

# Construction of a potentially functional long noncoding RNA-microRNA-mRNA network in diabetic cardiomyopathy

Qiwen Cao<sup>1</sup>, Zhihui Dong<sup>2</sup>, Yangbo Xi<sup>2</sup>, Jiana Zhong<sup>1</sup>, Jianzhong Huang<sup>3</sup>, Qunfeng Yang<sup>1</sup>

<sup>1</sup>Department of Endocrinology, Bin Hai Wan Central Hospital of Dongguan, Dongguan, China, <sup>2</sup>Department of Cardiology, Bin Hai Wan Central Hospital of Dongguan, Dongguan, China, <sup>3</sup>Central Laboratory, Bin Hai Wan Central Hospital of Dongguan, Dongguan, China

**Background:** Diabetic cardiomyopathy (DCM) is a severe complication among patients with Type 2 diabetes, significantly increasing heart failure risk and mortality. Despite various implicated mechanisms, effective DCM treatments remain elusive. This study aimed to construct a comprehensive competing endogenous RNA (ceRNA) network in DCM using bioinformatics analysis. **Materials and Methods:** Three expression profiles datasets (GSE161827, GSE161931, and GSE241166) were collected from gene expression omnibus database and then integrated for the identification of differentially expressed genes (DEGs). Gene Ontology, Kyoto Encyclopedia of Gene and Genome pathway analysis, and Gene set enrichment analysis (GSEA) were employed for functional analysis. Protein-protein interaction (PPI) network and hub genes were also identified. The ceRNA regulatory networks were constructed based on interaction between long noncoding RNA (lncRNA) and DEGs, microRNA (miRNA) and DEGs, as predicted by public available databases. **Results:** A total of 105 DEGs, including 44 upregulated and 61 downregulated genes were identified to be associated with DCM. Functional enrichment analysis showed that fatty acid metabolism pathway and inflammatory responses were significantly enriched in DCM. A total of 56 interactions between miRNA with DEGs, and 27 interactions between lncRNA with miRNA was predicted. Besides, a ceRNA network includes 9 mRNA, 17 miRNA and 10 lncRNA was constructed, among which *Cdh20* and *Cacna2d2* were hub genes in PPI network. **Conclusion:** The identified hub genes and ceRNA network components provide valuable insights into DCM biology and offer potential diagnostic biomarkers and therapeutic targets for further investigation. Further experimental validation and clinical studies are warranted to translate these findings into clinical applications.

**Key words:** Competing endogenous RNA, diabetic cardiomyopathy, long noncoding RNA, microRNA, mRNA

**How to cite this article:** Cao Q, Dong Z, Xi Y, Zhong J, Huang J, Yang Q. Construction of a potentially functional long noncoding RNA-microRNA-mRNA network in diabetic cardiomyopathy. J Res Med Sci 2024;29:74.

## INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by impaired glycemic control, estimated to affect 600 million individuals worldwide by 2040.<sup>[1]</sup> Diabetic cardiomyopathy (DCM) has emerged as a well-recognized complication of diabetes, significantly increasing the risk of hospitalization for heart failure (HF) and mortality among patients with Type 2 DM.<sup>[2]</sup> DCM is characterized by a progressive loss of cardiac cells through apoptosis and necrosis, as well as myocardial

fibrosis.<sup>[3]</sup> It leads to both systolic and diastolic dysfunction and eventually clinical HF, independently of coronary artery disease, hypertension, or valvular heart diseases.<sup>[4]</sup> Recent evidence has indicated that insulin resistance, oxidative stress, mitochondrial dysfunction, maladaptive immune responses, and impaired calcium homeostasis are all implicated in the onset of DCM.<sup>[2,5]</sup> Alterations in gene regulation, including the activation of transcription factors, microRNAs (miRNAs), and epigenetic mechanisms, have also been linked to the pathogenesis of DCM.<sup>[6,7]</sup> However, despite elucidation of various underlying pathogenic mechanisms of DCM,

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

**For reprints contact:** WKHLRPMedknow\_reprints@wolterskluwer.com

### Access this article online

Quick Response Code:



Website:

<https://journals.lww.com/jrms>

DOI:

10.4103/jrms.jrms\_205\_24

**Address for correspondence:** Dr. Qunfeng Yang, Department of Endocrinology, Bin Hai Wan Central Hospital of Dongguan, No. 111 Humen Avenue, Humen Town, Dongguan, China.

E-mail: 13925533020@163.com

**Submitted:** 18-Apr-2024; **Revised:** 02-Aug-2024; **Accepted:** 20-Sep-2024; **Published:** 31-Dec-2024

no effective treatment targets have yet been identified to prevent its progression. Thus, there is an urgent need for further exploration of potential mechanisms and identification of therapeutic targets for DCM.

The concept of competing endogenous RNA (ceRNA) was initially proposed by Salmena *et al.* in 2011.<sup>[8]</sup> This hypothesis suggests that both messenger RNA (mRNA) and noncoding RNAs, which contain miRNAs, long noncoding RNAs (lncRNAs), and circular RNAs, can engage in posttranscriptional regulation of protein expression by competitively binding miRNA response elements, thereby forming a complex regulatory network.<sup>[9]</sup> Disruption of the expression of RNA-like genes can perturb the balance of this regulatory network, potentially contributing to the development of various diseases.<sup>[10]</sup> Emerging evidence suggests a close association between ceRNA regulatory networks and DCM.<sup>[11–14]</sup> For instance, the lncRNA NORAD has been demonstrated to enhance cardiac function, mitigate fibrosis, and attenuate inflammatory responses in DCM mice through the ceRNA network involving NORAD/miR-125a-3p/Fyn.<sup>[12]</sup> The identification of interactions within the ceRNA network helps uncover disease mechanisms, thereby aiding in the understanding of pathogenesis. However, the ceRNA regulatory network in DCM remains poorly understood, warranting further investigation.

In this work, we utilized bioinformatics approaches to detect differentially expressed genes (DEGs) in the DCM datasets. The key biological functions of DEGs were further investigated by Gene Ontology (GO) enrichment, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, and Gene set enrichment analysis (GSEA) on DEGs. Finally, a lncRNA-miRNA-mRNA network regulating DCM was constructed based on the ceRNA theory to screen the functional lncRNAs.

