CircRNA_0263 and circRNA_1507 are Dysregulated in Atrial Fibrosis Rat Induced by Chronic Intermittent Hypoxia

Xue Liang

Tianjin Medical University Second Hospital

Yanhong Liu

Tianjin Medical University Second Hospital

Yu Liu

Tianjin Medical University Second Hospital

Weiding Wang

Tianjin Medical University Second Hospital

Wenfeng Shangguan

Tianjin Medical University Second Hospital

Manman Wang

Affiliated Hospital of Jining Medical University

Xiaowei Zhang

Tianjin Medical University Second Hospital

Shuai Miao

Tianjin Medical University Second Hospital

Enzhao Liu

Tianjin Medical University Second Hospital

Guangping Li (✉ tic_tjcardiol@126.com)

Tianjin Institute of cardiology, second hospital of Tianjin medical university <https://orcid.org/0000-0003-2424-8548>

Research Article

Keywords: chronic intermittent hypoxia, atrial fibrillation, atrial fibrosis, circRNA, ceRNA network

Posted Date: December 13th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1143676/v1>

License: (cc) (i) This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Aims: This study aimed to characterize circular RNA (circRNA) profiles associated with atrial fibrosis and identify critical circRNAs in a rat model of atrial fibrosis.

Methods: Sprague Dawley rats were randomly divided into control and atrial fibrosis groups (n=15 in each group). For rats in the atrial fibrosis group, atrial fibrosis was induced by chronic intermittent hypoxia. Atrial tissues were isolated for circRNA sequencing. The dysregulated circRNAs in atrial fibrosis were identified by DESeq. Subsequently, the potential functions of circRNAs in atrial fibrosis were investigated by Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis of the host genes. The interactions among circRNAs, microRNAs (miRNAs), and mRNAs were analyzed by constructing a competing endogenous RNA (ceRNA) network. Finally, the crucial circRNAs were validated by quantitative real-time polymerase chain reaction (qRT-PCR).

Results: Five upregulated and 11 downregulated circRNAs were identified in the atrial fibrosis group. These dysregulated circRNAs were primarily associated with “carbohydrate metabolism” and “cardiovascular diseases.” Two circRNAs (circRNA_0263 and circRNA_1507) were able to regulate target gene expression by interacting with corresponding miRNAs, including rno-miR-29b-5p, rno-miR-29b-3p, rno-miR-496-5p, rno-miR-136-5p, and novel123-mature. qRT-PCR successfully validated the differential expression of circRNA_0263 and circRNA_1507.

Conclusion: A series of circRNAs were identified as dysregulated in an atrial fibrosis rat model. The dysregulation of two circRNAs (circRNA_0263 and circRNA_1507) might be crucial for atrial fibrosis development by competing with several miRNAs.

1 Introduction

Atrial fibrillation (AF) is the most common type of cardiac arrhythmia disease, affecting an increasing number of individuals worldwide. The prevalence of AF is increasing sharply with the aging of the global population [1]. Aging and aging-related diseases result in cardiac electrical abnormalities and myocardial remodeling, which increase the occurrence of AF [2]. AF might also accelerate the aging process by causing ischemic or non-ischemic events [1]. Currently, the prevalence of AF is the highest among white populations, with an estimated 7 million individuals diagnosed with AF in Europe and the USA [3, 4]. In Asia, the incidence of AF was reported at 5.38 per 1,000 persons each year, according to a meta-analysis of data from three countries [5]. Multiple factors are associated with the increased risk of AF incidence, including age, arterial hypertension, obesity, diabetes, coronary artery disease, and heart failure. Progressive atrial fibrosis is the primary pathological change reported in AF, associated with disease recurrence and resistance to therapy [6]. However, the molecular mechanisms underlying atrial fibrosis remain poorly understood.

AF is well known to increase the risk of ischemic stroke, heart failure, and mortality, exacerbating the overall global health burden. Current treatment options for AF remain challenging, especially for the AF in

older patients. Available therapeutic strategies for AF primarily include antiarrhythmic drug therapy and catheter ablation [7, 8]. Guidelines recommend antiarrhythmic drugs as the initial therapy for symptomatic AF patients. However, recent randomized control trials have suggested catheter cryoballoon ablation as an initial therapy for AF instead of antiarrhythmic drug therapy due to significantly reduced rates of AF recurrence [8, 9]. Clinically, cardiac ultrasound, enhanced computed tomography, positron emission tomography, myocardial contrast echocardiography, enhanced nuclear magnetic field, myocardial biopsy, and other examinations can be used to evaluate the heart structure, pulmonary vein anatomy, and degree of myocardial fibrosis to guide preoperative evaluations and predict prognosis. Recently, the catheter-based ablation of atrial fibrosis, monitored by late gadolinium-enhancement magnetic resonance imaging, has been identified as an effective approach for improving clinical outcomes in AF [10]. Investigating the mechanisms that underlie AF will facilitate the exploration of novel approaches to AF prevention and therapy. Obstructive sleep apnea (OSA) has previously been associated with AF [11, 12], and several animal models of AF have been developed, including a rat model of AF induced by chronic intermittent hypoxia (CIH), which appears to closely model AF pathogenesis [13, 14]. However, limited data are available regarding the molecular mechanisms underlying atrial fibrosis in the CIH model.

Circular RNAs (circRNAs) are a type of non-coding RNA (ncRNA) that have attracted much attention for the major roles they play in transcriptional and post-transcriptional regulation. Due to their highly conserved sequences and stability in circulation, circRNAs have been extensively investigated in cancers, such as liver, colorectal, and lung cancer [15–17]. Recently, an integrative analysis of circRNA profiles in AF subjects was reported by Hu et al., which identified several dysregulated circRNAs associated with the inflammatory response process in AF, including hsa_circ_0007256 and hsa_circ_0003533 [18]. Zhang et al. suggested that circ_0000075 and circ_0082096 might be involved in AF development through the regulation of transforming growth factor- β (TGF- β) signaling [19]. However, the mechanisms through which circRNAs regulate AF have not yet been revealed, especially in AF progression.

High-throughput sequencing provides an effective method for the exploration of the pathogenic mechanisms that lead to AF at multiple levels. In this study, we generated an atrial fibrosis rat model induced by CIH to identify differentially expressed circRNAs compared with a control group. Functional enrichment analysis was performed, followed by competitive endogenous RNA (ceRNA) network analysis. The differential expression patterns of critical circRNAs were further validated by quantitative real-time polymerase chain reaction (qRT-PCR) assay.

