Construction and Investigation of a circRNA-Associated ceRNA Regulatory Network in Tetralogy of Fallot

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Research article

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Abstract

Background: As the most frequent type of cyanotic congenital heart diseases (CHD), Tetralogy of Fallot (TOF) has a relatively poor prognosis without the corrective surgery. Circular RNA (circRNA) represents a novel class of endogenous noncoding RNAs that regulate target gene expression post-transcriptionally in heart development. Here, we investigate the potential role of ceRNA network in the pathogenesis of TOF.

Methods: To identify circRNAs expression profiles in Tetralogy of Fallot, microarrays were used to screen the differentially expressed circRNA between 3 TOF and 3 control human myocardial tissue samples. Then, a dysregulated circRNA- associated ceRNA network was constructed via using the established multi-step screening strategy.

Results: In summary, total 276 differentially expressed circRNAs were identified, including 214 up-regulated and 62 down-regulated ones in TOF samples. By constructing the circRNA-associated ceRNA network based on the bioinformatics data, a total of 19 key circRNAs, 9 key miRNAs and 34 key mRNAs were further screened. Moreover, by enlarging the samples size, the qPCR results validated that the positive correlations between hsa_circ_0007798 and *HIF1A*.

Conclusions: The findings in this study provide a comprehensive understanding of the ceRNA network involved in TOF biology, such as hsa_circ_0007798/miR-199b-

5p/*HIF1A* signal axis, and may offer candidate diagnostic biomarkers or potential therapeutic targets for TOF. In addtion, we propose that the ceRNA network regulates TOF progression.

Background:

Congenital heart disease (CHD) is the most common congenital malformation, consisting of structural and functional abnormalities of the cardiovascular system during the embryonic development period that is present at birth. CHD affects 0.5–0.8% of all live births and is the main cause of neonatal death [1–3]. According to the changes in cardiac hemodynamic and pathophysiology, CHD can be divided into two main categories, cyanotic and noncyanotic form. Tetralogy of Fallot (TOF) is the most common type of cyanotic congenital heart diseases, accounting for 7–10% of all common CHD [4, 5]. TOF is composed of ventricular septal defect (VSD), pulmonary stenosis, right ventricular hypertrophy and aorta overriding the ventricular septum. With surgical correction has progressed in recent years since the initial successful repair of TOF, the attention of research community has shifted toward understanding causation [1, 6]. However, the exact pathogenesis of TOF still remains elusive. Several evidence had shown that miRNAs expression should be disordered in TOF heart tissues [7]. Up to date, there has been no relevant reports on comprehensive profiling of non-coding RNAs (ncRNAs) in TOF hearts.

Current knowledge and understanding have shown temporal and spatial expression patterns of heart development-related gene is essential in the regulation of cardiomyogenesis, which means both genetic and epigenetic factors play a critical role throughout development [8, 9]. Abnormal functional connectivity

in regulation network lead to failure of cardiac cell lineage specification, commitment, and differentiation [10, 11]. Unfortunately, the underlying molecular mechanisms remains relatively poorly understood. By using the next-generation sequencing technology, increasing evidence supports the participation of ncRNAs in the dysregulation of cardiomyogenesis [12, 13]. Different types of ncRNAs include microRNAs and a variety of long ncRNAs (lncRNAs), such as lincRNAs, antisense RNAs, pseudogenes and circular RNAs (circRNAs). For circular RNAs (circRNAs), using high-throughput circRNA microarray assay is beneficial for us to to detect and study circRNAs, compared with the circRNA-seq [14]. However, microarray can only explore known circRNAs. To identify novel circRNAs, next-generation sequencing technology should be an sensitive tool.

Recently, circRNAs, as hot topics and trends in scientific research, provide futher opportunities for a better understanding of biological mechanisms of heart diseases [15, 16]. CircRNAs, as a closed structure, are evolutionally conserved, tissue-specific and relatively stable. Besides, accumulating integrative analyses have demonstrated that circRNAs can participate in regulating gene transcription and biological process by acting as miRNA “sponge” to term competing endogenous RNAs (ceRNAs), including RNA transcripts, miRNAs and circRNAs. All of the observed characteristic features makes circRNAs have obvious advantages in the exploring of new clinical diagnostic biomarker and therapeutic targets [17]. However, there have been only a few reports exploring the circRNAs in human TOF, even though an enrichment for various diseases/functions of these promising circRNA findings were identified.

In the present study, we performed microarray analysis and evaluated circRNAs expression profiles in hearts from health controls and TOF samples. Moreover, by using bioinformatics analysis to these differentially expressed circRNAs, we screened key circRNAs and construct the ceRNA network. Our results may contribute to a mechanistic understanding of the pathogenesis and identify potential therapeutic targets for TOF in the future.

