Mathematical Biosciences and Engineering

MBE, 20(3): 5413–5429.
DOI: 10.3934/mbe.2023250
Received: 30 October 2022
Revised: 17 December 2022
Accepted: 25 December 2022
Published: 12 January 2023

http://www.aimspress.com/journal/MBE

Research article

Integrated whole transcriptome analysis for the crucial regulators and functional pathways related to cardiac fibrosis in rats

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Abstract: *Background:* Cardiac fibrosis has gradually gained significance in the field of cardiovascular disease; however, its specific pathogenesis remains unclear. This study aims to establish the regulatory networks based on whole-transcriptome RNA sequencing analyses and reveal the underlying mechanisms of cardiac fibrosis. *Methods:* An experimental model of myocardial fibrosis was induced using the chronic intermittent hypoxia (CIH) method. Expression profiles of long non-coding RNA (lncRNA), microRNA (miRNA), and messenger RNA (mRNA) were acquired from right atrial tissue samples of rats. Differentially expressed RNAs (DERs) were identified, and functional enrichment analysis was performed. Moreover, a protein-protein interaction (PPI) network and competitive endogenous RNA (ceRNA) regulatory network that are related to cardiac fibrosis were constructed, and the relevant regulatory factors and functional pathways were identified. Finally, the crucial regulators were validated using qRT-PCR. *Results:* DERs, including 268 lncRNAs, 20 miRNAs, and 436 mRNAs, were screened. Further, 18 relevant biological processes, such as "chromosome segregation," and 6 KEGG signaling pathways, such as "cell cycle," were significantly enriched. The regulatory relationship of miRNA–mRNA–KEGG pathways showed eight overlapping disease pathways, including "pathways in cancer." In addition,

crucial regulatory factors, such as *Arnt2*, *WNT2B*, *GNG7*, *LOC100909750*, *Cyp1a1*, *E2F1*, *BIRC5*, and *LPAR4*, were identified and verified to be closely related to cardiac fibrosis. *Conclusion:* This study identified the crucial regulators and related functional pathways in cardiac fibrosis by integrating the whole transcriptome analysis in rats, which might provide novel insights into the pathogenesis of cardiac fibrosis.

Keywords: cardiac fibrosis; whole-transcriptome RNA sequencing; functional pathways; competing endogenous RNA; regulatory network

1. Introduction

Cardiac fibrosis is regarded as the characteristic pathological end-stage of cardiovascular disease and remains the most common chronic disease worldwide [1]. Cardiac fibrosis leads to high morbidity and mortality, as well as a substantially increased global medical burden [2]. Defined as the accumulation of extracellular matrix (ECM) proteins, cardiac fibrosis results from an imbalance in its production and degradation, thus contributing to cardiac dysfunction [3,4]. However, the specific mechanism of cardiac fibrosis is not clear, and its biomedical effects have never been satisfactorily determined [5,6]. Therefore, efforts should be made to promote the translation of mechanistic knowledge on cardiac fibrosis into potential clinical therapeutic applications.

As indispensable non-coding RNAs (ncRNAs), both long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) play important roles in the regulation of messenger RNA (mRNA) expression levels [7–9]. Regulation of the lncRNA–miRNA–mRNA–competitive endogenous RNA (ceRNA) network is actively involved in the onset and progression of multiple diseases, including cancers [8,10,11]. An increasing number of studies have demonstrated that massive mRNAs and ncRNAs (lncRNAs and miRNAs) could serve as crucial biomarkers for cardiac fibrosis [12–14]. Various ceRNA networks have been reported to mediate the pathological process of cardiac fibrosis [14,15]. Gao *et al.* explored a cardiac fibroblast-related ceRNA network, which provided potential target genes in this field [12]. A pro-fibrotic lncRNA (PFL, also known as NONMMUT022555) has been reported to act as a ceRNA of the cardioprotective miRNA, let-7d [16]. The ceRNA network of NORAD/miR-125a-3p/Fyn has also been shown to improve cardiac fibrosis and reduce inflammatory responses [17]. LncRNA H19 has been reported to act as a ceRNA to mediate the connective tissue growth factor (CTGF) expression by sponging miR-455 in cardiac fibrosis [18], whereas another study found that H19 alleviates cardiac fibrosis by targeting miR-22-3p/lysine (K)-specific demethylase 3A (KDM3A) in myocardial infarction [19]. Dai et al. revealed that lncRNA nuclear-enriched abundant transcript 1 (NEAT 1) regulates atrial fibrosis via the miR-320/neuronal per Arnt-Sim domain protein 2 (NPAS2) axis in atrial fibrillation [20]. In addition, lncRNA taurine upregulation gene 1 (TUG1) mediates CTGF expression by sponging miR-133b in myocardial fibrosis after myocardial infarction [21]. However, there is limited knowledge from the perspective of RNA sequencing regarding the roles of the ceRNA regulatory network in the pathogenesis of cardiac fibrosis, which requires further clarification.

In this study, we constructed a rat model of cardiac fibrosis induced using the chronic intermittent hypoxia (CIH) method. Whole transcriptome sequencing was performed to identify differentially expressed RNAs (DERs) and the related functional pathways. Protein–protein

interaction (PPI) and ceRNA networks were constructed by integrating the whole transcriptome analysis. The present study aimed to reveal the crucial regulators and functional pathways related to cardiac fibrosis in rats and to further elucidate the underlying mechanisms of cardiac fibrosis.