2 Results

2.1 Comparison of Basic Parameters Between Two Groups by Echocardiography

After 8 weeks of CIH induction, no significant differences were observed in body weight ($P>0.05$) or heart mass ($P>0.05$) between rats in the CIH and control groups (Table 3). Cardiac ultrasound analysis of the

rat heart showed decreased cardiac systolic function in the CIH group compared with the control group (Fig. 1). The basic parameters of mPAP ($P<0.05$), LAD ($P<0.001$), left ventricular (LV) internal dimensions ($P<0.05$), and LV volumes ($P<0.05$) were significantly higher in the CIH group than in the control group (Table 3). The results for blood pressure and ECG monitoring performed in the two groups of rats are shown in Table 4. No significant differences were observed for any of the parameters measured during blood pressure and ECG monitoring between the two groups ($P>0.05$).

2.2 Pathological Changes of Atrial Tissue in CIH Rats Were Verified by Measuring Fibrosis Biomarkers

HE staining showed that the atrial muscle cells in CIH rats were larger than those in control rats. Masson's trichrome staining revealed that the atrial tissues of CIH rats presented colored collagen fibers within the interstitial area. The volume fraction of collagen in the atrial tissues exhibited a significant increase (246.47%) in the CIH group compared with that in the control group ($P<0.001$, Fig. 2).

Western blotting also revealed that fibrosis-related proteins were significantly overexpressed in atrial tissues from the CIH group compared with those from the control group ($P<0.05$, $P<0.01$; Fig. 3A). In the IHC assay, CIH induced the significant deposition of atrial interstitial collagen. The protein expression levels of COL-1, COL-3, CTGF, and TGF- β in the CIH group were significantly upregulated compared with those in the control group ($P<0.01$, $P<0.001$, Fig. 3B).

2.3 Characteristics of CircRNAs in Rat Atrial Tissue

Transcriptomic high-throughput sequencing (including circRNAs, mRNAs, and miRNAs) analysis was performed, and 6,911 circRNAs were identified as being highly enriched in atrial tissue. These circRNAs were derived from 2,875 host genes and consisted of five types: sense-overlapping (6,357; 95%), intronic (37; 1%), intergenic, (227; 3%), exonic (217; 3%), and antisense (73; 1%). Among these host genes, 1,509 (53%) host genes produced only 1 circRNA, whereas 54 host genes (2%) produced ten or more circRNAs (Fig. 4). Most circRNAs were primarily distributed between 200 bp and 1,000 bp in length, and 1,025 circRNAs contained over 2,000 nucleotides (Fig. 4). The characteristics of these circRNAs are shown in Fig. 4, including the chromosomal distribution and circRNA lengths.

2.4 Screening Differentially Expressed CircRNAs

Using the criteria of $\log_2|\text{fold change}| > 1$ and $P<0.05$, we identified 16 differentially expressed circRNAs from the atrial tissue in the CIH group compared with the control group, including 5 upregulated and 11 downregulated circRNAs (Fig. 5A and B). A volcano plot and an MA plot were used to visualize the differences in gene expression between the two groups (Fig. 5C and D). Among the dysregulated

circRNAs, circRNA_0263 and circRNA_1507 were the most upregulated and most downregulated circRNAs, respectively (Table 5).

2.5 Functional Enrichment Analysis

GO analysis demonstrated that the host genes of the dysregulated circRNAs were primarily associated with the “regulation of biological regulation,” “cellular process,” “metabolic process,” and “response to stimulus.” The KEGG pathway enrichment analysis revealed that the host genes of these circRNAs were primarily associated with the pathways “carbohydrate metabolism,” “cardiovascular diseases,” “immune system,” “excretory system,” “circulatory system,” “glycan biosynthesis and metabolism,” “amino acid metabolism,” “translation,” “substance dependence,” “signal transduction,” and “transport and catabolism” (Fig. 6).

2.6 Regulatory Network Involving CircRNA–miRNA–mRNA Interactions

Next, we constructed a ceRNA network comprised of circRNAs, miRNAs, and mRNAs (Fig. 7A). Within the network, the upregulated circRNA_0263 could regulate corresponding target genes by interacting with several miRNAs, such as rno-miR-29b-5p/3p, novel33-mature, and novel17-mature. The downregulated circRNA_1507 could regulate target genes by sponging rno-miR-136-5p, rno-miR-496-3p, rno-miR-337-3p, and novel123_mature. The analysis of the ceRNA network revealed complex and extensive interactions among circRNAs, miRNAs, and mRNAs in atrial fibrosis.

2.7 Validation of Critical CircRNAs

The expression levels of critical circRNAs were further verified via qRT-PCR analysis. Compared with the control group, circRNA_0263 was significantly upregulated in the atrial tissue of CIH model rats ($P < 0.05$, Fig. 7B), whereas circRNA_1507 was significantly downregulated ($P < 0.001$). The results of the qRT-PCR analysis were consistent with the high-throughput sequencing results and the bioinformatics prediction, indicating the reliability of our study.

3 Discussion

This study explored the characteristics of circRNAs in a CIH-induced rat model of AF. We identified 16 differentially expressed circRNAs in the CIH group compared with the normal control group. Functional enrichment analysis revealed that the host genes of these dysregulated circRNAs were primarily involved in “biological regulation,” “cellular process,” “carbohydrate metabolism,” and “cardiovascular diseases.” In the ceRNA network, circRNA_0263 and circRNA_1507 were identified as critical circRNAs involved in the gene regulation of AF through interactions with multiple miRNAs. qRT-PCR validated that circRNA_0263

was significantly upregulated in the CIH group, whereas circRNA_1507 was downregulated compared with the control group.

Clinical studies have shown that patients with OSA are vulnerable to the incidence of AF [24, 25]. CIH, which is a hallmark of OSA, has been associated with the development of fibrosis in multiple tissues, including the myocardium [20, 26], systemic vasculature [27], liver [28], and lungs [29]. Intermittent hypoxia for a period as short as 4–5 weeks can cause diffuse collagen fiber deposition in rats [29, 30]. In this study, CIH-induced atrial fibrosis was established, and the CIH environment was maintained for eight weeks. Although no significant differences were observed for body weight ($P>0.05$) or heart mass ($P>0.05$) between the rats in the CIH and control groups, fibrosis-related proteins were significantly overexpressed in atrial tissues from the CIH group compared with those from the control group ($P<0.05$). These results suggested that patients with OSA (asymptomatic) may begin to develop irreversible changes before they become aware of their diagnosis.

