNA-mRNA network. (A) circRNA-miRNA-mRNA competitive endogenous RNA network. Triangle pink indicates circRNA, V-shaped purple indicates miRNA, and parallelogram yellow indicates mRNA. (B) lncRNA-miRNA-mRNA competitive endogenous RNA network. Diamond green denotes lncRNA, V-shaped purple denotes miRNA, and parallelogram yellow denotes mRNA. (C) circRNA/lncRNA-miRNA-mRNA competitive endogenous RNA network. (D) Venn diagram of circRNA-miRNA, lncRNA-miRNA, and mRNA-miRNA intersections.

PLEKHO2 was associated with MAPK signaling pathway. There were consistent with our functional enrichment results.

3.5 | PPI network construction

The PPI network was constructed using the screened DEGs, which included 257 nodes and 2040 edges. We selected the top 10 genes in the network (Table 2) (Figure 4A) including JUN, FOS, JUNB, JUND, IL-10, IL-6, VEGFA, EGR1, MMP9, and FOSB as the pivotal genes in the PPI network. Functionally related and highly interconnected modules were extracted using MCODE (Figure 4B) to further screen key genes. Among them, the pivotal genes JUN, FOS, JUNB, JUND, IL-10, IL-6, VEGFA, EGR1, MMP9, and FOSB were contained in module 1 (Figure 4B). Finally, the lncRNA-circRNA-miRNA-hub mRNA (Figure 4C) network was constructed based on ceRNA theory, thus depicting the linkage between DElncRNAs, DEcircRNAs, DEmiRNAs, and DEmRNAs (Figure 5).

TABLE 2 Key differentially expressed genes in AF versus healthy controls

Gene	Rank	Score	logFoldChange	*p*-Value	FDR
JUN	1	253	1.592107322	2.14 E-09	5.33 E-07
FOS	2	211	2.192354316	3.37 E-05	0.002751
JUNB	3	174	1.571164608	0.001841	0.063396
JUND	4	168	1.076242341	0.000503	0.024643
IL-10	5	149	3.174554732	0.003669	0.099415
IL-6	6	140	2.07390269	0.030011	0.339182
VEGFA	7	136	1.05282663	0.000132	0.008479
EGFR1	8	130	2.834300372	0.003723	0.100058
MMP9	9	128	2.462719731	1.90 E-06	0.000229
FOSB	10	123	1.838558781	0.000339	0.018248