## MATERIALS AND METHODS

### Data sources and preprocessing

We searched the gene expression omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo/>) for RNA-sequence data on mice heart samples focusing on DCM. Datasets with only blood samples or cell lines of DCM were excluded in this study. Three expression profiles by array (GSE161931, GSE161827, and GSE241166) of DCM were collected from the GEO database.<sup>[15]</sup> The datasets from GSE161931<sup>[16]</sup> and GSE241166 were based on the GPL570 platform (Illumina NovaSeq 6000), while GSE161827<sup>[17]</sup> was based on the GPL19057 platform (Illumina NextSeq 500). GSE161931 dataset included 5 Db/db mice and 5 C57BLKS/J mice as control. GSE241166 dataset contained 3 DCM mice constructed by high fat diet feeding and streptozotocin injection, and 3 control mice with normal diet

feeding and only citrate acid buffer injection. GSE161827 dataset covered 4 mice with high fat and high sucrose to induce DCM, as well as 4 mice fed with control diet. The platform and series matrix files of above three datasets were downloaded for analysis.

Three datasets were combined and batch effect removal was performed using SVA R package to correct for technical batch effects. Principle component analysis was performed before and after batch-effect removal.

### Differential expression analysis

Background correction and normalization of the raw data were conducted using the R/Bioconductor affy tool. Subsequently, differential expression analysis was carried out employing the “DESeq2” R package, with adjustments made using the false discovery rate (FDR) to correct the *P* values. Genes exhibiting  $|\text{FoldChange}| > 1$  and an FDR-adjusted *P* < 0.05 were identified as DEGs. Visualization of the DEGs was performed using the “ggplot2” R package to generate volcano plots. In addition, the “pheatmap” R package was utilized for visualizing the DEGs, resulting in the generation of heatmaps.

### Functional enrichment analyses of the differentially expressed genes

GO enrichment including biological process, cellular component, molecular function (MF) and KEGG enrichment analyses of DEGs were performed using clusterProfiler R package.<sup>[18]</sup> FDR-adjusted *P* < 0.05 was considered statistically significant differences.

### Gene set enrichment analysis

GSEA was used to determine the key pathways and core genes during the development of DCM based on HALLMARK database (<https://www.gsea-msigdb.org/gsea/msigdb/>).<sup>[19]</sup> The default-weighted enrichment method was applied for enrichment analysis. The random combination was set for 1000 times. FDR-adjusted *P* < 0.05 and  $|\text{NES}| > 1$  were considered significant enrichment. GSEA package was applied to visualize the results, which presented the activated pathways in DCM compared with the normal.

### Construction of the protein-protein interaction network

The protein-protein interaction (PPI) network was built using the online tool STRING (<https://string-db.org/>).<sup>[20]</sup> Next, we used Cytoscape software to download the interaction data and improve the PPI network to find the important modules and hub genes. The top 20 hub genes were predicted using the cytoHubba plug-in.<sup>[21]</sup>

### Identification of upstream microRNA

Four database including miRanda (<http://www.microrna.org/microrna/home.do>),<sup>[22,23]</sup> miRDB (<https://mirdb.org/>)

org/),<sup>[24,25]</sup> PicTar (<https://pictar.mdc-berlin.de/>),<sup>[23]</sup> and TargetScan (<https://www.targetscan.org/>),<sup>[23,25]</sup> were used to predict upstream miRNA that can bind to DEGs. If a miRNA is predicted by all the four databases, it is considered the upstream miRNA of the DEG.

### Identification of upstream long noncoding RNA

ENCORI (<https://rnasysu.com/encori/>)<sup>[26]</sup> and RNAhybrid (<https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid/>)<sup>[27]</sup> were used to predict lncRNAs that binds to the predicted miRNA. If a lncRNA is predicted by both databases, it is considered the upstream lncRNA of the miRNA.

### Construction of the mRNA-microRNA-long noncoding RNA regulatory network

The DEGs, predicted mRNA-miRNA and predicted miRNA-lncRNA interaction were used to construct a regulatory network. Cytoscape was used to visualize mRNA-miRNA-lncRNA regulatory network to get ceRNA network.

## RESULTS

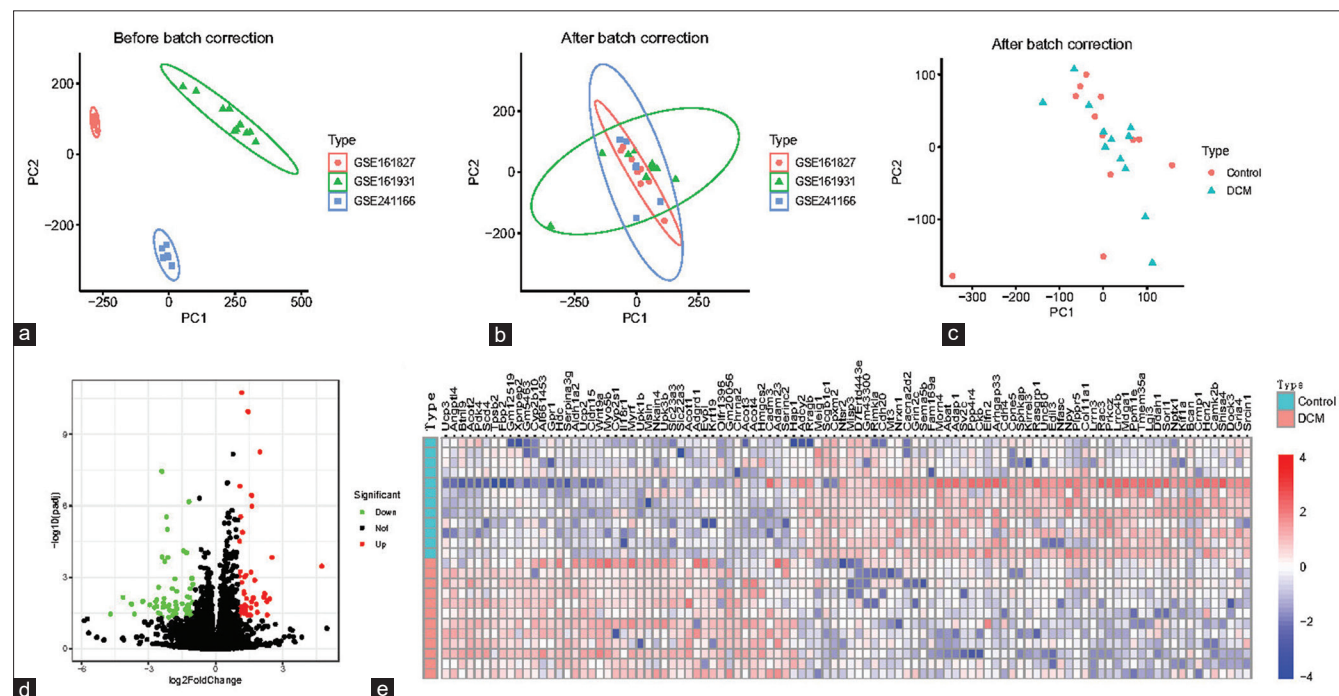
### Identification of significant differentially expressed genes in diabetic cardiomyopathy from gene expression omnibus database