Persistent AF leads to electrical remodeling and fibrosis of the atria, which serve as the primary hallmarks of atrial remodeling in patients with AF. Experimental and clinical data suggest that this process involves complex interactions among multiple factors, such as continuous hypoxia, inflammation, oxidative stress, calcium overload, miRNAs, and myofibroblast activation [31, 32]. Here, we examined the protein expression levels of fibrosis-related cytokines in CIH-induced rat atrial tissues. Atrial fibrosis was indicated by the increased expression of COL-1 and COL-3 proteins in both IHC and western blot assays, suggesting the successful establishment of the CIH model. COL-1 and COL-3 are the major heart collagens and comprise the most common components of the cardiac extracellular matrix (ECM). Alterations in collagen production or degradation have been observed in experimental canine models of AF, as well as in human patients with AF [33, 34]. CTGF is a potent profibrotic factor that promotes cellular adhesion and ECM synthesis. Increased tissue levels of CTGF have been identified in pathological fibrosis states for many organs in heart disease patients, such as the lungs, kidneys, liver, and myocardium [35–37]. TGF- β serves as a major mediator of CTGF signaling and has been reported as upregulated in the atrial tissue of patients with AF [38]. The CIH rat model in our study showed the significant upregulation of CTGF and TGF- β , which is in accordance with previous reports [35–38].

CircRNAs are covalently closed RNA molecules that are generated through a process known as back-splicing. Most circRNAs are evolutionarily conserved and are highly stable [39]. CircRNAs act as ceRNA molecules, involved in several intracellular signaling pathways, and circRNAs can regulate cellular apoptosis and fibrosis in organs. By sponging miRNAs in cells, circRNAs can competitively inhibit the binding of miRNAs with mRNAs, effectively increasing the expression levels of target genes [40]. The potential roles played by circRNAs were also preliminarily identified during cardiovascular disease development [41]. Based on the ceRNA network construction and the qRT-PCR analysis, our research identified two circRNAs (circRNA_0263 and circRNA_1507) that were dysregulated in rat atrial tissues obtained from the CIH model. The upregulated circRNA_0263 was predicted to be involved in AF progression through interactions with four miRNAs (Fig. 7A): no-miR-29b-5p/3p, novel33-mature, and novel17-mature. MiR-29 family members play vital roles during fibrosis in many organs through the

regulation of fibroblast activation, including lung, cardiac, and hepatic fibrosis [42–44]. MiR-29b is a member of the miR-29 family that was downregulated in a canine atrial fibrosis model, resulting in the increased expression of target ECM genes; TGF- β can induce miR-29b downregulation in atrial fibroblasts, indicating that miR-29b likely regulates the fibrosis processes through the TGF- β signaling pathway [45]. Furthermore, miRNA-29b overexpression alleviated myocardial fibrosis and cardiac dysfunction in post-myocardial infarction rats through the targeting of SH2B3 [46]. In mouse heart tissue, miR-29b prevented angiotensin II-mediated cardiac fibrosis and might represent a potential therapeutic agent for cardiac fibrosis by targeting the TGF- β /Smad3 pathway [19]. A recent study demonstrated that the miR-29b interaction with Smad3 regulated the expression of migration inhibitory factors, which exhibited an anti-fibrotic role in cardiac fibrosis [47]. Therefore, we suggest that the circRNA_0263/miR-29b axis could play a crucial role in AF progression. However, the potential roles played by the circRNAs predicted in our study should be further verified by experimental studies.

The downregulated circRNA_1507 could regulate the target genes by sponging rno-miR-136-5p, rno-miR-496-3p, rno-miR-337-3p, and novel123_mature. MiR-13-5p is expressed at low levels and acts as a tumor suppressor in many types of cancers [48–50]. MiR-337-3p was demonstrated to promote adipocyte browning by inhibiting TWIST1 in obesity [51] and serves as a tumor suppressor in cancers [52, 53]; however, its role in cardiovascular diseases has not been reported. By constructing the ceRNA network, we demonstrated that these miRNAs might participate in CIH-induced atrial fibrosis by regulating *Smim6*, *Gfra4*, *Sp6*, and *Efnb3* and could be sponged by circRNA_1507.

This study was associated with some limitations. First, this study was not conducted in aging AF rats; therefore, we were unable to provide a comprehensive description of changes in genes or molecules associated with AF in rats during the aging process. Second, although the differential expression of circRNA_0263 and circRNA_1507 was verified by qRT-PCR in this study, further research, including gain-of-function loss-of-function experiments, remains warranted to investigate the exact mechanisms through which these circRNAs compete with miRNAs in the ceRNA network during AF development.

In summary, we screened 16 differentially expressed circRNAs from a CIH-induced rat model of AF. Based on the ceRNAs network analysis and qRT-PCR verification, we identified circRNA_0263 and circRNA_1507 as candidate genes involved in the development of AF in rats, which might control target gene expression through interactions with corresponding miRNAs, such as miR-29b-5p/3p, novel33-mature, and rno-miR-136-5p. These regulatory networks, comprised of circRNAs, miRNAs, and target genes, are critical for carbohydrate metabolism and cardiovascular disease pathways, promoting AF progression. Our study promoted a better understanding of the circRNA functions during the fibrosis process in AF.

4 Materials And Methods

4.1 Reagents and Rats

Primary antibodies were purchased from Abcam, including antibodies against collagen I (COL-1), collagen III (COL-3), connective tissue growth factor (CTGF), TGF- β , and β -actin (details in Table 1). Secondary goat anti-rabbit IgG and mouse anti-rabbit IgG antibodies were obtained from Solarbio Company (Beijing, China).

Thirty healthy male Sprague Dawley (SD) rats (6–8 weeks old), weighing 200–250 g, were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd (Beijing, China).

4.2 Construction of a CIH-Induced Rat Model

All animal studies were conducted in compliance with the guidelines established by the Animal Administration Committee of Tianjin Medical University (Approval number: TMUaMEC2016012). The rats were randomly divided into a control group and a CIH group, with 15 rats in each group. The rats were allowed free access to food and water and provided with a normal diet. The atrial fibrosis rat model was established by exposure to a CIH environment, using a previously described protocol [20]. Briefly, the rats in the CIH group were placed in a chamber filled with alternating cycles of nitrogen and oxygen, whereas the control group rats were maintained under normal oxygen conditions. The intermittent hypoxia time was gradually increased from 4 hours each day to 8 hours each day during the first week (from 9:00 AM to 5:00 PM) and then fixed at 8 hours each day. After filling the chamber with nitrogen, the oxygen concentration decreased from the normal 21% to 8% and was maintained at 8% for 50 s. The oxygen level was then increased from 8% to 21% by aerating with oxygen and was maintained at 21% for 50 s. Each intermittent hypoxia cycle comprised 300 s, alternating between oxygen concentrations of 8% and 21%.