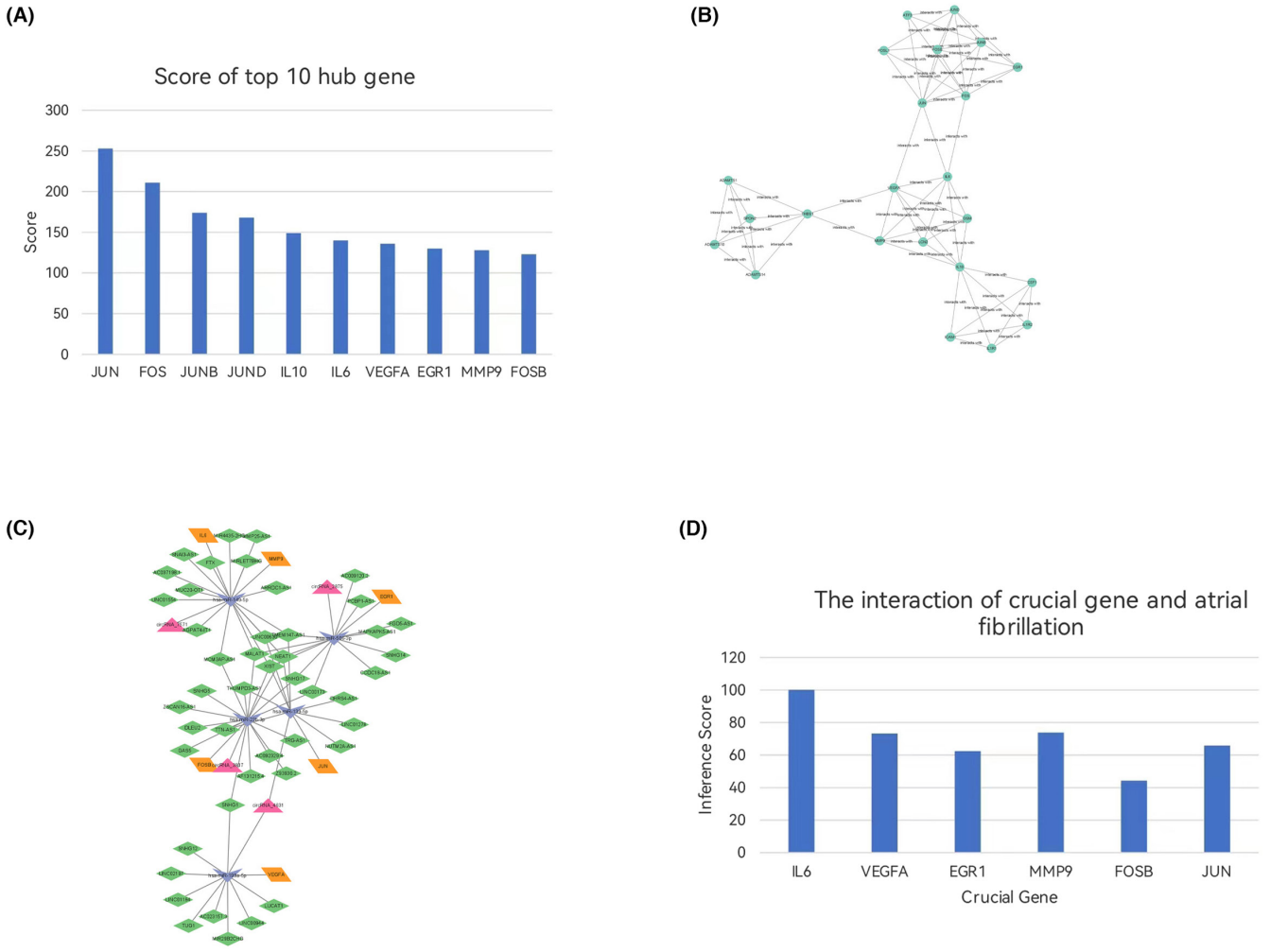

FIGURE 4 Establishment of circRNA/lncRNA-miRNA-hub mRNA network. (A) Scores of the top 10 hub genes. (B) Key modules. (C) The circRNA/lncRNA-miRNA-mRNA competitive endogenous RNA network constructed based on hubmRNA. triangle pink indicates circRNA, diamond green indicates lncRNA, V purple indicates miRNA, and parallelogram yellow indicates mRNA. (D) The interaction of crucial gene and atrial fibrillation.

Notably, upregulated LINC01554 or circRNA_7571 may act as ceRNAs to suppress hsa-miR-149-5p on IL-6 and MMP9, leading to their upregulation; IL-6 and MMP9 were chosen for the reason of their association with AF inflammatory and fibrotic mechanisms in AF.[18] In addition, the analysis of key genes and AF based on CTD database showed that IL-6 and MMP9 may be important in AF with higher scores of AF-related diseases (Figure 4D). Combined with the rank scores of hub genes (Table 2) and their expression in microarrays (Figure 6), JUN was selected as a potential novel marker in AF for further study.

3.6 | qRT-PCR and correlation analysis of differentially expressed mRNAs, circRNAs, and lncRNAs

To validate the microarray results, blood samples from 20 AF patients and 20 matched healthy individuals were selected for further study. Two mRNAs (MMP9 and CADM1), two circRNAs (circRNA_7571 and circRNA_2773), and two lncRNAs (FTX and XIST) were selected for qRT-PCR validation based on the constructed ceRNA network. Compared with the control group, circRNA_2773 (Figure 7A), XIST (Figure 7B), and CADM1 (Figure 7D) were downregulated in the AF group, hsa-miR-486-5p (Figure 7C), circRNA_7571 (Figure 7E), FTX (Figure 7F), and MMP9 (Figure 7H) were upregulated in the AF group, while hsa -miR-149-5p (Figure 7G) was also downregulated in AF, which was consistent with the microarray analysis. In addition, the expression level of circRNA_2773/XIST-hsa-miR-486-5p-CADM1 was analyzed. The results showed that hsa-miR-486-5 was negatively correlated with XIST, circRNA_2773, and CADM1, while circRNA_2773 and XIST were positively correlated with CADM1 (Figure 8A–E). This is consistent with the ceRNA theory that changing the expression level of one of the ceRNAs will cause another ceRNA, which shares the MRE, to have an effect and produce the same trend of change.