To explore the potential roles of molecular associated with the development of DCM, we first identified 3 expression

profiles (GSE161827, GSE161931, and GSE241166) downloaded from GEO databases [Figure 1a]. After batch normalization, the batch effects of these 3 data series were removed [Figure 1b]. We analyzed DEGs between 12 DCM and 12 healthy controls [Figure 1c]. According to the predefined cutoff criteria (FDR-adjusted  $P < 0.05$  and  $|\log_2FC| > 1$ ), as shown in the volcano plot and the heatmap, we found 44 up-regulated genes and 61 down-regulated genes [Figure 1d and e] in DCM compared to healthy controls.

### Functional enrichment analysis of differentially expressed genes

In order to analyze the potential molecular mechanisms of these genes, we conducted the GO function enrichment analysis for the DEGs. The three distinct categories of GO function enrichment analysis are shown in Figure 2. The results of GO enrichment analysis showed that the DEGs were mainly enriched in cell junction assembly, postsynapse organization, regulation of synapse organization, and regulation of synapse structure or activity. The DEGs mainly focused on the actin cytoskeleton, transmembrane transporter complex, transporter complex, and postsynaptic density. The MF of DEGs involved acyl-CoA hydrolase activity, CoA hydrolase activity, thiolester hydrolase activity and PDZ domain binding [Figure 2a]. KEGG enrichment analysis revealed that these DEGs could participate in fatty acids metabolism pathway and cAMP signaling pathway [Figure 2b].



**Figure 1:** Differentially expressed genes identified in the three datasets GSE161827, GSE161931 and GSE241166. (a and b) Principal component analysis before and after batch correction. (c) Principal component analysis after batch correction grouped by diabetic cardiomyopathy (DCM) and control. (d) Volcanomaps of differentially expressed genes. Black is a gene with no difference, red is an up-regulated gene, and green is a down-regulated gene. (e) Heatmap of differentially expressed genes. The ordinate is the samples grouped by DCM and control, the abscissa is the differentially expressed genes, red is high expression, and blue is low expression. DCM: Diabetic cardiomyopathy

GSEA analysis was performed to explore potential molecular mechanisms suggested by hallmark pathways. As shown in Figure 2c, fatty acid metabolism pathway and inflammatory responses, including interleukin 6-JAK-STAT signaling pathway and interferon alpha response were upregulated in DCM.

### Protein-protein interaction network construction and identification of hub genes

Using the STRING online database and Cytoscape software, the DEGs were mapped into the PPI network complex [Figure 3a]. In the network, nodes with the top 20° were chosen as hub genes based on the radiality [Figure 3b and Table 1]. The color depth of the nodes indicated the scores calculated by plug-in cytoHubba. A darker color means a higher score of the hub gene.

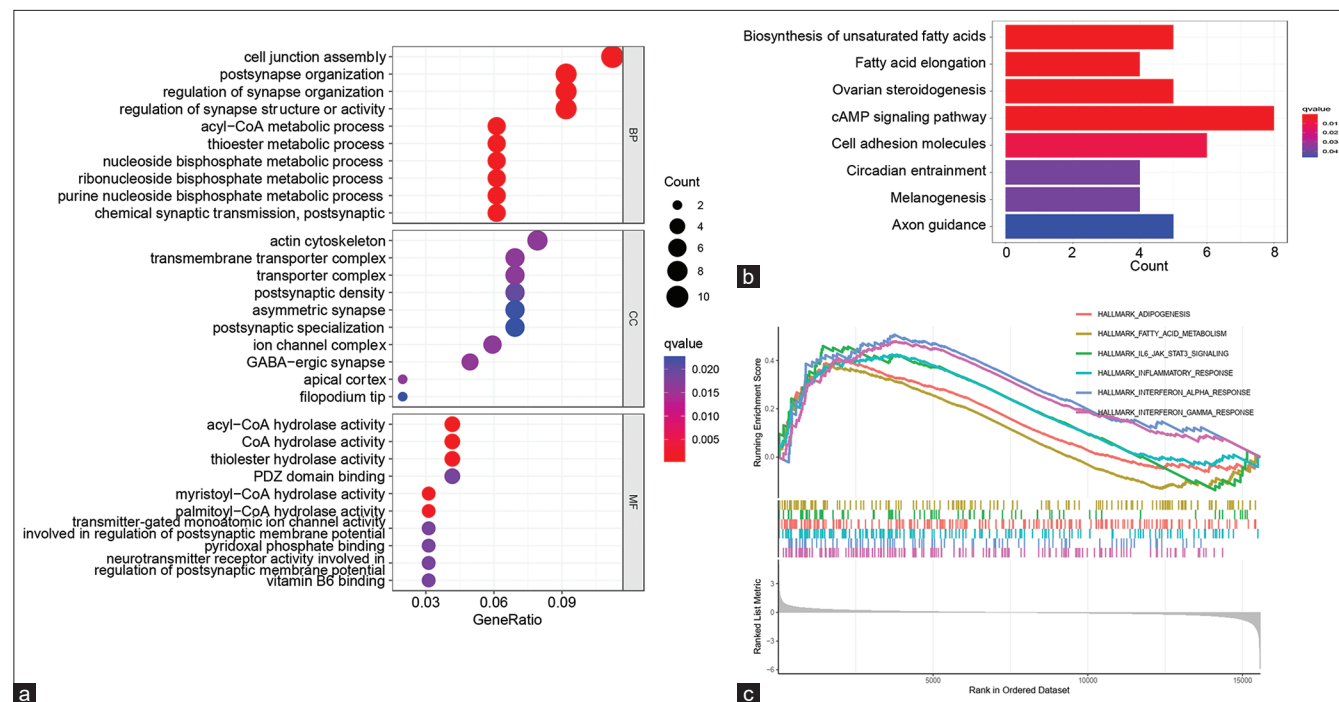
### Construction of the long noncoding RNA-microRNA-mRNA network