4.3 Assessment of Echocardiography (ECG)

Intermittent hypoxia exposure lasted for 8 weeks, after which all rats were weighed and anesthetized with ether to perform the ECG assay. Animals were fixed on a platform and assessed by a GE Vivid-7 Doppler ultrasonic machine (General Electric, USA). Interventricular septal thickness (IVS), left ventricular end-diastolic dimension (LVEDD), left ventricular end-systolic dimension (LVESD), left atrial diameter (LAD), and mean pulmonary artery pressure (mPAP) were monitored.

We performed a carotid artery intubation for the blood pressure and ECG monitoring of all rats in both groups. The right common carotid artery was separated from the vagus nerve without damage. Distal ligation was performed with a non-absorbable suture, and a single vascular clamp was fixed on the proximal carotid artery. After a wedge-shaped resection was performed in the middle area, the artery was inserted into a polystyrene catheter (1.5-mm-diameter, injecting heparin sodium) connected to a biological signal acquisition system. ECG data and arterial blood pressure variables were recorded in 10 cardiac cycles, including heart rate, RR interval, PR interval, QRS interval, QT interval, systolic blood pressure, diastolic blood pressure, mean blood pressure, and pulse pressure.

At the end of ECG monitoring, the hearts from both groups were collected, weighed, and washed with pre-cooled phosphate-buffered saline three times. The left atrial tissue of five rats in each group was used for hematoxylin-eosin (HE) staining, Masson's trichrome staining, and immunohistochemistry (IHC); all other rat samples were frozen at -80°C for molecular biological analysis.

4.4 HE Staining

The left atrial tissues were immersed in 4% paraformaldehyde for two weeks. After paraffin-embedding, these tissues were cut into 5- μm sections. The slices were dewaxed in xylene for 10 min, three times, and then moved through a 95%, 80%, and 70% alcohol gradient for gradual dehydration, for 5 min each. The specimens were then successively stained with hematoxylin for 3 min and eosin for 5 min, followed by reimmersion in alcohol and xylene. Morphological changes were observed under an Olympus CX-21FSI microscope system (Olympus, Japan), and Image-Pro Plus 7.0 software was applied for imaging analysis. Three fields of view were taken from each slide.

4.5 Masson's Trichrome Staining

Masson's trichrome staining was performed using a kit from Nanjing Jiancheng (Jiangsu, China). Briefly, the left atrial tissues were cut into 5- μm sections. The slides were dewaxed, rehydrated, and immersed in Bouin's solution at 56°C for 15 min. Then, the slides were dyed with Wiegert's hematoxylin, Biebrich scarlet-acid fuchsin, and aniline blue, followed by fixation in 1% acetic acid. Finally, the slides were dehydrated and mounted. Three fields of view were taken from each slide.

4.6 IHC

The expression levels of fibrosis-related proteins, including COL-1, COL-3, CTGF, and TGF- β 1, were detected using IHC assay. The baked slices were dewaxed with xylene and hydrated with an ethanol gradient (100%–70%). After successively incubating with citrate buffer and 3% H_2O_2 for 30 min, the slides were sealed with blocking solution and incubated with the primary antibodies overnight at 4°C . On the second day, the slides were rinsed and incubated with the corresponding secondary antibody (Zhongshan Jinqiao Biotechnology Company; Beijing, China) for 15 min, followed by 3,3'-diaminobenzidine (DAB) and HE staining. The slides were then examined and photographed (400 $\times$) using an Olympus BX53 fluorescence microscope (Tokyo, Japan). The DAB staining was analyzed by Image-Pro Plus 7.0 software (Media Cybernetics, Rockville, MD, USA). Three fields of view were taken for each slide. The mean optical density was calculated as the integral optical density/the total brown area.

4.7 Western Blotting

To further explore the expression of fibrosis-related proteins, we performed western blotting assay. Frozen artery tissue samples were homogenized in radioimmunoprecipitation assay lysis buffer and centrifuged (12,000 rpm for 15 min). Protein samples (10 µl) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred onto polyvinylidene difluoride membranes, and incubated with primary antibodies at 4°C overnight (Table 1). After washing with Tris-buffered saline containing Tween-20 3 times, the membranes were incubated with secondary antibody for 2 h. The signal was visualized using a chemiluminescence kit (Millipore, USA) and exposed using an autoradiographic system.

4.8 Identifying Differentially Expressed CircRNAs

High-throughput sequencing was performed by Shanghai OE Biotech Company. We used Trimmomatic [21] software to perform the original data preprocessing, including quality control, read number counts, and summarization. The CircBase database [22] contains numerous circRNA sequences from six species: humans, mice, nematodes, speartail, *Drosophila melanogaster*, and coelacanth. Here, we used CIRI software [23] to perform circRNA prediction. The prediction results were compared with the CircBase database to obtain known and novel circRNAs.

In addition, circRNA expression was quantified using the reads per million (RPM) method, and the RPM files for circRNAs in each sample were obtained, as were the expression boxplots.

The number of circular reads represents the read number compared with the circRNA back-spliced junction area, which is derived from the circRNA prediction software. The number of total reads (in millions) represents the read number for each sample (clean_data).

DESeq software was used to normalize the junction read counts of circRNA in each sample. A negative binomial distribution test was conducted to identify the differentially expressed circRNAs, based on the criteria of $\log_2[\text{fold change}] > 1$ and $P\text{-value} < 0.05$. Cluster analysis was performed for the differentially expressed circRNAs. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed for the host genes of the differentially expressed circRNAs.

4.9 Prediction of CircRNA–miRNA Interactions

Miranda database was used to predict microRNAs (miRNAs) that interact with differentially expressed circRNAs. The miRNAs with greater connections to circRNAs were screened using a hypergeometric distribution test. The circRNA–miRNA target interaction network was visualized using R software.

4.10 qRT-PCR Analysis

Critical circRNA expression levels were further investigated by qRT-PCR analysis. TRIzol reagent (Invitrogen, USA) was used to isolate total RNA from atrial tissue samples. Total RNA was reverse transcribed into cDNA following the instruction of the reverse transcription kit (TIANGEN, Beijing, China). The obtained cDNAs were used for qRT-PCR analysis using a qRT-PCR kit (TransGen Biotech, Beijing, China) and a 7500 RT-PCR System (Thermo, USA). Primers were generated by Beijing BGI Company (Beijing, China), and the primer sequences are shown in Table 2. The cDNA levels were analyzed by the $2^{-\Delta\Delta Ct}$ method, and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as an internal control.