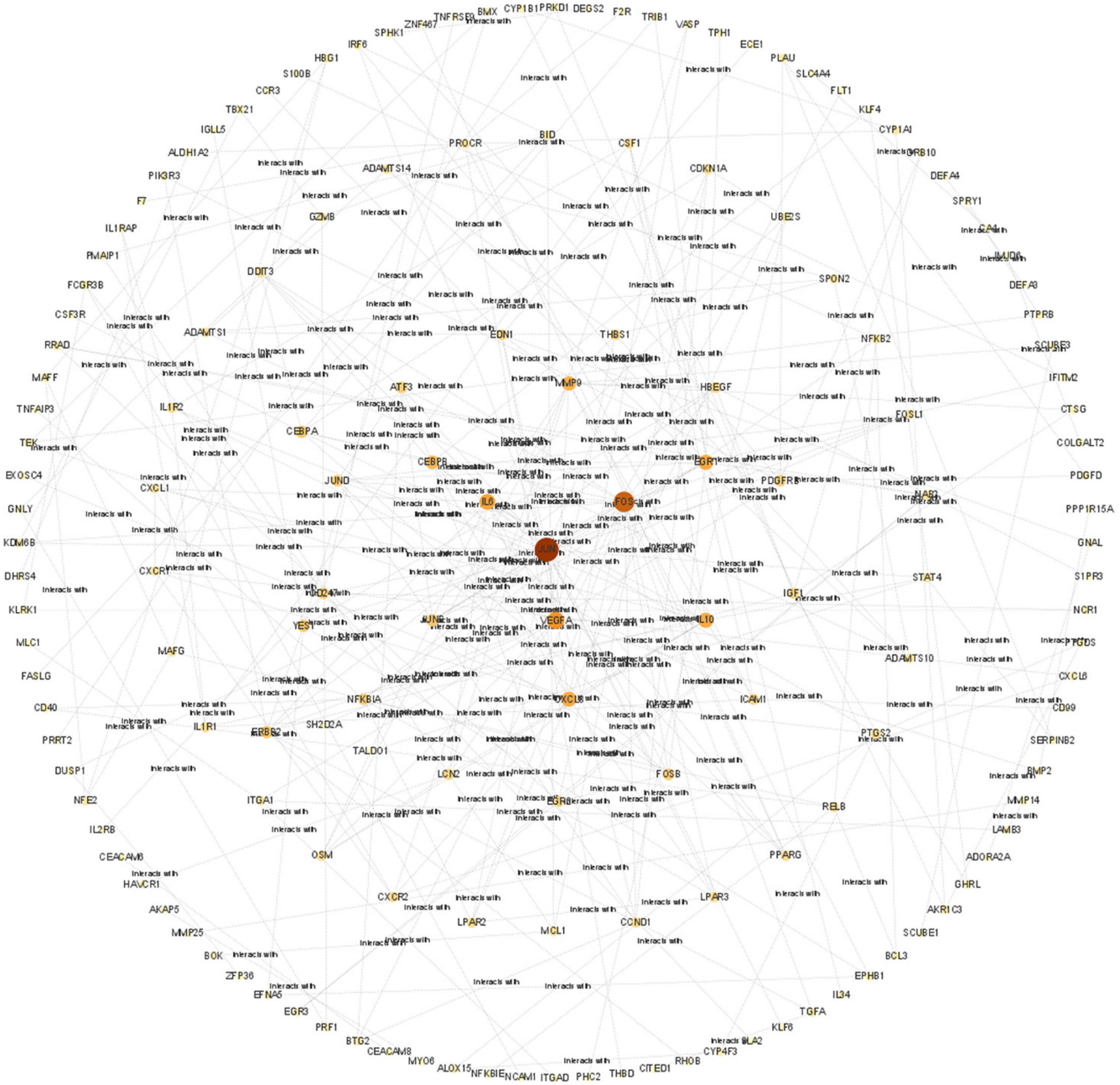

FIGURE 5 Protein and protein interaction network of differentially expressed genes