To explore the upstream miRNAs of DEGs, potential miRNAs binding to the DEGs were predicted using the miRanda, miRDB, PicTar, and TargetScan databases. A total of 49 miRNAs were identified for DEGs, with 56 miRNA-DEGs interactions predicted [Table 2]. On the other hand, potential binding lncRNAs to these miRNAs were predicted using the ENCORI and RNAhybrid databases, reporting 27 lncRNA-miRNA interactions [Table 3]. The lncRNA-miRNA-mRNA network is displayed in Figure 4. This network includes 9 mRNA, 17 miRNA, and 10

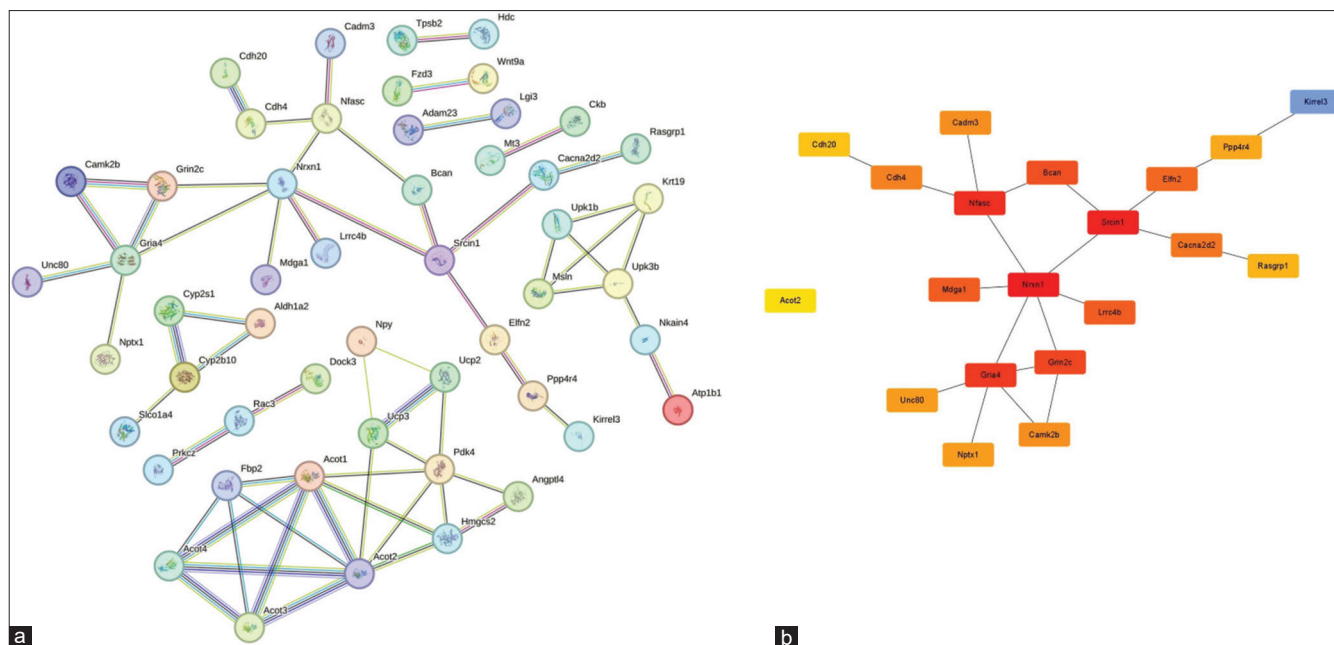
lncRNA. *Cdh20* and *Cacna2d2* were identified as hub genes in the PPI network, indicating the important roles of the *Malat1-Cdh20* and *Mir181a-1 hg-Cacna2d2* interactions in DCM pathogenesis.

**Table 1: Top 20 differentially expressed genes in network ranked by connectivity degree with Cytoscape cytoHubba plug-in software using radiality method**

Rank	Name	Score
1	<i>Nrxn1</i>	2.42
2	<i>Srcin1</i>	2.32
3	<i>Nfasc</i>	2.24
4	<i>Gria4</i>	2.21
5	<i>Grin2c</i>	2.17
6	<i>Bcan</i>	2.13
7	<i>Lrrc4b</i>	2.07
8	<i>Mdga1</i>	2.07
9	<i>Elnf2</i>	2.05
10	<i>Cacna2d2</i>	2.01
11	<i>Cdh4</i>	1.92
12	<i>Cadm3</i>	1.88
13	<i>Camk2b</i>	1.88
14	<i>Nptx1</i>	1.86
15	<i>Unc80</i>	1.86
16	<i>Ppp4r4</i>	1.74
17	<i>Rasgrp1</i>	1.66
18	<i>Cdh20</i>	1.57
19	<i>Kirrel3</i>	1.39
20	<i>Acot2</i>	0.67



**Figure 2:** Enrichment analysis of differentially expressed genes. (a) Gene ontology (GO) enrichment. The abscissa represents the ratio of the genes annotated to the entry to the total number of genes annotated, and the ordinate is the result of GO enrichment. (b) Kyoto Encyclopedia of Gene and Genome (KEGG) pathway enrichment, the abscissa is the number of enriched differential genes, and the ordinate is the result of KEGG enrichment. (c) Pathway-based gene set enrichment analysis (GSEA) of mRNAs. The abscissa is the rank of genes in the ordered datasets, and the ordinate is the enrichment score



**Figure 3:** The protein-protein interaction (PPI) networks that were obtained by using String tool. (a) PPI network to show the interaction between differentially expressed genes. Circles represent genes, and lines represent protein interactions between the genes. The symbols inside the circles represent the protein structures. The color of the lines indicates the different types of evidence supporting the protein interactions. Green line: Neighborhood evidence; pink line: Experimental evidence; yellow line: Text mining evidence; blue line: Co-occurrence evidence; black line: Co-expression evidence. (b) The interaction between top 20 hub genes. The nodes color changes gradually from yellow to red in ascending order according to the Degree ranking

## DISCUSSION

In the current study, our objective was to conduct an extensive bioinformatics analysis to elucidate the regulatory network involving mRNAs, miRNAs, and lncRNAs in the pathogenesis of DCM. By harmonizing data from three RNA-seq experiments conducted on heart tissue samples obtained from DCM mouse model, we identified 20 hub genes among the 105 DEGs associated with DCM. Furthermore, employing the “mRNA-miRNA-lncRNA” framework, our investigation revealed a ceRNA regulatory network consisting of 9 mRNAs, 17 miRNAs, and 10 lncRNAs.