4.11 Statistical Analysis

All experimental data are presented as the mean $\pm$ standard deviation and were analyzed by two-tailed *t*-tests in SPSS 19.0 software (SPSS Inc., USA). $P < 0.05$ was considered a significant difference.

Abbreviations

AF: atrial fibrillation; CIH: chronic intermittent hypoxia; circRNAs: circular RNAs; ceRNA: competing endogenous RNAs; COL-1, collagen I; COL-3, collagen III; CTGF, connective tissue growth factor; DAB, 3,3'-diaminobenzidine; Echocardiography; ECM, extracellular matrix; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GO: gene ontology; HE, hematoxylin-eosin; IHC, immunohistochemistry; IVS, Interventricular septal thickness; KEGG: Kyoto Encyclopedia of Genes and Genomes; LAD, left ventricular end-systolic dimension; log₂ FC, log₂ fold change; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension miRNAs: microRNAs; mPAP, mean pulmonary artery pressure; ncRNA, non-coding RNA; OSA, Obstructive sleep apnea; qRT-PCR, quantitative real-time polymerase chain reaction; RPM, reads per million; SD, Sprague Dawley; TGF- β , transforming growth factor- β .

Declarations

Ethics approval and consent to participate

All animal studies were conducted in compliance with the guidelines established by the Animal Administration Committee of Tianjin Medical University (Approval number: TMUaMEC2016012).

Competing Interests

The authors declare that they have no conflicts of interest.

Consent to publish

Not applicable.

Funding

This work was supported by the National Natural Science Foundation of China Youth Science Foundation Project [No. 81700304 and No. 82000313]; The Scientific Research Fund Project of Key Laboratory of Second Hospital of Tianjin Medical University [No. 2019ZDSYS11, No. 2019ZDSYS10, No. 2019ZDSYS02, No. 2019ZDSYS07]; Tianjin Natural Science Foundation [No. 18JCYBJC92200]; The Research Fund for Central Laboratory of Second Hospital of Tianjin Medical University [No. 2020ydey05]; The Science & Technology Development Fund of Tianjin Education Commission for Higher Education [No. 2020KJ168]; and The PhD Research Foundation of Affiliated Hospital of Jining Medical University [No. 2021-BS-005].

Author Contributions

All authors contributed to the study conception and design. Conception and design of the research: GPL and EZL; Acquisition of data: XL, YHL, and YL; Analysis and interpretation of data: XL and WDW; Statistical analysis: MMW; Drafting the manuscript: XL, WFSG, and XWZ; Revision of manuscript for important intellectual content: SM, GPL and EZL.

Data Availability Statement

I confirm I have included a Data Availability Statement in my manuscript.

BioProject:PRJNA786466

References

1. Ravassa S, Ballesteros G, and Díez J. Aging and atrial fibrillation: a matter of fibrosis. *Aging* (Albany NY). 2019; 11(22): 9965–9966.
2. Lin YK, Chen YA, Lee TI, Chen YC, Chen SA, and Chen YJ. Aging modulates the substrate and triggers remodeling in atrial fibrillation. *Circ J*. 2018; 82(5): 1237–1244.
3. Kallistratos MS, Poulimenos LE, and Manolis AJ. Atrial fibrillation and arterial hypertension. *Pharmacol Res*. 2018; 128: 322–326.
4. Go AS, Hylek EM, Phillips KA, Chang Y, Henault LE, Selby JV, and Singer DE. Prevalence of diagnosed atrial fibrillation in adults: national implications for rhythm management and stroke prevention: the AnTicoagulation and Risk Factors in Atrial Fibrillation (ATRIA) Study. *JAMA*. 2001; 285(18): 2370–2375.
5. Bai Y, Wang YL, Shantsila A, and Lip GYH. The global burden of atrial fibrillation and stroke: A systematic review of the clinical epidemiology of atrial fibrillation in Asia. *Chest*. 2017; 152(4): 810–820.
6. Nattel S. Molecular and cellular mechanisms of atrial fibrosis in atrial fibrillation. *JACC Clin Electrophysiol*. 2017; 3(5): 425–435.

7. Baher A and Marrouche NF. Management of atrial fibrillation in patients with heart failure: Time to implement ablation control. *Curr Cardiol Rep*. 2019; 21(2): 10.
8. Wazni OM, Dandamudi G, Sood N, Hoyt R, Tyler J, Durrani S, Niebauer M, Makati K, Halperin B, Gauri A, Morales G, Shao M, Cerkenvenik J, et al. Cryoballoon ablation as initial therapy for atrial fibrillation. *N Engl J Med*. 2021; 384(4): 316–324.
9. Andrade JG, Wells GA, Deyell MW, Bennett M, Essebag V, Champagne J, Roux JF, Yung D, Skanes A, Khaykin Y, Morillo C, Jolly U, Novak P, et al. Cryoablation or drug therapy for initial treatment of atrial fibrillation. *N Engl J Med*. 2021; 384(4): 305–315.
10. Fochler F, Yamaguchi T, Kheirkahan M, Kholmovski EG, Morris AK, and Marrouche NF. Late gadolinium enhancement magnetic resonance imaging guided treatment of post-atrial fibrillation ablation recurrent arrhythmia. *Circ Arrhythm Electrophysiol*. 2019; 12(8): e007174.
11. Linz D, McEvoy RD, Cowie MR, Somers VK, Nattel S, Lévy P, Kalman JM, and Sanders P. Associations of obstructive sleep apnea with atrial fibrillation and continuous positive airway pressure treatment: A review. *JAMA Cardiol*. 2018; 3(6): 532–540.
12. Marulanda-Londoño E and Chaturvedi S. The interplay between obstructive sleep apnea and atrial fibrillation. *Front Neurol*. 2017; 8: 668.
13. Linz D, Schotten U, Neuberger HR, Böhm M, and Wirth K. Negative tracheal pressure during obstructive respiratory events promotes atrial fibrillation by vagal activation. *Heart Rhythm*. 2011; 8(9): 1436–1443.
14. Miao S, Yang Y, Li R, Yin L, Zhang K, Cheng L, Xu X, Wang W, Zhao Z, and Li G. The potential effects of aliskiren on atrial remodeling induced by chronic intermittent hypoxia in rats. *Drug Des Devel Ther*. 2020; 14: 3755–3764.
15. Hu W, Bi ZY, Chen ZL, Liu C, Li LL, Zhang F, Zhou Q, Zhu W, Song YY, Zhan BT, Zhang Q, Bi YY, Sun CC, et al. Emerging landscape of circular RNAs in lung cancer. *Cancer Lett*. 2018; 427: 18–27.
16. Qiu LP, Wu YH, Yu XF, Tang Q, Chen L, and Chen KP. The emerging role of circular RNAs in hepatocellular carcinoma. *J Cancer*. 2018; 9(9): 1548–1559.
17. Taborda MI, Ramirez S, and Bernal G. Circular RNAs in colorectal cancer: Possible roles in regulation of cancer cells. *World J Gastrointest Oncol*. 2017; 9(2): 62–69.
18. Hu X, Chen L, Wu S, Xu K, Jiang W, Qin M, Zhang Y, and Liu X. Integrative analysis reveals key circular RNA in atrial fibrillation. *Front Genet*. 2019; 10: 108.
19. Zhang Y, Huang XR, Wei LH, Chung AC, Yu CM, and Lan HY. miR-29b as a therapeutic agent for angiotensin II-induced cardiac fibrosis by targeting TGF-beta/Smad3 signaling. *Mol Ther*. 2014; 22(5): 974–985.
20. Ma Z, Zhang K, Wang Y, Wang W, Yang Y, Liang X, Zhang Y, and Li G. Doxycycline improves fibrosis-induced abnormalities in atrial conduction and vulnerability to atrial fibrillation in chronic intermittent hypoxia rats. *Med Sci Monit*. 2020; 26: e918883.
21. Bolger AM, Lohse M, and Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014; 30(15): 2114–2120.