2. Materials and methods

2.1. Experimental animals and model of cardiac fibrosis

All the animal experiments were conducted on Sprague–Dawley rats (adult, male) using an experimental model of cardiac fibrosis induced by the CIH method [22]. The experimental rats were obtained and maintained as previously described [23]. The rat model of CIH adopted in this study was described in our previous study, where the establishment of cardiac fibrosis was successfully verified [22,23]. All the animal experiments and study protocols were approved by the hospital's Medical Ethics Committee.

2.2. Whole transcriptomics analysis

After successful establishment of the cardiac fibrosis model, the right atrial tissue samples of the control and CIH groups (n = 3/group) were collected and subjected to whole transcriptome sequencing as previously described [23]. Preprocessing, quality control, and normalization of the raw data was also described in our previous study [23]. Subsequently, the whole transcriptome data of lncRNAs, miRNAs, and mRNA were used for bioinformatics analyses of differential expression, functional and pathway enrichments, and construction of PPI and ceRNA regulatory networks in R.

2.3. DERs selection

The expression levels of lncRNA, miRNA, and mRNA between the control and CIH groups were compared to screen the DERs, including differentially expressed mRNAs (DEmRNAs), differentially expressed lncRNAs (DElncRNAs), and differentially expressed miRNAs (DEmiRNAs), by using the "limma" package in R3.6.1 [24]. $|\log_2(\text{fold change})| > 1$ and $P < 0.05$ were selected as the screening thresholds. All three types of DERs are shown in volcano plots. Moreover, the expression of DERs was presented using bidirectional hierarchical clustering heatmaps based on the Pearson correlation algorithm [25,26].

2.4. Functional and pathway enrichment analysis

The screened DEmRNAs were then subjected to functional and pathway enrichment analyses using the Database for Annotation, Visualization, and Integrated Discovery (DAVID), version 6.8 [27]. Biological processes of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted [28]. A false discovery rate (FDR) value < 0.05 was considered as the threshold for enrichment significance.

2.5. Construction of PPI network

A PPI network was built to obtain the interactions and relationships among proteins encoded by DEmRNAs using the STRING version 11.0 database [29]. Interacting pairs were retained when the interaction scores were higher than 0.4. The PPI network was visualized using Cytoscape version 3.6.1 [30]. Next, functional and pathway enrichment analyses were performed for the nodes in the PPI network. The set threshold of enrichment significance was $P < 0.05$.

2.6. Construction of ceRNA regulatory network

The interactions of these DERs were predicted to construct a ceRNA regulatory network. In brief, the DElncRNA–DEmiRNA interactions were predicted using miRanda software. The parameters were set as Gap Extend = 0, Score Threshold = 80, Energy Threshold = −20, and Matched Seq% Threshold = 80%. Only connections with opposite directions between the DElncRNAs and DEmiRNAs were retained.

The target genes of DEmiRNAs in the DElncRNA–DEmRNA network were predicted using the miRWalk 3.0 database [31]. The DEmRNAs among the target genes were filtered. Only connections with opposite directions between the DEmiRNAs and DEmRNAs were retained.

The regulatory relationships in lncRNA–miRNA and miRNA–mRNA interactions were integrated to construct the ceRNA regulatory network, which was visualized using Cytoscape software Version 3.6.1 [30]. In addition, functional and pathway enrichment analyses were conducted based on the DEmRNAs in the ceRNA regulatory network. The threshold for enrichment significance was set at $P < 0.05$.

2.7. Identification of the crucial regulators in the related ceRNA regulatory network

According to the DEmRNAs involved in the KEGG pathways of the ceRNA regulatory network and miRNA–mRNA interactions of the ceRNA regulatory network, a Sankey diagram was generated to illustrate the regulatory relationships and relevant pathways. Moreover, the Comparative Toxicogenomics Database (CTD) was used to search for disease-relevant KEGG pathways and genes [32], and "atrial fibrillation" was used as the keyword. By comparing the screened genes and the important KEGG pathways involved in them, which were obtained from the previously constructed ceRNA regulatory network, the overlapping crucial regulators and relevant KEGG pathways were identified.

2.8. Quantitative reverse transcription PCR (qRT-PCR)

Total RNA was extracted from atrial specimens of rats using TRIzol reagent (Promega, Beijing, China), which was then reverse-transcribed routinely to cDNA (Transgen Biotech, Beijing, China). Next, qRT-PCR analysis was conducted using real-time PCR software with the SYBR Green PCR kit (Transgen Biotech, Beijing, China). The quantification of mRNA was measured using the $2^{-\Delta\Delta CT}$ method, and the level of lncRNA/genes and miRNA was separately normalized to GAPDH and U6. The primer sequences used in this study are listed in Table 1.

Table 1. Primer sequences used in qRT-PCR.

Primer	species	Sequence (5'-3')	Tm (°C)
GAPDH	Rat	F: AGTGCCAGCCTCGTCTCATA	58.0
		R: ACCAGCTTCCCATTCTCAGC	
NONRATT011877.2	Rat	F: GCTACCAACCAGCCCTTCTT	58.0
		R: CCTTCACCTTCTCAGGCACC	
NONRATT019720.2	Rat	F: GGTGCCTGTCATTTGCCCTTTATC	58.0
		R: AGCAGTGCGTTTCTCTCCTTTGA	
Arnt2	Rat	F: GCATATAAGCCTTCCTTCCTCACT	58.0
		R: CAGCCACCACAAACAGAAATCC	
BIRC5	Rat	F: CACTGCCCTACCGAGAATGAG	58.0
		R: TTCCACCTGCTTCTTGACTGTAA	
GDF6	Rat	F: GTTCCCACCAAATTGACTCCCATC	58.0
		R: CTCCACCACCATGTCCTCGTA	
U6	Rat	F: CTCGCTTCGGCAGCACA	60.0
		R: AACGCTTCACGAATTTGCGT	
miR-3577	Rat	F: TCTGTCCCTCTTGGCCCTTAG	60.0
		R: GTATCCAGTGCAGGGTCCGAGGT	

2.9. Statistical analysis

R software (R version 3.6.1) and Statistical Product and Service Solutions (SPSS 21.0) were used to conduct statistical analysis. $P < 0.05$ or FDR value < 0.05 was considered as a significant difference.