Currently, there are no established effective treatments for the long-term management of DCM.<sup>[28]</sup> CeRNA regulation may offer a novel perspective for better understanding the pathogenesis of DCM, providing a new theoretical basis for its diagnosis and treatment. However, most previous studies have focused on individual ceRNA interaction rather than taking a comprehensive approach to explore the entire ceRNA networks and its associated modules. In this study, we systematically screened differential mRNAs and predicted lncRNA-miRNA, miRNA-mRNA interaction based on several databases, then constructed a ceRNA network. The results of this study are based on bioinformatic predictions and require further experimental and clinical validation. Specifically, our research identifies novel targets for the development of noninvasive diagnostic methods for

DCM. It is essential to conduct diagnostic efficacy analyses on the identified targets in cohorts of DCM patients to improve early detection of the disease. Moreover, functional validation of predicted pathways, such as *Malat1-Cdh20* and *Mir181a-1 hg-Cacna2d2* associated with hub genes, will be a key focus of our future research. For example, the loss/gain-of-function study, both *in vivo* and *in vitro*, should be performed to explore the effect of the identified targets on DCM. This effort aims to provide new strategies for the development of DCM treatment approaches.

Two novel hub genes for DCM, *Cdh20* and *Cacna2d2*, were identified using Cytoscape software. Cui *et al.* identified *Cacna2d2* as a novel diagnostic biomarker for DCM. The mRNA expression of *Cacna2d2* in heart tissue and peripheral blood mononuclear cell showed promising diagnostic performance for DCM, with area under the curve values of 0.68 and 0.87, respectively.<sup>[29]</sup> *Cacna2d2* encodes a subunit of the voltage-dependent calcium channel complex, which plays a crucial role in complex assembly, membrane localization, and regulation of calcium current and channel dynamics.<sup>[29]</sup> Another study reported that cardiac *Prdm16*-specific knockout induced the dysregulated expression of ion channel genes, including *Kcne1*, *Scn5a*, *Cacna1 h*, and *Cacna2d2*, ultimately leading to cardiomyopathy.<sup>[30]</sup> However, whether *Cacna2d2* is involved in the pathogenesis of DCM remains unclear, as there are currently no studies elucidating the specific mechanisms.

**Table 2: Identification of upstream microRNA of differentially expressed genes in diabetic cardiomyopathy with miRanda, miRDB, PicTar and TargetScan**

mRNA	miRNA
<i>Adam23</i>	<i>mmu-miR-25-3p, mmu-miR-367-3p, mmu-miR-92a-3p, mmu-miR-363-3p, mmu-miR-153-3p, mmu-miR-32-5p, mmu-miR-92b-3p</i>
<i>Aldh1a2</i>	<i>mmu-miR-137-3p</i>
<i>Angptl4</i>	<i>mmu-miR-672-5p</i>
<i>Arhgap33</i>	<i>mmu-miR-3072-5p</i>
<i>Atp1b1</i>	<i>mmu-let-7c-1-3p, mmu-miR-467h, mmu-miR-223-3p, mmu-miR-217-5p, mmu-miR-323-3p, mmu-miR-1953</i>
<i>Cacna2d2</i>	<i>mmu-miR-466k</i>
<i>Cdh20</i>	<i>mmu-miR-96-5p, mmu-miR-200c-3p, mmu-miR-139-5p, mmu-miR-429-3p, mmu-miR-200b-3p, mmu-miR-18a-3p</i>
<i>Col11a1</i>	<i>mmu-miR-29c-3p, mmu-miR-29b-3p</i>
<i>Ddah1</i>	<i>mmu-miR-219a-5p</i>
<i>Elfn2</i>	<i>mmu-miR-323-5p</i>
<i>Fam169a</i>	<i>mmu-miR-708-5p</i>
<i>Mdga1</i>	<i>mmu-miR-218-5p</i>
<i>Morn4</i>	<i>mmu-miR-139-5p</i>
<i>Nptx1</i>	<i>mmu-miR-148a-3p, mmu-miR-182-5p, mmu-miR-152-3p, mmu-miR-148b-3p, mmu-miR-881-3p</i>
<i>Nrxn1</i>	<i>mmu-miR-669a-5p, mmu-miR-669l-5p</i>
<i>Pdk4</i>	<i>mmu-miR-148a-3p, mmu-miR-152-3p, mmu-miR-148b-3p</i>
<i>Ppp4r4</i>	<i>mmu-miR-154-3p</i>
<i>Rragb</i>	<i>mmu-miR-361-5p, mmu-miR-883a-5p</i>
<i>Ucp3</i>	<i>mmu-miR-148a-3p, mmu-miR-152-3p, mmu-miR-148b-3p</i>
<i>Wnt9a</i>	<i>mmu-let-7f-5p, mmu-miR-140-5p, mmu-let-7b-5p, mmu-let-7c-5p, mmu-miR-98-5p, mmu-let-7e-5p, mmu-let-7i-5p, mmu-let-7d-5p, mmu-let-7a-5p, mmu-let-7g-5p</i>