Methods:

Participants

All microarray analyses were run on samples from 3 fetuses with nonsyndromic TOF (ie, no 22q11.2 deletion) and 3 normally developing hearts of inevitable abortion fetuses. Additionally, we recruited an additional 20 subjects (10 nonsyndromic TOF and 10 health controls) to expand the sample size in the independent validation via qPCR.

We obtained the fetal hearts (≈ 150 days gestation) from the Fujian Provincial Maternity and Children’s Hospital. The fetal hearts were dissected by a surgeon who also performed many of the reconstructions of the conotruncal defects (Hua Cao), to ensure that the analyzed myocardial tissue samples were taken from a similar location (right ventricle outflow tract) [18]. After the sample was removed, the blood stains were washed with pre-cooled physiological saline, then immediately stored into RNALater at − 80 °C for subsequent processing.

RNA extraction and Quantitative PCR (qPCR)

Total RNA content was extracted from the tissues by using Trizol Reagent (Invitrogen, CA, USA) and reversely transcribed into cDNA by using the PrimeScript RT reagent Kit (TaKaRa, Tokyo, Japan) according to manufacturer's instructions. The relative expression levels of mRNA (normalized to β-actin) was analyzed by the $2^{-\Delta\Delta Ct}$ relative quantification method, the relative circRNA expression was calculated with the $2^{-\Delta Ct}$ method [19]. QPCR were performed using SYBE Green qPCR kit (TOYOBO, Tokyo, Japan) in the StepOne PCR system (Applied Biosystems). Primer sequences are shown in Table 1.

Profiling of CircRNA expression by Arraystar Human Circular RNA V2.0

The Arraystar Human Circular RNA Microarray V2.0 (Arraystar, Inc.), performed by KangChen Biotech (Shanghai, China), is designed for the purpose of profiling both circRNAs in the human genome. Scanned images processing were analyzed by using the Agilent Feature Extraction Software Version 11.0.1.1. The circRNAs ($|foldchange| \geq 2.0$ and $Pvalue < 0.05$) were selected as remarkably differentially expressed circRNAs. The microarray data produced in this study has been uploaded to the NCBI/GEO repositor (Accession Number: GSE145610).

Construction of the ceRNA (circRNA-miRNA-mRNA) regulatory network

Several online bioinformatics platforms were used in conjunction to predict circRNA-miRNA-target gene associations. The targeted miRNAs and corresponding miRNA response elements of circRNAs were firstly obtained by performing Arraystar`s home-made miRNA target prediction software. In addition, we extracted differentially expressed DE miRNAs from GSE35490, which including 16 TOFs and 8 health controls[18]. Target genes of integrated miRNAs were detected by targetScan (http://www.targetscan.org/vert_72/) and miRTar-Base (http://mirtarbase.mbc.nctu.edu.tw/php/index.php) and miRDB (http://mirdb.org/) [20]. The circRNA-miRNA-mRNA regulatory network was constructed utilizing a integration of circRNA-miRNA pairs and miRNA-mRNA pairs. Finally, the regulatory network of TOF was visualized using R package "ggalluvial" [21].

Functional GO terms and pathway enrichment analyses

Gene Ontology (GO) and pathway enrichment analyses of genes involved in ceRNA network were carried out using the database for integrated discovery bioinformatics resources (Enrichr, http://amp.pharm.mssm.edu/Enrichr/enrich) [22]. Identification of genes involved in ceRNA network more relevant to the CHD. The comparative toxicogenomics database (http://ctdbase.org/) was used to integrated chemical-gene, chemical-disease, and gene-disease interactions, so as to predict novel associations and generate expanded networks [23]. The relationships between gene products and congenital heart defects were analysed through these data. Here, relationships between genes extracted from ceRNA network and diseases were shown by radar chart.

Statistical Analysis

Data was presented as the mean ± standard deviation. All statistical data were analyzed using Statistical Program for Social Sciences (SPSS) 23.0 software (SPSS, Chicago, IL, USA). Comparison between different groups were performed using the independent Student's t test (two-tailed) or one-way ANOVA followed by Tukey's post-hoc analysis [24]. Pearson's correlation analyses were used to identify the relationships between circRNAs and mRNA [25]. In all cases, p-value < 0.05 was taken to be a statistically significant difference.

Results:

Screening of differentially expressed circRNAs

We employed the Arraystar Human circRNA Array Analysis to identify TOF-related circRNAs. The box plot showed the distribution of circRNA expression profiling. After the normalization, the distributions of log2 ratios across different samples were displayed in Figure. 1A. We then used the scatter-plot to identify differentially expressed circRNAs between TOF group and control group (Figure. 1B). Unsupervised hierarchical clustering analysis shows the dysregulated circRNA expression pattern in clinical samples (Figure. 1C).