22. Glazar P, Papavasileiou P, and Rajewsky N. circBase: a database for circular RNAs. *RNA*. 2014; 20(11): 1666–1670.
23. Gao Y, Wang J, and Zhao F. CIRI: an efficient and unbiased algorithm for de novo circular RNA identification. *Genome Biol*. 2015; 16: 4.
24. Gami AS, Pressman G, Caples SM, Kanagala R, Gard JJ, Davison DE, Malouf JF, Ammash NM, Friedman PA, and Somers VK. Association of atrial fibrillation and obstructive sleep apnea. *Circulation*. 2004; 110(4): 364–367.
25. Gami AS, Hodge DO, Herges RM, Olson EJ, Nykodym J, Kara T, and Somers VK. Obstructive sleep apnea, obesity, and the risk of incident atrial fibrillation. *J Am Coll Cardiol*. 2007; 49(5): 565–571.
26. Ramos P, Rubies C, Torres M, Batlle M, Farre R, Brugada J, Montserrat JM, Almendros I, and Mont L. Atrial fibrosis in a chronic murine model of obstructive sleep apnea: mechanisms and prevention by mesenchymal stem cells. *Respir Res*. 2014; 15(1): 54.
27. Marcus JA, Pothineni A, Marcus CZ, and Bisognano JD. The role of obesity and obstructive sleep apnea in the pathogenesis and treatment of resistant hypertension. *Curr Hypertens Rep*. 2014; 16(1): 411.
28. Kang HH, Kim IK, Lee HI, Joo H, Lim JU, Lee J, Lee SH, and Moon HS. Chronic intermittent hypoxia induces liver fibrosis in mice with diet-induced obesity via TLR4/MyD88/MAPK/NF- κ B signaling pathways. *Biochem Biophys Res Commun*. 2017; 490(2): 349–355.
29. Braun RK, Broytman O, Braun FM, Brinkman JA, Clithero A, Modi D, Pegelow DF, Eldridge M, and Teodorescu M. Chronic intermittent hypoxia worsens bleomycin-induced lung fibrosis in rats. *Respir Physiol Neurobiol*. 2018; 256: 97–108.
30. Wei Q, Bian Y, Yu F, Zhang Q, Zhang G, Li Y, Song S, Ren X, and Tong J. Chronic intermittent hypoxia induces cardiac inflammation and dysfunction in a rat obstructive sleep apnea model. *J Biomed Res*. 2016; 30(6): 490–495.
31. Jalife J and Kaur K. Atrial remodeling, fibrosis, and atrial fibrillation. *Trends Cardiovasc Med*. 2015; 25(6): 475–484.
32. Corradi D. Atrial fibrillation from the pathologist's perspective. *Cardiovasc Pathol*. 2014; 23(2): 71–84.
33. Cardin S, Libby E, Pelletier P, Le Bouter S, Shiroshita-Takeshita A, Le Meur N, Leger J, Demolombe S, Ponton A, Glass L, and Nattel S. Contrasting gene expression profiles in two canine models of atrial fibrillation. *Circ Res*. 2007; 100(3): 425–433.
34. Polyakova V, Miyagawa S, Szalay Z, Risteli J, and Kostin S. Atrial extracellular matrix remodelling in patients with atrial fibrillation. *J Cell Mol Med*. 2008; 12(1): 189–208.
35. Dean RG, Balding LC, Candido R, Burns WC, Cao Z, Twigg SM, and Burrell LM. Connective tissue growth factor and cardiac fibrosis after myocardial infarction. *J Histochem Cytochem*. 2005; 53(10): 1245–1256.
36. Kato T, Yamashita T, Sekiguchi A, Tsuneda T, Sagara K, Takamura M, Kaneko S, Aizawa T, and Fu LT. AGEs-RAGE system mediates atrial structural remodeling in the diabetic rat. *J Cardiovasc*