miRNA=MicroRNA; mRNA=Messenger RNA

**Table 3: Identification of upstream long noncoding RNA of microRNA with ENCORI and RNAhybrid**

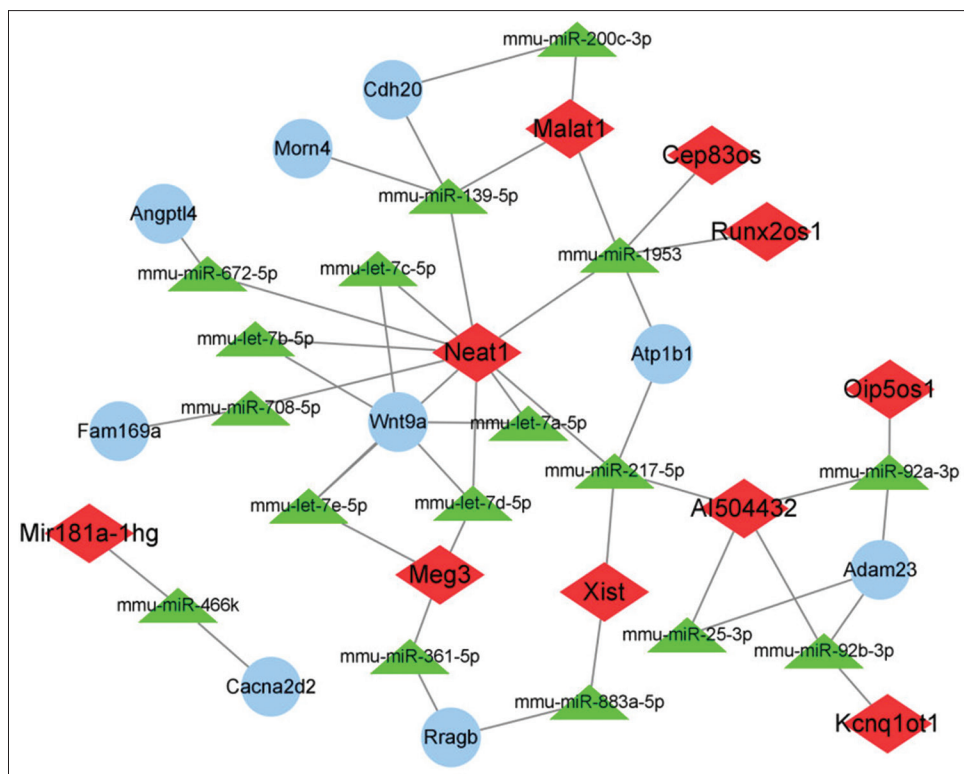
lncRNA	miRNA
<i>AI504432</i>	<i>mmu-miR-25-3p, mmu-miR-92a-3p, mmu-miR-92b-3p, mmu-miR-217-5p</i>
<i>Cep83os</i>	<i>mmu-miR-1953</i>
<i>Kcnq1ot1</i>	<i>mmu-miR-92b-3p</i>
<i>Malat1</i>	<i>mmu-miR-139-5p, mmu-miR-1953, mmu-miR-200c-3p</i>
<i>Meg3</i>	<i>mmu-let-7d-5p, mmu-let-7e-5p, mmu-miR-361-5p</i>
<i>Mir181a-1hg</i>	<i>mmu-miR-466k</i>
<i>Neat1</i>	<i>mmu-let-7a-5p, mmu-let-7b-5p, mmu-let-7c-5p, mmu-let-7d-5p, mmu-let-7e-5p, mmu-miR-139-5p, mmu-miR-1953, mmu-miR-217-5p, mmu-miR-672-5p, mmu-miR-708-5p</i>
<i>Oip5os1</i>	<i>mmu-miR-92a-3p</i>
<i>Runx2os1</i>	<i>mmu-miR-1953</i>
<i>Xist</i>	<i>mmu-miR-217-5p, mmu-miR-883a-5p</i>

lncRNA=Long noncoding RNA; miRNA=MicroRNA

*Cadherins* are a crucial family of cell adhesion molecules essential for organ development and maintaining tissue integrity, comprising more than 20 subfamilies.<sup>[31]</sup> Among them, *Cdh11* has been identified as a key promoter of cardiac

fibrosis.<sup>[32]</sup> Specifically, the engagement of *Cdh11* promotes the production of pro-inflammatory and pro-fibrotic mediators, which leads to increased extracellular matrix deposition and fibrosis. However, the role of *Cadherin-20* in DCM is less well-studied. Li *et al.* found that *Cdh20/β-catenin* suppressed transforming growth factor-β (TGF-β)-induced epithelial-to-mesenchymal transition.<sup>[33]</sup> Activation of the TGF-β-related signaling pathway is a key mechanism in the pathogenesis of DCM.<sup>[34]</sup> Our data indicate downregulation of *Cdh20* in DCM hearts. Whether the downregulation of *Cdh20* reduces inhibition of TGF-β, thereby promoting the occurrence of DCM, requires further experimental validation.

As previously discussed, miRNAs and lncRNAs play pivotal roles in gene expression regulation through the mechanism known as ceRNA. In the current study, a ceRNA network was established by predicting interactions between DE miRNAs and both lncRNAs and mRNAs. Within this network, 10 lncRNAs were identified, among which 5 have been shown to be associated with the onset of DCM. Notably, studies have demonstrated that inhibition of lncRNA-MALAT1 mitigates DCM by attenuating cardiomyocyte apoptosis and cardiac fibrosis.<sup>[35-37]</sup> Wang *et al.* reported that lncRNA-MALAT1 sequesters *miR-185-5p*, thereby mediating oxidative stress and mitochondrial injury in diabetic cardiomyocytes.<sup>[36]</sup> Chen *et al.* showed that lncRNA-MEG3 acts as a ceRNA to promote cardiomyocyte apoptosis under conditions of high glucose stimulation.<sup>[38]</sup> In addition, under DCM conditions, lncRNA-KCNQ1OT1 modulates *miR-181a-5p* to upregulate PDCD4, thereby inducing cardiomyocyte apoptosis, while silencing lncRNA-KCNQ1OT1 mitigated pyroptosis and fibrosis.<sup>[39,40]</sup> Furthermore, upregulation of lncRNA-NEAT1 in DCM enhances its onset by recruiting EZH2 to the Smad7 promoter region.<sup>[41]</sup> Chen *et al.* identified *miR-424-5p* and *miR-497-5p* as the primary miRNAs regulated by lncRNA-XIST in the ceRNA network of DCM.<sup>[11]</sup> In summary, the roles of these 5 lncRNAs in DCM encompass the modulation of cardiomyocyte apoptosis, oxidative stress, and cardiac fibrosis, aligning closely with the principal pathogenic mechanisms of DCM. These prior observations lend support to the validity of our present analytical findings. However, the specific lncRNA-miRNA-mRNA axis of each of the 5 lncRNAs, as predicted by our study, has not yet been reported and remains to be validated in future. For the remaining 5 lncRNAs in the ceRNA network, *Oip5os1* was related with left atrial dysfunction but not with ventricular function in diabetes mice.<sup>[42]</sup> lncRNA AI504432 was upregulated in the aging eWAT and modulated lipogenesis through the miR-1a-3p/Fasn signaling pathway.<sup>[43]</sup> Tye *et al.* reported that lncRNA MIR181A1HG is a novel epigenetic regulator in the early stages of mesenchymal stromal cell differentiation.<sup>[44]</sup>



**Figure 4:** The potential mRNA-microRNA (miRNA)-long noncoding RNA (lncRNA) regulatory network correlated with diabetic cardiomyopathy. The red rhombus represents lncRNAs, the blue circle represents differentially expressed genes, and the green triangle represents miRNAs. A line between two nodes indicates a potential regulatory relationship between them

Currently, there is a lack of research literature on CEP83OS and RUNX2OS1.