The general data, including the functional classification and chromosome location of these differentially expressed circRNAs were summarized. Overall, 276 circRNAs were found to be significantly differentially expressed (|logFC|-value > 2.0, P-value < 0.05) (Supplementary Table 1). Compared to control group, 214 circRNAs, including 183 exonic, 13 intronic, 16 sense overlapping, 1 antisense and 1 intergenic were up-regulated and 62 circRNAs, including 53 exonic, 3 intronic, 5 sense overlapping and 1 antisense were down-regulated in CHD group (Figure. 2A & 2B). Figure. 2C and 2D demonstrated that the up and down-regulated circRNAs were located in human chromosomes.

CircRNA-miRNA-mRNA network in TOF

A total of 793 miRNA response elements (MREs) of the differentially expressed circRNAs were identified by utilizing the miRNA target prediction tool derived from Arraystar (Figure.3A). Next, to consolidate the identified MREs that are associated with TOF and screen the more significant miRNA, we used GSE35490 from the NCBI/GEO database to screen 123 differentially expressed miRNAs in TOF group compared with health groups. (|logFC|-value > 1.5, P-value < 0.05) (Figure.3B). Using venn diagram analysis, 25 shared miRNAs (key miRNA signature) were obtained and presented in Venn diagram (Figure.3C).

In order to predict the key target mRNAs, we futher uploaded the abovementioned 25 key miRNAs to the miRDB, miRTarBase and TargetScan databases for searching. 34 key mRNAs that interacted with 8 of the 25 key miRNAs in all 3 datasets were selected. After removing the remaining 17 key miRNA and the corresponding circRNAs, 19 circRNAs, 8 miRNAs and 34 mRNAs were finally obtained to construct the ceRNA network related to TOF (Figure 4, Supplementary Table 2).

Functional GO terms and pathway enrichment analyses

To characterize the functional consequences of our identified genes, we performed an enrichment analysis of the ChIP-X (ChEA) database with Enrichr for all of the 34 key genes involved in the ceRNA network. The results revealed that GO terms with the most statistical significance was heart development (P-value = 0.002538) (Figure.5A).

In addition, we characterized the putative pathogenic gene of TOF based on the CTD database. A total of 34 genes had curated or inferred association with “Congenital Heart Defects” ranked by inference score. Among these genes, *HIF1A* was the most significant marker gene (Figure.5B), confirmed by three studies [11, 26, 27]. To make our results more reliable, we futher assessed the association of 34 genes with TOF by using public databases GSE35776 [28]. Unsupervised clustering resulted in two distinct subgroups according to the genes expression model (Figure.5C).

Up-regulation of hsa_circ_0007798 expression in TOF heart tissues

To validate the previous study results, we expanded the sample size. The expression levels of hsa_circ_0007798 in 10 TOF heart tissues and 10 health heart tissues were measured by qPCR method, which showed that hsa_circ_0007798 expression was significantly up-regulated in TOF group ($P < 0.001$, Figure.6A). By means of inquiring from circBase (http://www.circbase.org), we knew that hsa_circ_0007798 was located at chromosome 6 and was composed of 6 exons ontaining the full 805-bp sequence (Figure.6B). CircRNAs mostly act by competitively sponging miRNAs to regulate the expression of their target genes according to ceRNA theory. Therefore, basd on our screening strategy, we firstly consider hsa_circ_0007798/miR-199b-5p/*HIF1A* (Figure.6C). In addition, the results of the correlation analysis of qPCR verified the positvie linear relationship between hsa_circ_0007798 and *HIF1A* (pearson r coefficient = 0.6291) (Figure.6D). These results could make it possible for us to understand how predicted and screened key circRNAs-miRNA-mRNA are related to TOF progression.

Discussion:

TOF is one of the most common cyanotic CHD worldwide that develops as a result of crossroad between genetic, epigentic and environmental risk factors [29]. At present, treatment of TOF is poorly effective, also implying long-term economic burden to families and society. Although surgical repair has allowed survival into adulthood, the lack of efficient biomarkers and the unclear mechanisms underlying TOF is still a challenge in the field of cardiology [5, 6, 29]. Previous research have demonstrated the genetic and epigenetic mechanisms, including mutaion, histone modification, DNA/RNA methylation, ncRNA modifications and others, play vital roles in cardiac development [8, 12, 18]. Among these major events, ncRNAs may be potential biomarkers for better prognosis and diagnosis. However, the study of circRNAs is just beginning.

circRNAs were regarded as sequencing artifacts in early research and only continuous improvement of bioinformatics analysis algorithms led to the recent discovery [15]. As we known, endogenous circRNA expression patterns are extensive tissue-specific and cell-specific, indicating a crucial role of of circRNAs in regulation of transcriptomics and biological processes, including heart related diseases [30–32]. circRNAs carries out multiple functions. The emerging competing endogenous RNA (ceRNA), suggesting large-scale regulatory networks among a variety of types of RNAs (circRNAs, mRNAs, and miRNAs) at transcriptome level, becomes a novel field of RNA biology [17]. Accumulating evidence has revealed circRNAs act as sponge RNAs to compete with miRNAs for the binding sites of miRNAs and influence the expression of corresponding miRNA target genes [33].