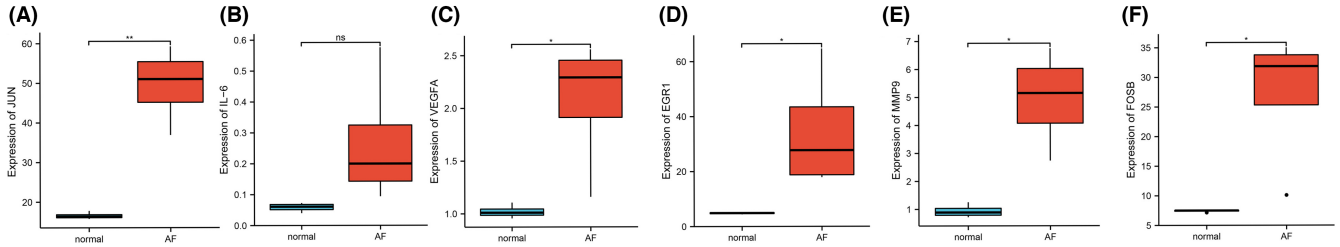

FIGURE 6 Expression of hub genes in microarrays

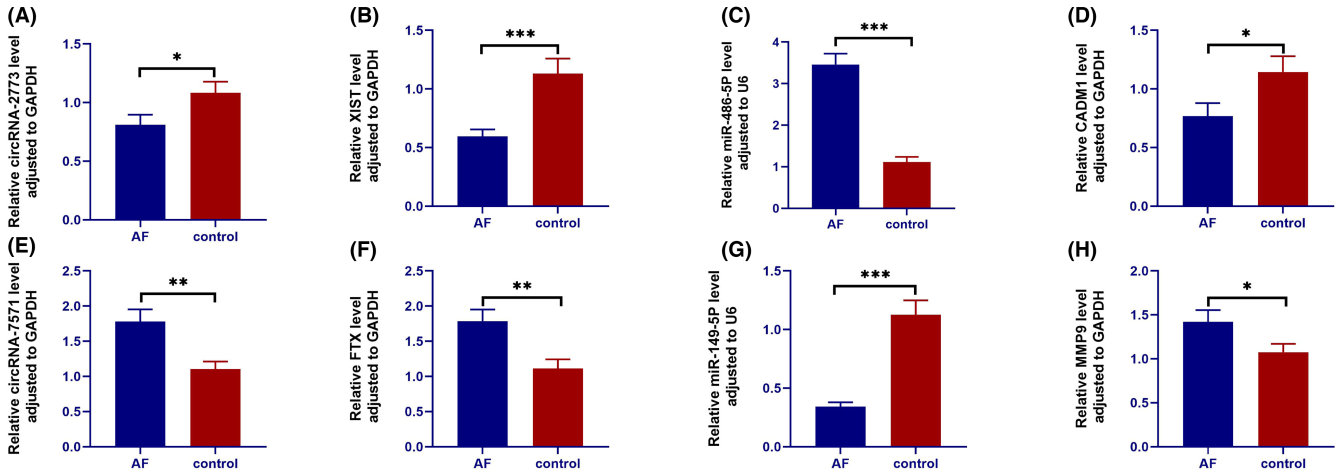

FIGURE 7 Differential expression of ceRNA-qRT-PCR validation

4 | DISCUSSION

With the rapid development of high-throughput technologies and bioinformatics analysis, more and more efforts have been devoted to reveal AF-related pathogenesis, and the ceRNA hypothesis is of increasing interest. To our knowledge, there are few studies to elucidate the pathogenesis of AF by constructing circRNA/lncRNA-miRNA-mRNA-related networks.