However, our study has some limitations that should be acknowledged. The ceRNA network was predicted based on differential gene expression without functional validation. Although our study systematically screened potential ceRNA networks, subsequent in-depth *in vitro* and *in vivo* functional studies are warranted to verify their roles. Each tool used for identifying upstream miRNAs has its own limitations. miRanda may fail to identify interactions with low complementarity or in complex contexts, miRDB might miss novel interactions due to limitations in its data, PicTar may overlook species-specific targets due to its conservation-based approach, and TargetScan may not capture nonconserved interactions because of its focus on conserved motifs.<sup>[45-47]</sup> For upstream lncRNA prediction, ENCORI could be limited by the quality and breadth of its curated data, which may affect the accuracy of lncRNA-miRNA binding predictions, while RNAhybrid might not detect interactions with noncanonical binding patterns or in complex scenarios due to its focus on thermodynamic stability.<sup>[27,48]</sup> To ensure prediction accuracy, only miRNAs predicted by all four tools and lncRNAs predicted by both tools were used for analysis. In addition, our research was conducted using a mouse model of DCM, which remains to be validated in human studies.

## CONCLUSION

In current research, we identified 20 hub genes related to DCM pathogenesis and elucidated potential pathways contributing to DCM. Besides, by utilizing integrated bioinformatics analysis, a novel mRNA-miRNA-lncRNA regulating network associated with DCM was constructed, particularly focusing on the *Malat1-Cdh20* and *Mir181a-1hg-Cacna2d2* networks. Our study offers valuable insights and evidence into the development of diagnostic biomarkers and treatment strategies for DCM.

## Financial support and sponsorship

This work was supported by the Dongguan Science and Technology of Social Development Program (20221800901152, 20231800935492), and Guangdong Basic and Applied Basic Research Foundation (21202107190000195).

## Conflicts of interest

There are no conflicts of interest.

## REFERENCES

1. Ng AC, Delgado V, Borlaug BA, Bax JJ. Diabetes: The combined burden of obesity and diabetes on heart disease and the role of imaging. *Nat Rev Cardiol* 2021;18:291-304.

2. Murtaza G, Virk HU, Khalid M, Lavie CJ, Ventura H, Mukherjee D, et al. Diabetic cardiomyopathy – A comprehensive updated review. *Prog Cardiovasc Dis* 2019;62:315-26.
3. Katare R, Oikawa A, Cesselli D, Beltrami AP, Avolio E, Muthukrishnan D, et al. Boosting the pentose phosphate pathway restores cardiac progenitor cell availability in diabetes. *Cardiovasc Res* 2013;97:55-65.
4. Jia G, Whaley-Connell A, Sowers JR. Diabetic cardiomyopathy: A hyperglycaemia- and insulin-resistance-induced heart disease. *Diabetologia* 2018;61:21-8.
5. Jia G, DeMarco VG, Sowers JR. Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy. *Nat Rev Endocrinol* 2016;12:144-53.
6. Grubić Rotkvić P, Planinić Z, Liberati Pršo AM, Šikić J, Galić E, Rotkvić L. The mystery of diabetic cardiomyopathy: From early concepts and underlying mechanisms to novel therapeutic possibilities. *Int J Mol Sci* 2021;22:5973.
7. Barwari T, Joshi A, Mayr M. MicroRNAs in cardiovascular disease. *J Am Coll Cardiol* 2016;68:2577-84.
8. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: The Rosetta stone of a hidden RNA language? *Cell* 2011;146:353-8.
9. Ala U. Competing endogenous RNAs, Non-coding RNAs and diseases: An intertwined story. *Cells* 2020;9:1574.
10. Karreth FA, Pandolfi PP. ceRNA cross-talk in cancer: When ce-bling rivalries go awry. *Cancer Discov* 2013;3:1113-21.
11. Chen K, Ma Y, Wu S, Zhuang Y, Liu X, Lv L, et al. Construction and analysis of a lncRNA-miRNA-mRNA network based on competitive endogenous RNA reveals functional lncRNAs in diabetic cardiomyopathy. *Mol Med Rep* 2019;20:1393-403.
12. Liu Y, Zhu Y, Liu S, Liu J, Li X. NORAD lentivirus shRNA mitigates fibrosis and inflammatory responses in diabetic cardiomyopathy via the ceRNA network of NORAD/miR-125a-3p/Fyn. *Inflamm Res* 2021;70:1113-27.
13. Hou J, Liang WY, Xiong S, Long P, Yue T, Wen X, et al. Identification of hub genes and potential ceRNA networks of diabetic cardiomyopathy. *Sci Rep* 2023;13:10258.
14. Pezhouman A, Nguyen NB, Kay M, Kanjilal B, Noshadi I, Ardehali R. Cardiac regeneration – Past advancements, current challenges, and future directions. *J Mol Cell Cardiol* 2023;182:75-85.
15. Clough E, Barrett T. The gene expression omnibus database. *Methods Mol Biol* 2016;1418:93-110.
16. Huang X, Zhang KJ, Jiang JJ, Jiang SY, Lin JB, Lou YJ. Identification of crucial genes and key functions in type 2 diabetic hearts by bioinformatic analysis. *Front Endocrinol (Lausanne)* 2022;13:801260.
17. Croteau D, Luptak I, Chambers JM, Hobai I, Panagia M, Pimentel DR, et al. Effects of sodium-glucose linked transporter 2 inhibition with ertugliflozin on mitochondrial function, energetics, and metabolic gene expression in the presence and absence of diabetes mellitus in mice. *J Am Heart Assoc* 2021;10:e019995.
18. Yu G, Wang LG, Han Y, He QY. ClusterProfiler: An R package for comparing biological themes among gene clusters. *OMICS* 2012;16:284-7.
19. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* 2005;102:15545-50.
20. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res* 2019;47:D607-13.
21. Su W, Zhao Y, Wei Y, Zhang X, Ji J, Yang S. Exploring the pathogenesis of psoriasis complicated with atherosclerosis via microarray data analysis. *Front Immunol* 2021;12:667690.
22. Zhu B, Gao J, Zhang Y, Liao B, Zhu S, Li C, et al. CircRNA/miRNA/mRNA axis participates in the progression of partial bladder outlet obstruction. *BMC Urol* 2022;22:191.
23. Ying H, Lyu J, Ying T, Li J, Jin S, Shao J, et al. Risk miRNA screening of ovarian cancer based on miRNA functional synergistic network. *J Ovarian Res* 2014;7:9.
24. Chen Y, Wang X. miRDB: An online database for prediction of functional microRNA targets. *Nucleic Acids Res* 2020;48:D127-31.
25. Wei C, Wang B, Peng D, Zhang X, Li Z, Luo L, et al. Pan-cancer analysis shows that ALKBH5 is a potential prognostic and immunotherapeutic biomarker for multiple cancer types including gliomas. *Front Immunol* 2022;13:849592.
26. Balasundaram A, GPD C. *In silico* analysis revealed the potential circRNA-miRNA-mRNA regulative network of non-small cell lung cancer (NSCLC). *Comput Biol Med* 2023;152:106315.
27. Krüger J, Rehmsmeier M. RNAhybrid: MicroRNA target prediction easy, fast and flexible. *Nucleic Acids Res* 2006;34:W451-4.
28. Yi T, Cheema Y, Tremble SM, Bell SP, Chen Z, Subramanian M, et al. Zinc-induced cardiomyocyte relaxation in a rat model of hyperglycemia is independent of myosin isoform. *Cardiovasc Diabetol* 2012;11:135.
29. Cui M, Wu H, An Y, Liu Y, Wei L, Qi X. Identification of important modules and biomarkers in diabetic cardiomyopathy based on WGCNA and LASSO analysis. *Front Endocrinol (Lausanne)* 2024;15:1185062.
30. Nam JM, Lim JE, Ha TW, Oh B, Kang JO. Cardiac-specific inactivation of Prdm16 effects cardiac conduction abnormalities and cardiomyopathy-associated phenotypes. *Am J Physiol Heart Circ Physiol* 2020;318:H764-77.
31. Vielmuth F, Radeva MY, Yeruva S, Sigmund AM, Waschke J. cAMP: A master regulator of cadherin-mediated binding in endothelium, epithelium and myocardium. *Acta Physiol (Oxf)* 2023;238:e14006.
32. Fang G, Li Y, Yuan J, Cao W, Song S, Chen L, et al. Cadherin-11-Interleukin-6 signaling between cardiac fibroblast and cardiomyocyte promotes ventricular remodeling in a mouse pressure overload-induced heart failure model. *Int J Mol Sci* 2023;24:6549.
33. Li C, Ao H, Chen G, Wang F, Li F. The interaction of CDH20 With  $\beta$ -catenin inhibits cervical cancer cell migration and invasion via TGF- $\beta$ /Smad/SNAIL mediated EMT. *Front Oncol* 2019;9:1481.
34. Yue Y, Meng K, Pu Y, Zhang X. Transforming growth factor beta (TGF- $\beta$ ) mediates cardiac fibrosis and induces diabetic cardiomyopathy. *Diabetes Res Clin Pract* 2017;133:124-30.
35. Zhang M, Gu H, Xu W, Zhou X. Down-regulation of lncRNA MALAT1 reduces cardiomyocyte apoptosis and improves left ventricular function in diabetic rats. *Int J Cardiol* 2016;203:214-6.
36. Wang T, Li N, Yuan L, Zhao M, Li G, Chen Y, et al. MALAT1/miR-185-5p mediated high glucose-induced oxidative stress, mitochondrial injury and cardiomyocyte apoptosis via the RhoA/ROCK pathway. *J Cell Mol Med* 2023;27:2495-506.
37. Che H, Wang Y, Li H, Li Y, Sahil A, Lv J, et al. Melatonin alleviates cardiac fibrosis via inhibiting lncRNA MALAT1/miR-141-mediated NLRP3 inflammasome and TGF- $\beta$ 1/Smads signaling in diabetic cardiomyopathy. *FASEB J* 2020;34:5282-98.
38. Chen Y, Zhang Z, Zhu D, Zhao W, Li F. Long non-coding RNA MEG3 serves as a ceRNA for microRNA-145 to induce apoptosis of AC16 cardiomyocytes under high glucose condition. *Biosci Rep* 2019;39:BSR20190444.
39. Zhao SF, Ye YX, Xu JD, He Y, Zhang DW, Xia ZY, et al. Long non-coding RNA KCNQ1OT1 increases the expression of PDCD4