3. Results

3.1. Identification of DERs and functional enrichment analysis

A total of 25,332 lncRNAs, 1204 miRNAs, and 22,601 mRNAs were identified. After the routine analysis for comparison and screening, 268 lncRNAs, 20 miRNAs, and 436 mRNAs were identified as DERs. All DERs are displayed in volcano plots (Figure 1A–C) and clustering heatmaps (Figure 1D–F). As shown in Figure 1D–F, the expression patterns of DERs were distinctly separated based on the different sample groups, which indicated that the identified DERs were expressed characteristically and specifically in cardiac fibrosis.

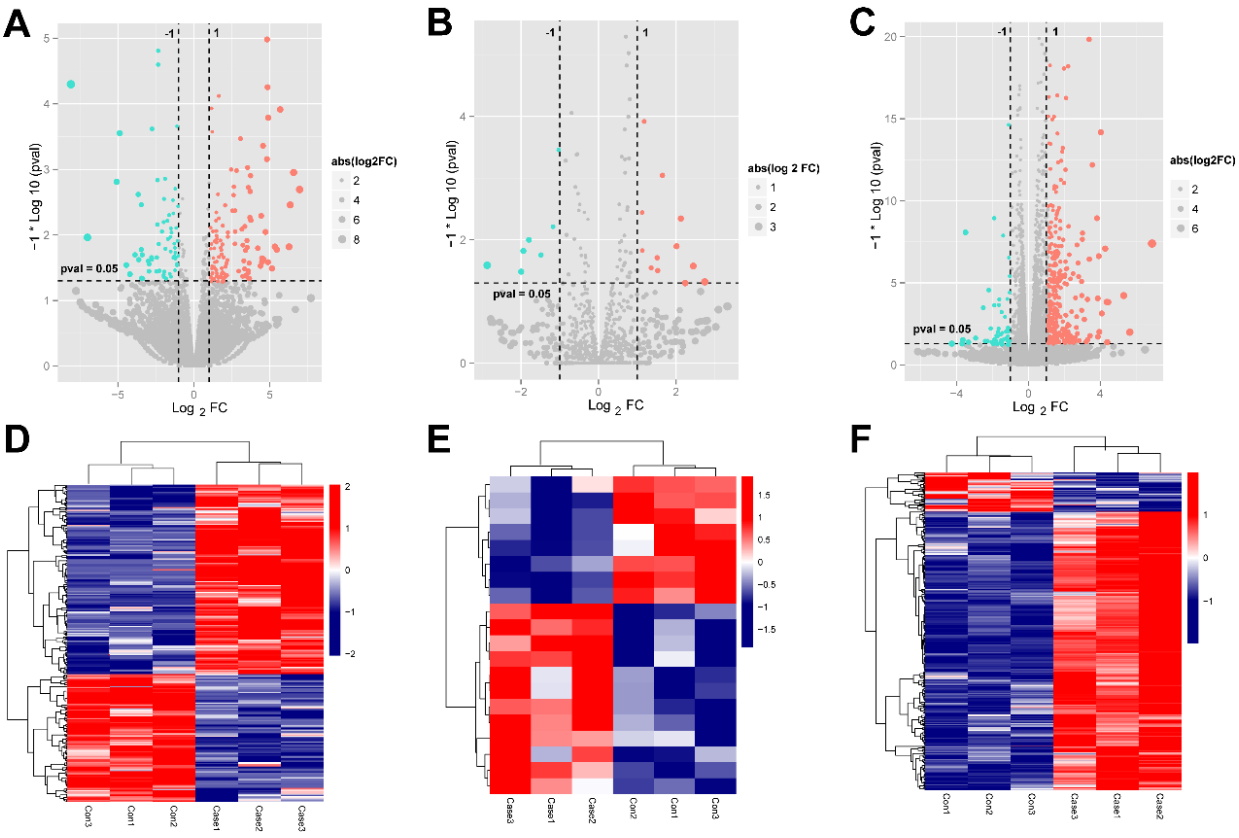

Figure 1. Identification of differentially expressed RNAs for the transcriptomics analysis in experimental cardiac fibrosis in rats. Volcano plots of the differentially expressed lncRNAs (A), miRNAs (B), and mRNAs (C). Heatmap of all the significant differentially expressed lncRNAs (D), miRNAs (E), and mRNAs (F) among all samples based on hierarchical cluster analysis.

Next, DEmRNAs were subjected to functional and pathway enrichment analyses. As shown in Figure 2, a total of 18 relevant biological processes such as "chromosome segregation," "cell division," and "microtubule-based movement" were significantly enriched, and 6 KEGG signaling pathways such as "cell cycle," "alcoholism," and "viral carcinogenesis" were significantly enriched.