In this study, high-throughput sequencing of peripheral venous blood samples from four AF patients and healthy controls was performed to analyze differentially expressed lncRNAs, circRNAs, and mRNAs, which showed 912 DElncRNAs and 120 DEcircRNAs, and 813 DEmRNAs. The interaction network of lncRNA, circRNA, miRNA, and mRNA was further constructed based on ceRNA theory. In the circRNA/lncRNA-miRNA-mRNA network, CCNL1, TNFAIP3, BTG2, CADM1, MYBL1, ENPP5, and PLEKHO2 were identified as key genes among the differential mRNAs, and KEGG correlation analysis showed that BTG2 and CADM1 were involved in PI3K/AKT pathway, while ENPP5 was associated with phosphorylation and PLEKHO2 was associated with MAPK signaling pathway. There were consistent with the relevant reports.[19,20] Based on our constructed ceRNA network, only the gene CAMD1 has been reported to be associated with AF fibrosis.[21] CAMD1 is the upstream gene of PI3K/AKT, and PI3K/AKT is a pathway associated with fibrosis, In this study, KEGG showed that PI3K/AKT was a related pathway of AF, based on the *p*-value intensity and point size, and PI3K/AKT signaling pathway contains about 30 genes. CAMD1 has been reported to be associated with multiple diseases, but its potential role in the pathogenesis of AF has not been fully explored. Therefore, we selected XIST- circRNA_2773- hsa-miR-486-5p-CADM1 for further study. The expression of CADM1 was significantly higher in AF patients than in controls, which was consistent with the results for wei cao et al. According to Bo Yuyan et al., XIST was reported to inhibit inflammatory necrosis in cardiac myocytes,[22] and in addition a related study suggested that overexpression of XIST may increase the proliferation and expression levels of fibrosis-related proteins in human cardiac fibroblasts.[23] However, there are few related studies and the exact mechanism is unclear. Wang JG et al found a link between miR-486-5p and the regulation of electrical remodeling in AF.[24] Other studies have explored miR-486-5p-related networks, and Sun et al.[25] demonstrated that miR-486-5p can target CADM1 and thus promote nonsmall-cell lung cancer metastasis. Xiong et al.[26] showed that XIST can act on miR-486-5p to regulate brain ischemia–reperfusion injury. In addition, miR-486 could participate in the biological functions of ovarian cancer cells by interacting with CADM1 in an ovarian cancer study.[27] Based on our network, XIST/circRNA_2773-miR-486-5p-CADM1 and other related pathways involved in the occurrence and development of AF, the down-regulated XIST, circRNA_2773, and CADM1 were negatively correlated with miR-486-5p expression and had a targeting relationship with miR-486-5p. That is, XIST/circRNA_2773 could competitively bind miR-486-5p to act as a "sponge," thus diminishing the inhibitory effect of miR-486-5p on CADM1. Therefore, it can be inferred that XIST/circRNA_2773-miR-486-5p-CADM1 may play