- Electrophysiol. 2008; 19(4): 415–420.
37. Liu BC, Sun J, Chen Q, Ma KL, Ruan XZ, and Phillips AO. Role of connective tissue growth factor in mediating hypertrophy of human proximal tubular cells induced by angiotensin II. *Amer J Nephrol.* 2003; 23(6): 429–437.
 38. Tsai CT, Tseng CD, Hwang JJ, Wu CK, Yu CC, Wang YC, Chen WP, Lai LP, Chiang FT, and Lin JL. Tachycardia of atrial myocytes induces collagen expression in atrial fibroblasts through transforming growth factor beta1. *Cardiovasc Res.* 2011; 89(4): 805–815.
 39. Patop IL, Wüst S, and Kadener S. Past, present, and future of circRNAs. *EMBO J.* 2019; 38(16): e100836.
 40. Zheng Q, Bao C, Guo W, Li S, Chen J, Chen B, Luo Y, Lyu D, Li Y, Shi G, Liang L, Gu J, He X, et al. Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. *Nat. Commun.* 2016; 7: 11215.
 41. Li Y, Zhang J, Huo C, Ding N, Li J, Xiao J, Lin X, Cai B, Zhang Y, and Xu J. Dynamic organization of lncRNA and circular RNA regulators collectively controlled cardiac differentiation in humans. *EBioMedicine.* 2017; 24: 137–146.
 42. Cushing L, Kuang PP, Qian J, Shao F, Wu J, Little F, Thannickal VJ, Cardoso WV, and Lü J. miR-29 is a major regulator of genes associated with pulmonary fibrosis. *Am J Respir Cell Mol Biol.* 2011; 45(2): 287–294.
 43. Deng Z, He Y, Yang X, Shi H, Shi A, Lu L, and He L. MicroRNA-29: A crucial player in fibrotic disease. *Mol Diag Ther.* 2017; 21(3): 285–294.
 44. He Y, Huang C, Lin X, and Li J. MicroRNA-29 family, a crucial therapeutic target for fibrosis diseases. *Biochimie.* 2013; 95(7): 1355–1359.
 45. Li J, Cen B, Chen S, and He Y. MicroRNA-29b inhibits TGF-beta1-induced fibrosis via regulation of the TGF-beta1/Smad pathway in primary human endometrial stromal cells. *Mol Med Rep.* 2016; 13(5): 4229–4237.
 46. Wang Y, Jin BJ, Chen Q, Yan BJ, and Liu ZL. MicroRNA-29b upregulation improves myocardial fibrosis and cardiac function in myocardial infarction rats through targeting SH2B3. *Eur Rev Med Pharmacol Sci.* 2019; 23(22): 10115–10122.
 47. Liang JN, Zou X, Fang XH, Xu JD, Xiao Z, Zhu JN, Li H, Yang J, Zeng N, Yuan SJ, Pan R, Fu YH, Zhang M, et al. The Smad3-miR-29b/miR-29c axis mediates the protective effect of macrophage migration inhibitory factor against cardiac fibrosis. *Biochim Biophys Acta Mol Basis Dis.* 2019; 1865(9): 2441–2450.
 48. Li J, Huang C, Zou Y, Ye J, Yu J, and Gui Y. CircTLK1 promotes the proliferation and metastasis of renal cell carcinoma by sponging miR-136-5p. *Mol Cancer.* 2020; 19(1): 103.
 49. Niu J, Li Z, and Li F. Overexpressed microRNA-136 works as a cancer suppressor in gallbladder cancer through suppression of JNK signaling pathway via inhibition of MAP2K4. *Am J Physiol Gastrointest Liver Physiol.* 2019; 317(5): G670–G681.

50. Jeong JY, Kang H, Kim TH, Kim G, Heo JH, Kwon AY, Kim S, Jung SG, and An HJ. MicroRNA-136 inhibits cancer stem cell activity and enhances the anti-tumor effect of paclitaxel against chemoresistant ovarian cancer cells by targeting Notch3. *Cancer Lett.* 2017; 386:168–178.
51. Vonhögen IGC, El Azzouzi H, Olieslagers S, Vasilevich A, de Boer J, Tinahones FJ, da Costa Martins PA, de Windt LJ, and Murri M. MiR-337-3p promotes adipocyte browning by inhibiting TWIST1. *Cells.* 2020; 9(4): 1056.
52. Zhang Z, Zhang L, Wang B, Wei R, Wang Y, Wan J, Zhang C, Zhao L, Zhu X, Zhang Y, Chu C, Guo Q, Yin X, et al. MiR-337-3p suppresses proliferation of epithelial ovarian cancer by targeting PIK3CA and PIK3CB. *Cancer Lett.* 2020; 469: 54–67.
53. Wang Z, Yao L, Li Y, Hao B, Wang M, Wang J, Gu W, Zhan H, Liu G, and Wu Q. miR-337-3p inhibits gastric tumor metastasis by targeting ARHGAP10. *Mol Med Rep.* 2020; 21(2): 705–719.

Tables

Table 1

Primary and secondary antibodies used in western blotting and immunohistochemical assay.

Primary antibodies	molecular weight	dilution (WB/IHC)	solvent (WB/IHC)
Rabbit Anti-Collagen I antibody (ab34710)	130kDa	1:7500/1: 200	TBST/PBS
Rabbit Anti-Collagen III antibody (ab7778)	138kDa	1:5000/1:200	TBST/PBS
Rabbit Anti-CTGF (ab6992)	36kDa	1:4000/1:100	TBST/PBS
Rabbit anti-TGF β 1 (ab170874)	44kDa	1:1000/1:150	TBST/PBS
Mouse anti β -actin (ab32572)	42kDa	1:5000	TBST/PBS
Secondary antibodies	-	dilution	solvent
Goat anti-rabbit IgG-HRP (SE00002-1)	-	1:5000	TBST
Mouse anti-rabbit IgG-HRP (SE131)	-	1:5000	TBST

Table 2
Primers used in real-time PCR.

Primer	Sequence (5'-3')	Tm(°C)
GAPDH	F: AGTGCCAGCCTCGTCTCATA	58.0
	R: ACCAGCTTCCCATTCTCAGC	
circRNA_1507 Chr12:41157218_41200958_+	F: TGAAGGAGAGATGCTTCCGA	58.0
	R: GACTTGCCCTTGAGTGTG	
circRNA_0263 Chr1:131552706_131639910_+	F: ACGCCTTCATGTCTGAGTA	58.0
	R: AGCTTTCCTTAAGACCACATTG	

Table 3
Echocardiography assessment for control group and chronic intermittent hypoxia (CIH) induced rats.

Parameter	Control (n=15)	CIH (n=15)	P value
Body weight(g)	417.75±30.79	401.38±31.27	0.146
Heart weight(g)	1.10±0.12	1.08±0.20	0.724
IVSd, mm	2.40±0.21	2.32±0.30	0.833
IVSs, mm	3.79±0.35	3.36±0.54	0.012
LA; mm	3.55±0.32	4.11±0.26	0.001
LVIDd, mm	6.79±0.54	7.05±0.60	0.209
LVIDs, mm	3.46±0.57	4.13±0.81	0.012
LVPWd, mm	2.55±0.30	2.38±0.41	0.201
LVPWs, mm	3.74±0.30	3.48±0.41	0.048
EF, %	78.73±6.90	69.23±14.72	0.026
FS; diameter, %	49.06±6.78	41.32±11.44	0.027
LV Vold, mm ³	240.31±44.17	261.80±49.03	0.203
LV Vols, mm ³	51.57±20.42	79.36±36.60	0.014
mPAP, mmHg	64.00±1.20	65.81±2.42	0.013
IVSd: Interventricular septal thickness in diastole; IVSs: Interventricular septal thickness in systole; LA, left atrial diameter; LVIDd: left ventricular internal diameter in diastole; LVIDs: left ventricular internal diameter in systole; LVPWd: left ventricular posterior wall thickness in diastole; LVPWs: left ventricular posterior wall thickness in systole; EF: Ejection fraction; FS: fractional shortening; LV Vold: left ventricular volume in diastole; LV Vols: left ventricular volume in systole; mPAP, mean pulmonary artery pressure; *: P<0.05.			