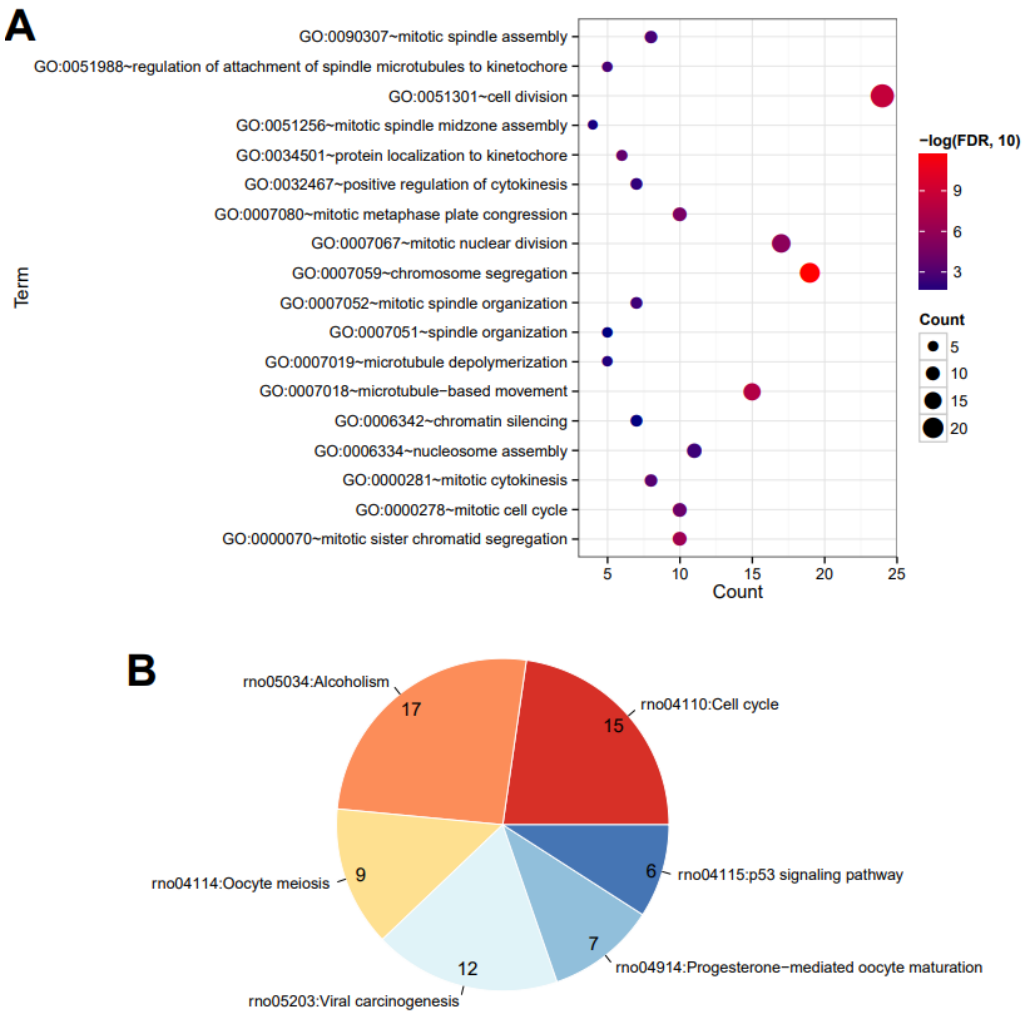

Figure 2. Functional and pathway enrichment analysis for the differentially expressed mRNAs (DEmRNAs) in experimental cardiac fibrosis in rats. (A) Gene Ontology terms of the DEmRNAs in experimental cardiac fibrosis in rats displayed by the bubble plot. (B) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of the DEmRNAs in experimental cardiac fibrosis in rats displayed by the pie diagram.

3.2. Construction of PPI network

The interactions and relationships among proteins encoded by DEmRNAs were predicted using the STRING database to construct a PPI network. A total of 3936 interacting pairs were obtained when interaction scores were higher than 0.4. A PPI network with 306 nodes was established (Figure 3A). Accordingly, the topological structural properties of all the 306 nodes in the PPI network were analyzed. The top 20 nodes are listed in Table 2 in the order of their degrees (from high to low), such as Cdk1, Ccnb1, Cdca8, and Aurkb. Functional and pathway enrichment analyses were also performed. As presented in Figure 3B,C, the relevant biological processes, such as "chromosome segregation," "cell division," and "microtubule-based movement," were significantly enriched; in addition, KEGG signaling pathways, such as "pathways in cancer," "alcoholism," and "oocyte meiosis" were significantly enriched.