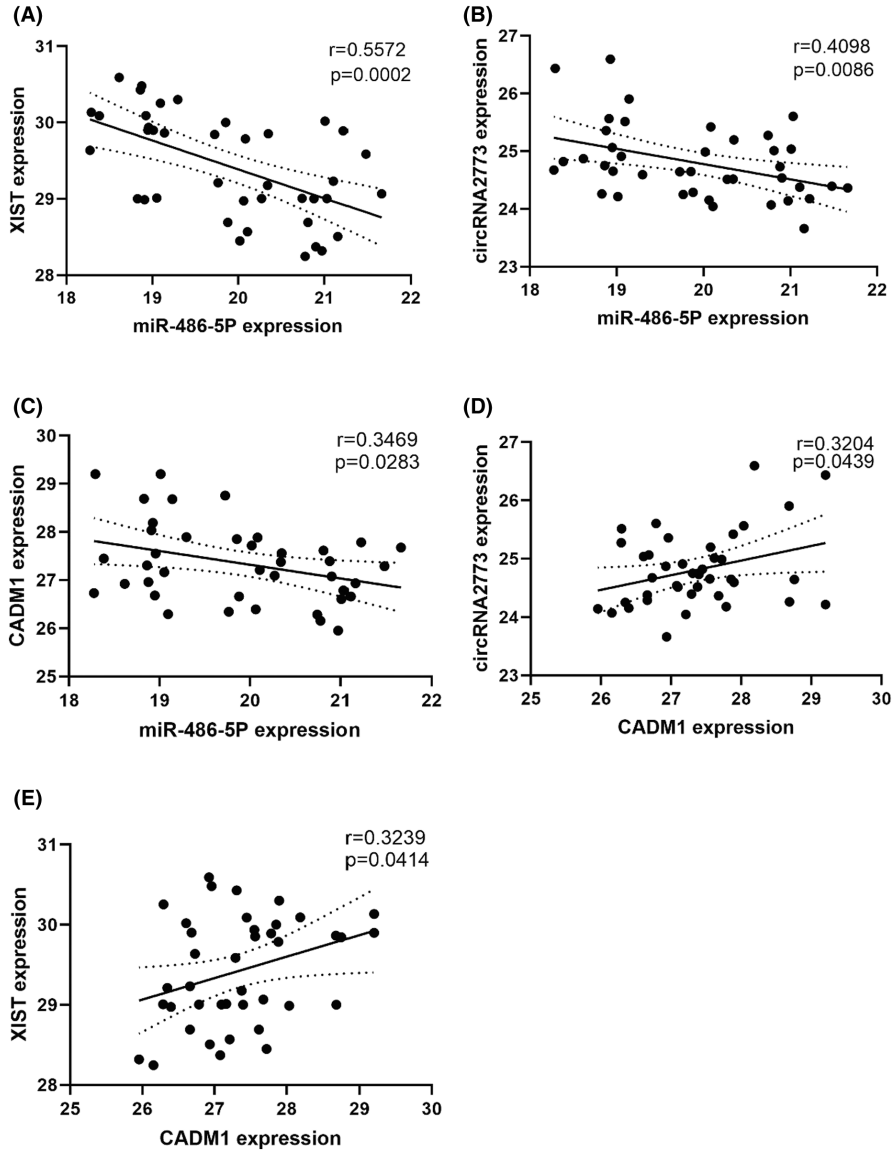

FIGURE 8 Correlation of ceRNA expression levels with differential expression

an important role in the development of AF. This study provides a new perspective on the potential pathogenesis of AF. Although RNA quantitative expression correlations suggest that they may have potential ceRNA relationships, their targeting relationships can be further clarified by subsequent relevant experiments.

In addition, PPI networks, and JUN, FOS, JUNB, JUND, IL-10, IL-6, VEGFA, EGR1, MMP9, and FOSB were identified as key genes in AF, which were identified by topological characterization and modular screening of genes in the PPI networks. Subsequently, five key DEmiRNAs (hsa-miR-149-5p, hsa-miR-506-3p, hsa-miR-23b-3p, hsa-miR-139-5p, and hsa-miR-199a-5p) were identified by constructing ceRNA networks and six key genes (JUN, IL-6, VEGFA, EGR1, MMP9, and FOSB), JUN, EGR1, and FOSB were found to be involved in PI3K/AKT pathway, MMP9, IL-6 were associated with MAPK, Rap1 signaling pathway, VEGFA was associated with TNF signaling pathway. A large number of studies have shown that MMP9 is associated with fibrosis and remodeling in AF and plays an important role in the development of AF.[28,29]

Previous studies also confirmed that related profibrotic markers such as MMP9 are associated with the development and progression of AF[30]; in addition, there are relevant studies showing the role of hsa-miR-149-5p in regulating cardiovascular disease providing relevant support for the role of hsa-miR-149-5 in the regulation of cardiovascular disease, and Qiuyue Li et al.[31] showed that hsa-miR-149-5 mediates inflammatory responses through MAPK and NF-κB signaling pathways. Previous studies have shown that the hsa-miR-149-5p-IL-6 axis is involved in various diseases. In abdominal aortic aneurysms, the overexpressed hsa_circ_0087352 binds to has-miR-149-5p to promote IL-6 release.[32] Another study on chronic obstructive pulmonary disease reported that IL6-AS1 (lncRNA) can competitively bind to miR-149-5p to promote the expression of IL-6.[33] In addition, hsa_circ_0000479-hsa-miR-149-5p-RIG-I and IL-6 interaction axis play a role in COVID-19 infection.[34] Different circRNAs / lncRNAs can act on the hsa-miR-149-5p-IL-6 axis. Our network further suggested that the upregulated FTX, circRNA_7571, may be particularly important for AF due to their roles as ceRNA