Table 4
Blood pressure and ECG monitoring for two groups of rats.

	Control (n=15)	CIH (n=15)	<i>P</i> value
Blood pressure:			
Systolic Pressure (mmHg)	153.60±12.01	151.83±22.71	0.814
Diastolic Pressure (mmHg)	119.75±12.10	117.28±18.11	0.699
Mean Pressure (mmHg)	136.06±11.47	134.53±19.41	0.816
Pulse Pressure (mmHg)	33.85±8.07	34.55±5.89	0.811
ECG:			
RR Interval (s)	0.16±0.02	0.15±0.02	0.443
Heart Rate (BPM)	370.34±42.05	390.90±55.32	0.316
PR Interval (s)	0.045±0.002	0.047±0.007	0.456
P Duration (s)	0.016±0.001	0.015±0.003	0.748
QRS Interval (s)	0.018±0.003	0.017±0.001	0.507
QT Interval (s)	0.064±0.008	0.071±0.010	0.080
P Amplitude (mV)	0.148±0.031	0.166±0.030	0.184
Q Amplitude (mV)	-0.030±0.022	-0.037±0.022	0.463
R Amplitude (mV)	0.850±0.194	0.852±0.181	0.974
ST Height (mV)	0.041±0.062	0.054±0.039	0.542
T Amplitude (mV)	0.139±0.051	0.179±0.046	0.058

Table 5

The list of differentially expressed circRNAs between the two groups.

circRNAs	P value	type
circRNA_0263 Chr1:131552706_131639910_+	0.033776083	UP
circRNA_1856 Chr14:37251286_37262083_-	0.027084150	UP
circRNA_2007 Chr14:106381229_106384412_-	0.047278831	UP
circRNA_2108 Chr15:33611316_33637880_-	0.042367506	UP
circRNA_4760 Chr4:147527096_147528435_+	0.013073357	UP
circRNA_1507 Chr12:41157218_41200958_+	0.040819746	DOWN
circRNA_1674 Chr13:83636128_83660292_-	0.030138812	DOWN
circRNA_1879 Chr14:38434024_38454336_+	0.013745937	DOWN
circRNA_1939 Chr14:81267948_81280976_-	0.024104189	DOWN
circRNA_2450 Chr17:5514017_5548199_+	0.040256919	DOWN
circRNA_2527 Chr17:27571479_27602936_-	0.031667857	DOWN
circRNA_4260 Chr3:97349444_97419275_+	0.007670028	DOWN
circRNA_4735 Chr4:127663314_127673795_-	0.044042436	DOWN
circRNA_4748 Chr4:133205267_133215619_+	0.030243209	DOWN
circRNA_5285 Chr5:156454276_156463365_+	0.038325511	DOWN
circRNA_6282 Chr8:84443616_84461546_-	0.039550736	DOWN

Figures

Figure 1

The basic parameters of mPAP ($P<0.05$), LAD ($P<0.001$), left ventricular (LV) internal dimensions ($P<0.05$), and LV volumes ($P<0.05$) were significantly higher in the CIH group than in the control group (Table 3). The results for blood pressure and ECG monitoring performed in the two groups of rats are shown in Table 4. No significant differences were observed for any of the parameters measured during blood pressure and ECG monitoring between the two groups ($P>0.05$).

Figure 2

HE staining showed that the atrial muscle cells in CIH rats were larger than those in control rats. Masson's trichrome staining revealed that the atrial tissues of CIH rats presented colored collagen fibers within the interstitial area. The volume fraction of collagen in the atrial tissues exhibited a significant increase (246.47%) in the CIH group compared with that in the control group

Figure 3

Western blotting also revealed that fibrosis-related proteins were significantly overexpressed in atrial tissues from the CIH group compared with those from the control group ($P < 0.05$, $P < 0.01$; Fig. 3A). In the IHC assay, CIH induced the significant deposition of atrial interstitial collagen. The protein expression levels of COL-1, COL-3, CTGF, and TGF-β in the CIH group were significantly upregulated compared with those in the control group ($P < 0.01$, $P < 0.001$, Fig. 3B).

Figure 4

Transcriptomic high-throughput sequencing (including circRNAs, mRNAs, and miRNAs) analysis was performed, and 6,911 circRNAs were identified as being highly enriched in atrial tissue. These circRNAs were derived from 2,875 host genes and consisted of five types: sense-overlapping (6,357; 95%), intronic (37; 1%), intergenic, (227; 3%), exonic (217; 3%), and antisense (73; 1%). Among these host genes, 1,509 (53%) host genes produced only 1 circRNA, whereas 54 host genes (2%) produced ten or more circRNAs

Figure 5

Using the criteria of $\log_2|\text{fold change}| > 1$ and $P < 0.05$, we identified 16 differentially expressed circRNAs from the atrial tissue in the CIH group compared with the control group, including 5 upregulated and 11 downregulated circRNAs (Fig. 5A and B). A volcano plot and an MA plot were used to visualize the differences in gene expression between the two groups (Fig. 5C and D).

Figure 6

GO analysis demonstrated that the host genes of the dysregulated circRNAs were primarily associated with the “regulation of biological regulation,” “cellular process,” “metabolic process,” and “response to stimulus.” The KEGG pathway enrichment analysis revealed that the host genes of these circRNAs were primarily associated with the pathways “carbohydrate metabolism,” “cardiovascular diseases,” “immune

system," "excretory system," "circulatory system," "glycan biosynthesis and metabolism," "amino acid metabolism," "translation," "substance dependence," "signal transduction," and "transport and catabolism"

Figure 7

Next, we constructed a ceRNA network comprised of circRNAs, miRNAs, and mRNAs (Fig. 7A). Within the network, the upregulated circRNA_0263 could regulate corresponding target genes by interacting with several miRNAs, such as rno-miR-29b-5p/3p, novel33-mature, and novel17-mature. The downregulated circRNA_1507 could regulate target genes by sponging rno-miR-136-5p, rno-miR-496-3p, rno-miR-337-3p, and novel123_mature. The analysis of the ceRNA network revealed complex and extensive interactions among circRNAs, miRNAs, and mRNAs in atrial fibrosis.