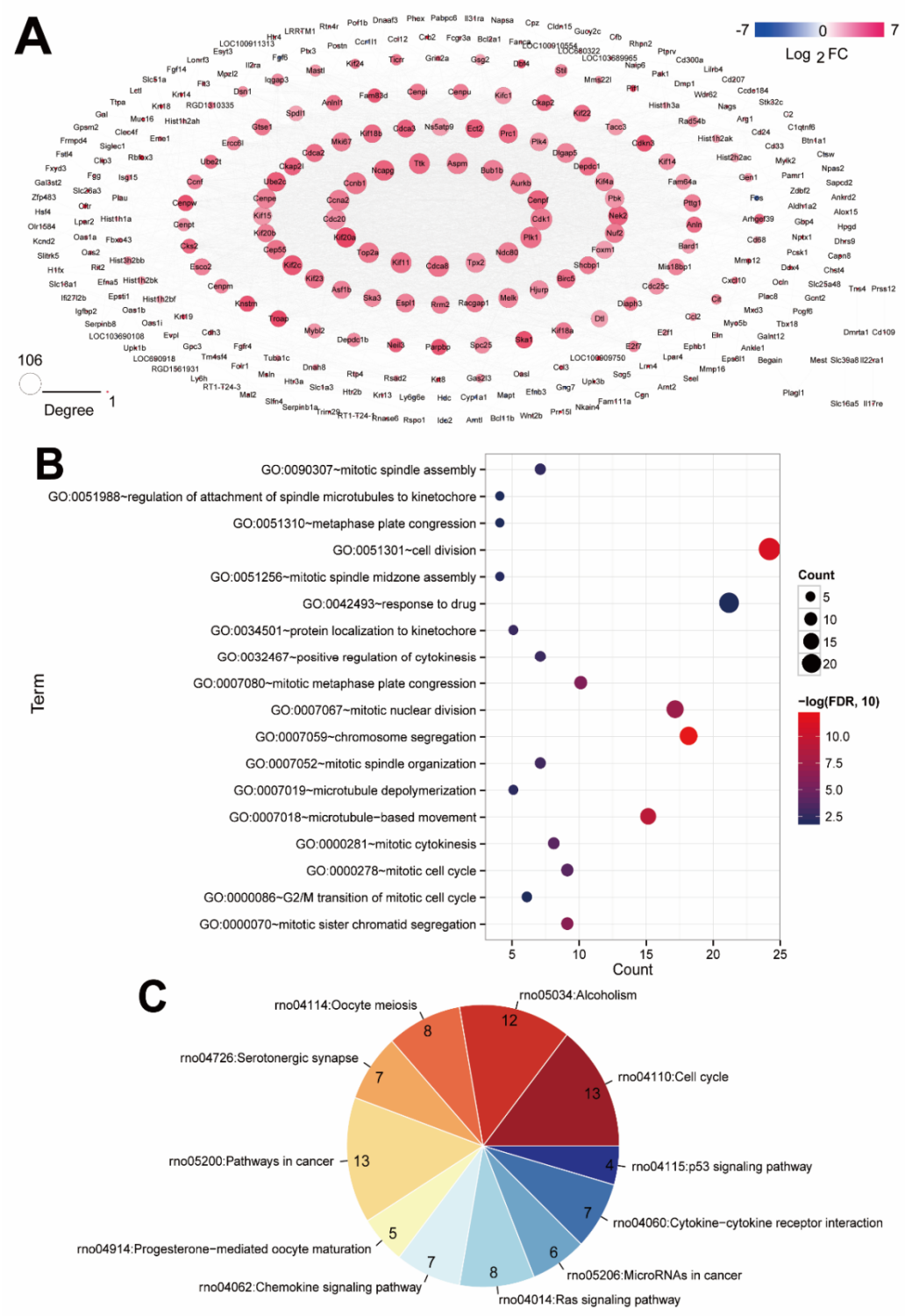

Figure 3. Construction of protein-protein interaction (PPI) network for the differentially expressed mRNAs (DEmRNAs) in experimental cardiac fibrosis in rats and its functional and pathway enrichment analysis. (A) PPI network of the DEmRNAs in experimental cardiac fibrosis in rats. (B) Gene Ontology terms of the DEmRNAs in the PPI network displayed by the bubble plot. (C) KEGG pathways of the DEmRNAs consisting of the PPI network expressed by the pie diagram.

Table 2. The information for the top 20 nodes of the PPI network.

Symbol	Average Shortest Path Length	Betweenness Centrality	Closeness Centrality	log FC	Degree
Cdk1	2.29794521	0.03042122	0.43517139	1.458608048	106
Ccnb1	2.31849315	0.02793644	0.43131462	1.539594873	104
Cdca8	2.32191781	0.0516753	0.43067847	1.595155253	103
Aurkb	2.32191781	0.04407768	0.43067847	1.39788571	103
Plk1	2.32191781	0.02828696	0.43067847	1.633557392	102
Aspm	2.30479452	0.04814318	0.43387816	1.466839476	100
Kif11	2.35616438	0.00910023	0.4244186	1.606132126	99
Ccna2	2.33561644	0.01376403	0.42815249	1.457514154	99
Bub1b	2.3630137	0.00572088	0.42318841	1.418276991	99
Ndc80	2.3630137	0.02016646	0.42318841	1.434083887	98
Ttk	2.35616438	0.01567416	0.4244186	1.705593536	98
Ncapg	2.36986301	0.00456672	0.42196532	1.780442727	96
Cdc20	2.35958904	0.01193459	0.42380261	1.24250715	96
Top2a	2.37328767	0.00618188	0.42135642	1.585737948	96
Tpx2	2.39383562	0.00280374	0.41773963	1.256355204	93
Kif20a	2.3869863	0.00298192	0.41893831	2.207109516	93
Kif2c	2.39383562	0.00275871	0.41773963	1.948591736	92
Cenpf	2.33561644	0.04132747	0.42815249	1.749162922	92
Cenpe	2.39383562	0.00406084	0.41773963	1.376675991	92
Melk	2.38356164	0.00471963	0.41954023	1.379361709	91
Nek2	2.3869863	0.02614422	0.41893831	1.806189323	91

Note: PPI, protein-protein interaction; FC, Fold Change.

3.3. Construction of a ceRNA regulatory network

The communication between DERs was predicted by constructing a ceRNA regulatory network. Consequently, 262 DElncRNA–DEmRNA pairs and 125 DEmiRNA–DEmRNA pairs were obtained. By integrating these pairs, two ceRNA regulatory networks were established (Figure 4A,B). lncRNA–miRNA–mRNA and ceRNA interactions, such as NONRATT012985.2-rno–miR-3577–*Arnt2*, NONRATT000861.2-rno–miR-3577–*BIRC5*, and NONRATT019720.2-rno–miR-3577–*GDF6*, were identified from these networks.