binding to hsa-miR-149-5p, which leads to the upregulation of IL-6, MMP9, respectively. Therefore, further studies are needed to determine how IL-6 and MMP9 affect the development of AF. Our findings provided a theoretical basis for subsequent studies. In addition, Wang et al.[35] showed that plasma VEGF-A levels were significantly elevated in AF patients, suggesting that endothelial dysfunction may be the cause of AF. However, relevant studies have been performed only at the protein level, and our network shows that upregulated LUCAT1 and circRNA_4631 simultaneously target hsa-miR-199a-5p to upregulate the expression of VEGFA, which further affects the occurrence of AF. There are many crosstalk modes between ncRNAs, and targeting multiple genes may be a new direction for AF treatment. Therefore, it is necessary to further explore the detailed information of these RNA crosstalk modulation modes.

In particular, JUN is the most important key hub gene in PPI network, and enrichment analysis shows that it is involved in MAPK pathway. It is a transcription complex of activated protein-1 (AP-1),[36,37] which is mainly expressed in fibroblasts. A number of studies have shown that many fibrosis diseases are induced by high expression of JUN,[38] such as JUN related to pulmonary fibrosis and liver fibrosis.[39–41] Other reports have shown that JUN leads to myocardial fibrosis by inducing the transcription activity of AP-1,[42] and myocardial fibrosis leads to the occurrence of AF.[43] As we know, there is no direct report on JUN and AF at present. In summary, it can be speculated that JUN is likely to be related to AF fibrosis and can be regarded as a potential new biomarker.

There were some limitations in this study. First, the sample size was not large. Validation in a larger number of patients was necessary to explore its clinical value. Second, this was a preliminary screening study, and further experimental studies were needed to validate the interactions in the identified ceRNA axes in AF.

In conclusion, for the first time, we constructed the circRNA/lncRNA-miRNA-mRNA network in AF. Several lncRNA/circRNA-miRNA-mRNA interaction axes were identified (LINC01554/circRNA_7571-hsa-miR-149-5p-IL-6/MMP9, circRNA_2875/SNHG14-hsa-miR-506-3p-EGR1, LINC00173/circRNA_4631-hsa-miR-139-5p-JUN, DLEU2/circRNA_3807-hsa-miR-23b-3p-FOSB, LUCAT1/circRNA_4631-hsa-miR-199a-5p-VEGFA, XIST/circRNA_2773-miR-486-5p-CADM1), XIST/circRNA_2773-miR-486-5p-CADM1 was selected for further study, indicating that XIST, circRNA_2773, CADM1, and miR-486-5p have potential targeting relationship and correlation. In the future, it is still necessary to explore its specific mechanism of action in AF by basic experiments such as luciferase assay, gene knockout, or overexpression. The crosstalk of various RNA forms a complex regulatory network, which mediates the occurrence and development of AF. It provides a new direction for understanding the treatment of AF and provides data and basis for later experiments. In the future, a large number of clinical and basic experiments are still needed to verify the determined expression pattern and interaction.

AUTHOR CONTRIBUTIONS

JW, ZR, FW, JH, GC, JZ, YR, and LZ contributed to the conception and design of the study. JW and ZR searched the relevant literature. ZR, JW, and GC helped in experimentation and data acquisition. FWand JZ contributed to clinical evaluation and sample provision. JW wrote the manuscript. ZR provided advice and were responsible for revising the manuscript. All authors have read and approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors indicated no potential conflicts of interest.

DATA AVAILABILITY STATEMENT

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

INFORMED CONSENT

Written informed consent was obtained from all patients.

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