- by targeting miR-181a-5p, contributing to cardiomyocyte apoptosis in diabetic cardiomyopathy. *Acta Diabetol* 2021;58:1251-67.
40. Yang F, Qin Y, Lv J, Wang Y, Che H, Chen X, *et al.* Silencing long non-coding RNA Kcnq1ot1 alleviates pyroptosis and fibrosis in diabetic cardiomyopathy. *Cell Death Dis* 2018;9:1000.
  41. Ge Z, Yin C, Li Y, Tian D, Xiang Y, Li Q, *et al.* Long noncoding RNA NEAT1 promotes cardiac fibrosis in heart failure through increased recruitment of EZH2 to the Smad7 promoter region. *J Transl Med* 2022;20:7.
  42. Zhuang A, Tan Y, Liu Y, Yang C, Kiriazis H, Grigolon K, *et al.* Deletion of the muscle enriched lncRNA Oip5os1 induces atrial dysfunction in male mice with diabetes. *Physiol Rep* 2023;11:e15869.
  43. Wang S, Wang R, Hu Y, Zhang Y, Yuan Q, Luo Y, *et al.* Long noncoding RNA AI504432 upregulates FASN expression by sponging miR-1a-3p to promote lipogenesis in senescent adipocytes. *Cell Signal* 2024;120:111232.
  44. Tye CE, Ghule PN, Gordon JA, Kabala FS, Page NA, Falcone MM, *et al.* LncMIR181A1HG is a novel chromatin-bound epigenetic suppressor of early stage osteogenic lineage commitment. *Sci Rep* 2022;12:7770.
  45. Wang X. miRDB: A microRNA target prediction and functional annotation database with a wiki interface. *RNA* 2008;14:1012-7.
  46. Marín RM, Vaníček J. Efficient use of accessibility in microRNA target prediction. *Nucleic Acids Res* 2011;39:19-29.
  47. Krek A, Grün D, Poy MN, Wolf R, Rosenberg L, Epstein EJ, *et al.* Combinatorial microRNA target predictions. *Nat Genet* 2005;37:495-500.
  48. Li JH, Liu S, Zhou H, Qu LH, Yang JH. StarBase v2.0: Decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. *Nucleic Acids Res* 2014;42:D92-7.