Functional and pathway enrichment analyses were also performed. The results indicated that the relevant biological processes, such as "chromosome segregation," "cell division," and "spermatogenesis" were significantly enriched; in addition, KEGG signaling pathways, such as "microRNAs in cancer," "pathways in cancer," and "metabolic pathways" were significantly enriched (Figure 4C,D).

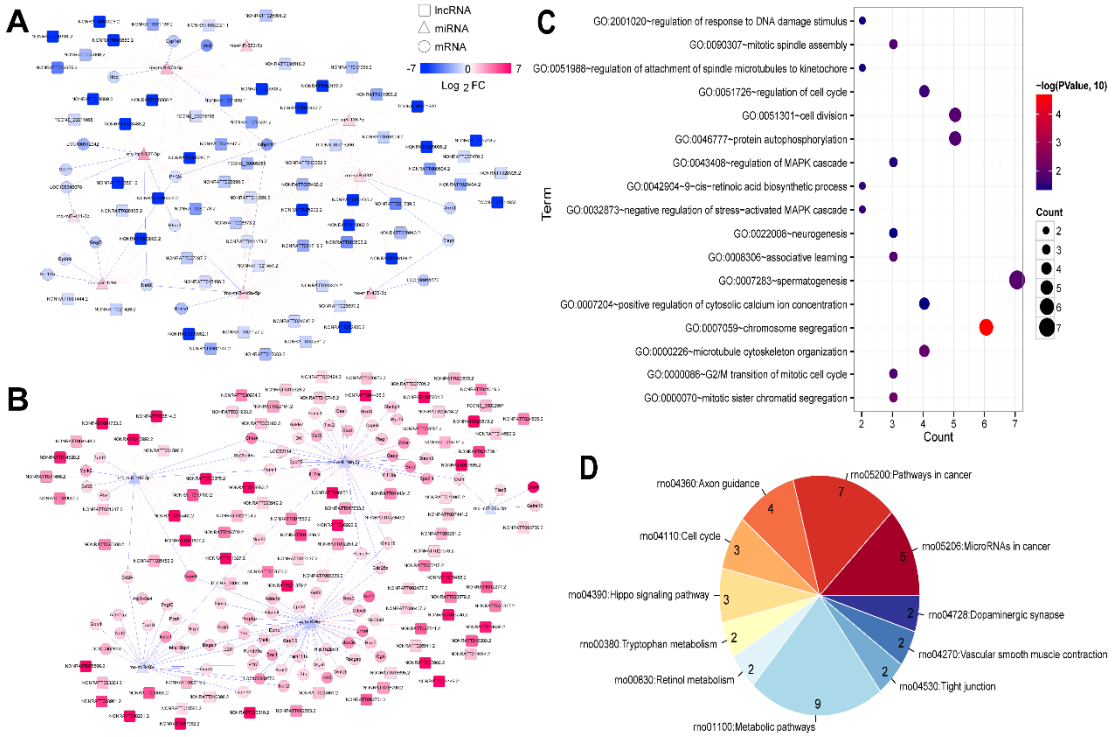

Figure 4. Competitive endogenous RNA (ceRNA) regulatory network for the differentially expressed lncRNAs, miRNAs, and mRNAs in experimental cardiac fibrosis in rats and its functional and pathway enrichment analysis. (A) ceRNA regulatory network for the differentially downregulated-expressed lncRNAs and mRNAs as well as the upregulated miRNAs in experimental cardiac fibrosis in rats. (B) ceRNA regulatory network for the differentially upregulated-expressed lncRNAs and mRNAs as well as the downregulated miRNAs in experimental cardiac fibrosis in rats. (C) Gene Ontology terms of the DEmRNAs in the ceRNA regulatory network displayed by the bubble plot. (D) KEGG pathways of the differentially expressed mRNAs in the ceRNA regulatory network displayed by the pie diagram.

3.4. Identification of the crucial regulators in the related ceRNA regulatory network

According to the DEmRNAs involved in the KEGG pathways and miRNA–mRNA interactions, a Sankey diagram was drawn (Figure 5). Regulatory relationships and relevant pathways, such as miR-3577–*Arnt2*-pathways in cancer and the miR-3577–*BIRC5*–Hippo signaling pathway, were identified.

By searching the CTD database for "atrial fibrillation," 154 KEGG pathways and 168 relevant

genes were identified. After comparing the genes and KEGG pathways in the ceRNA regulatory network, 8 overlapping disease pathways and 1 directly involved gene, *Arnt2*, were identified. *Arnt2* is involved in the disease pathway rno05200: Pathways in cancer. We speculated that the genes involved in the eight disease pathways might be important regulators of cardiac fibrosis. The relevant regulatory lncRNAs and miRNAs, possibly upstream of these genes, might also be closely associated with cardiac fibrosis. In particular, other genes, such as *WNT2B*, *GNG7*, *LOC100909750*, *Cyp1a1*, *E2F1*, *BIRC5*, and *LPAR4*, which are also involved in rno0520: pathways in cancer along with *Arnt2,* might be closely related to the pathogenesis of cardiac fibrosis. Finally, three mRNAs, *Arnt2*, *BIRC5*, and *GDF6*, one miRNA, miR-3577, and two lncRNAs, NONRATT011877.2 and NONRATT019720.2, were identified as crucial regulators in the ceRNA regulatory network.

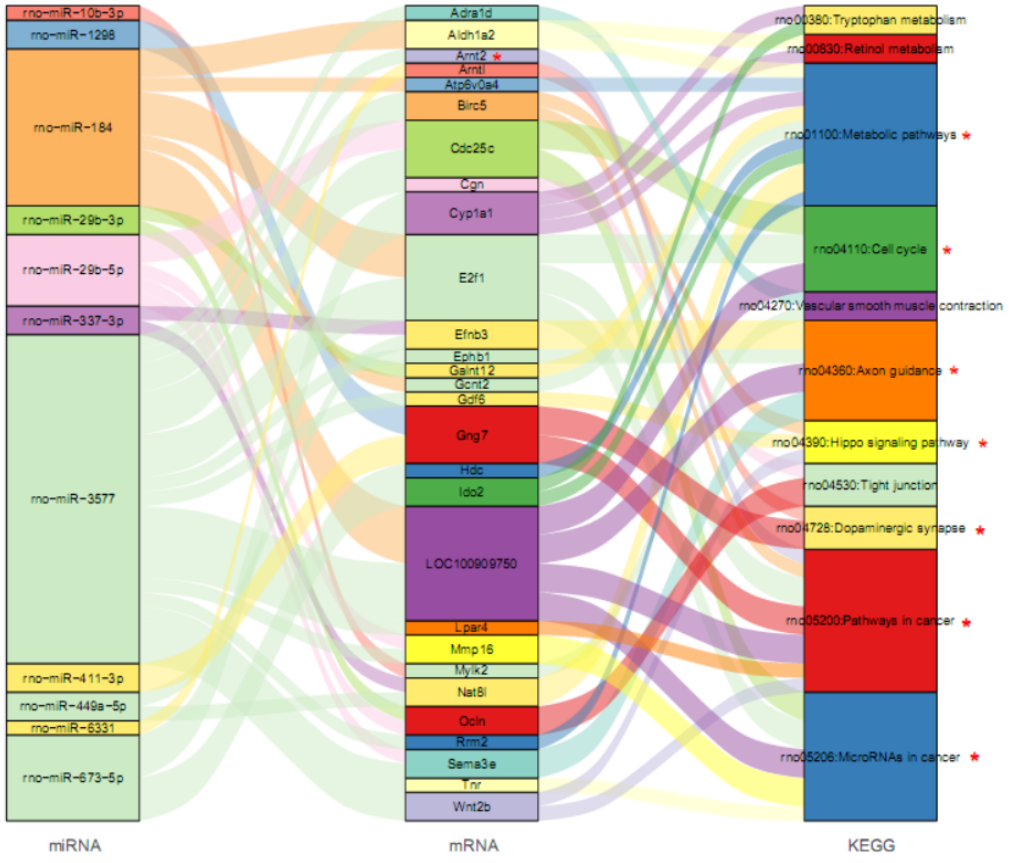

Figure 5. Sankey diagram of miRNA–mRNA–KEGG pathway. Regulatory relationships showing the co-occurrences of miRNAs, mRNAs, and KEGG pathways in the competitive endogenous RNA (ceRNA) regulatory network expressed by a Sankey diagram.

3.5. Verification of the crucial regulators in the related ceRNA regulatory network by qRT-PCR

The above crucial regulators in the ceRNA regulatory network were selected for verification based on qRT-PCR experiments. As shown in Figure 6, the relative levels of mRNAs, *Arnt2*, *BIRC5*, and *GDF6*, were all remarkably upregulated in the CIH group compared with the control group ($P < 0.05$), the relative level of miRNA, miR-3577, was notably downregulated in the CIH group ($P < 0.05$), and

the relative levels of lncRNAs, NONRATT011877.2 and NONRATT019720.2, were both significantly upregulated in the CIH group ($P < 0.05$). These results validated the consistency of the qRT-PCR experiments and RNA sequencing analyses.

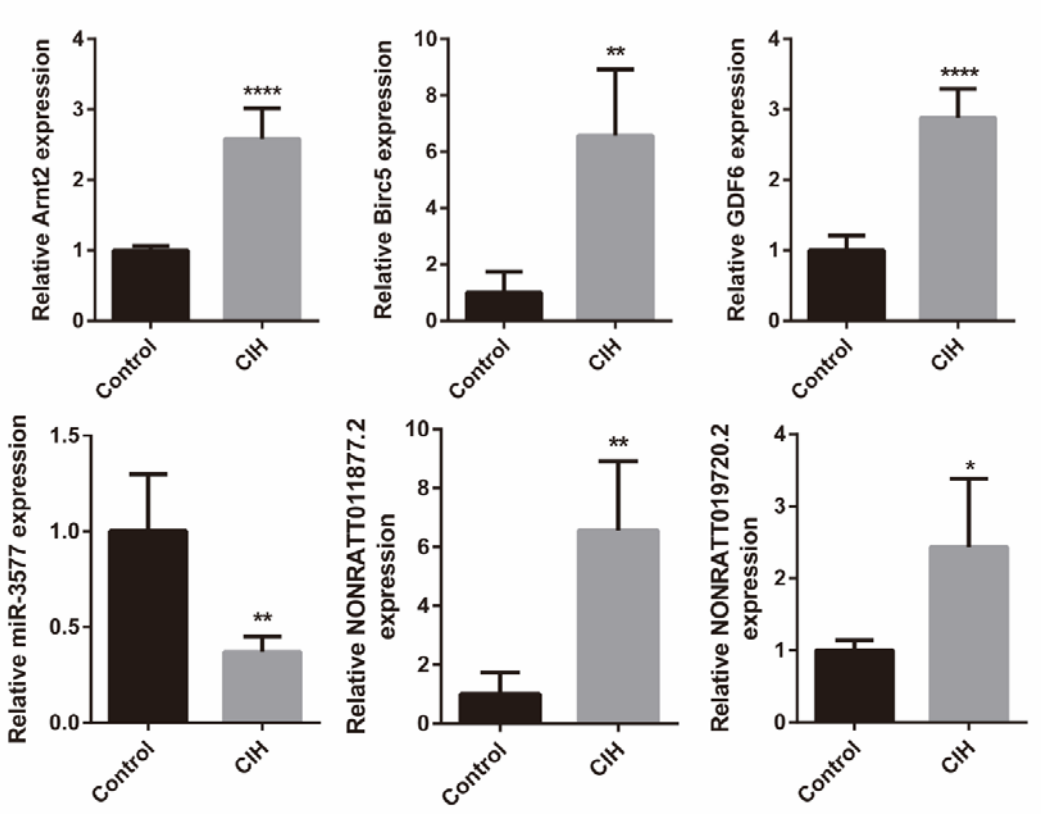

Figure 6. Verification of the crucial regulators in the related competitive endogenous RNA (ceRNA) regulatory network by qRT-PCR in experimental cardiac fibrosis in rats. CIH, chronic intermittent hypoxia; * indicates $P < 0.05$, ** indicates $P < 0.01$, **** indicates $P < 0.001$.

4. Discussion

Recent studies have shown that mRNAs and ncRNAs, including lncRNAs and miRNAs, play important roles in cardiac fibrosis [13]. Nevertheless, integrated whole-transcriptome analysis of cardiac fibrosis is still indispensable for the lncRNA–miRNA–mRNA-mediated ceRNA regulatory network [33].

In this study, we identified DERs, including 268 lncRNAs, 20 miRNAs, and 436 mRNAs, as well as the related functional pathways involved in cardiac fibrosis. Subsequently, a PPI network containing 306 nodes was constructed and the top 5 nodes were *Cdk1*, *Ccnb1*, *Cdca8*, *Aurkb*, and *Plk1*, all of which were upregulated in the myocardial tissues of cardiac fibrosis. A study in 2014 already proved that targeting *Cdk1* may inhibit fibrosis and subsequently confer protection against cardiac fibrosis [34]. Chen et al. found that *Cdk1* could promote atrial fibrosis by phosphorylating paxillin at Ser244 CDK1 and plays a key role in fibroblast differentiation [35]. However, other proteins of the top5 nodes have not yet been studied in cardiac fibrosis.

The results of the ceRNA regulatory network demonstrated specific lncRNA–miRNA–mRNA interactions in this study. For example, *BIRC5* could be regulated by rno–miR-3577 as well as its upstream lncRNAs, including NONRATT012985.2 and NONRATT000861.2; *Cyp1a1* could be regulated by rno–miR-673-5p and its upstream lncRNAs, including NONRATT006306.2 and TCONS_00006085; and *E2F1* could be regulated by rno–miR-184 and its upstream lncRNAs, including NONRATT008981.2 and NONRATT019720.2. For these crucial regulators in the present study, previous studies have revealed that *BIRC5* is expressed in cardiac progenitor cells (CPCs), and survivin (encoded by *BIRC5*) can directly induce CPCs proliferation and enhance cardiomyocyte survival. Transgenic *Cyp1a1* is closely associated with hypertension and cardiac fibrosis. Liao *et al.* found that *E2F1* may be a potential therapeutic target for cardiac fibrosis [36–39]. However, the underlying mechanisms have not been elucidated. The ceRNA relationships identified in this study may provide hints for further mechanistic investigations.

Meanwhile, the enriched functional pathways including "cell division" and "cell cycle" have also been reported by international studies as potential pathological factors of cardiac fibrosis [40–42]. For instance, the overlapping disease pathways in this study, including "pathways in cancer" and "Hippo signaling pathway," have also been previously reported to be related with cardiac regeneration and proliferation, arbitrating the cardiac fibroblast identity and activation [43–47]. Finally, the identified crucial regulators in the related ceRNA regulatory network, three mRNAs, *Arnt2*, *BIRC5*, and *GDF6*, one miRNA, miR-3577, and two lncRNAs—NONRATT011877.2 and NONRATT019720.2—were screened out, and their differential expression was successfully validated by the qRT-PCR experiment. Nevertheless, only *BIRC5* plays a role in the biological regulation of cardiac fibrosis. Therefore, this evidence could be regarded as the experimental foundation for these potential regulators of the ceRNA regulatory network of cardiac fibrosis.

However, there are still some limitations in this study, such as the lack of in vitro data, depletion of specific genes, and the need of further experiments for mechanism exploration and pathway validation. More importantly, how the ceRNA regulatory network impacts the advancement of knowledge in clinical patients subjected to cardiac fibrosis, how to prove medically the effectiveness of these crucial regulators and the functional pathways, and how to practice the significance of clinical transformation would need more investigations and efforts in future medical practice.

Compared with the results of a previous study, this work provides a comprehensive understanding of the ceRNA network-based regulation of cardiac fibrosis. In this study, different crucial regulators and functional pathways were identified as closely related to cardiac fibrosis in a newly established ceRNA regulatory network based on an experimental rat model. In conclusion, the present study mainly indicates the integration of whole-transcriptome data to construct the ceRNA network and identify the crucial regulators, including *Arnt2*, *BIRC5*, and *GDF6*, to construct networks such as NONRATT012985.2-rno–miR-3577–*Arnt2*, NONRATT000861.2-rno–miR-3577–*BIRC5*, and NONRATT019720.2-rno–miR-3577–*GDF6*, and trace the related functional pathways in cardiac fibrosis in rats, thus suggesting the potential underlying mechanisms of cardiac fibrosis.

Acknowledgments

This work was supported by the Science & Technology Development Fund of Tianjin Education Commission for Higher Education [No. 2020KJ168].

Conflict of interest

The authors declare that they have no conflicts of interest.

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