Up to the present moment, this study is the first time to provide the systematic profiling of circRNA expression in TOF and to investigate further characterization of the role of ceRNA netwrok in the pathogenesis of TOF in early life stage. Moreover, we identified and screened 214 significantly up-regulated and 62 down-regulated circRNAs through the microarray analysis. Then, 19 circRNAs were chosen according to the established multi-step screening strategy and the circRNA-miRNA-mRNA regulatory module was constructed in an attempt to better explore TOF occurrence and development. We analyzed the functions and pathways of key mRNAs participating in the ceRNA network by using ChIP-X (ChEA) and CTD database. These key mRNAs were mainly enriched in functions of “heart development” and “notch signaling pathway” etc., which are closely associated with TOF [8]. In addition, we found *HIF1A* is closely related to human CHD consistent with previous studies.

Hypoxia is the typical and inevitable pathophysiological process of TOF. As a master transcription factor associated with cellular and developmental responses to hypoxia, *HIF1A* was linked to cardiac differentiation promotion in embryonic stem cells [26]. Besides, there is evidence to demonstrate that hypoxia-inducible factor 1-alpha (HIF-1α) regulated the expression of cardiac transcription factor genes, including Tbx5 and Nkx2-5, in a bidirectional way [34]. In the current study, we rescreened the most promising ceRNA module by targeting *HIF1A* and performed correlation analysis to reveal that hsa_circ_0007798 has the positive correlation with *HIF1A*. Among the module, miR-199b-5p serves as a bridge between the circRNA and the mRNA. Coincidentally, miR-199b-5p was identified to participate in left ventricular remodeling, associated with pathologic cardiac hypertrophy [35–37]. These studies are in accordance with our finding showing hsa_circ_0007798/miR-199b-5p/*HIF1A* regulatory module is associated with the cardiac defects, including the TOF.

Conclusions:

In summary, we established a ceRNA network mediated by differential expression circRNAs in TOF on the basis of the “ceRNA hypothesis”. This study provides new insight for mechanistic investigation and may offer candidate diagnostic biomarkers or potential therapeutic targets for TOF. In addtion, we propose that the hsa_circ_0007798/miR-199b-5p/*HIF1A* signal axis regulates TOF progression. Futher studies are still needed to validate the sponge effect of the specific circRNAs, such as hsa_circ_0007798.

Abbreviations

CHD
Congenital heart diseases; TOF:Tetralogy of Fallot; circRNA:circular RNA; VSD:ventricular septal defect; NcRNAs:non-coding RNAs; LncRNAs:long ncRNAs; HIF1α:Hypoxia-inducible factor 1-alpha; CeRNAs:competing endogenous RNAs; MREs:miRNA response elements; qPCR:Quantitative PCR; GO:Gene Ontology;SPSS:Statistical Program for Social Sciences.

Declarations

Acknowledgements

Not applicable.

Authors'contributions

This study was conceived and designed by HC, HY and XW. HY is responsible for anatomical observation and collection of heart specimens. HY and XW did the collection of data or analysis. HY draft the manuscript. XW and HC checked it and revised critically. All authors read and approved the final manuscript.

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Availability of data and materials

The microarray data produced in this study has been uploaded to the NCBI/GEO repositor, all data generated or analysed during this study are included in this published article.

Ethical approval and consent to participate

This study had been approved by the Ethics Committee of the Fujian Maternity and Child Health Hospital, and conformed to the principles of the Declaration of Helsinki. The written informed consent was obtained from each parent or legal guardian after reading the consent document and solving their queries. All proper institutional review board approvals were obtained for this study.

Consent for publication

Not applicable.

Declarations:

Competing interests

The authors declare that they have no competing interests.

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References

1. Simmons MA, Brueckner M. The genetics of congenital heart disease… understanding and improving long-term outcomes in congenital heart disease: a review for the general cardiologist and primary care physician. Curr Opin Pediatr. 2017;29:520.
2. Hinton RB, Ware SM. Heart Failure in Pediatric Patients With Congenital Heart Disease. Circulation Research. 2017;120:978–94.
3. Abouk R, Grosse SD, Ailes EC, Oster ME. Association of US State Implementation of Newborn Screening Policies for Critical Congenital Heart Disease With Early Infant Cardiac Deaths. Jama. 2017;318:2111.
4. Seckeler M, Lawson E, Barber B, Klewer S. Percutaneous management of complex acquired aortic coarctation in an adult with tetralogy of Fallot and pulmonary atresia. Annals of Pediatric Cardiology. 2017;10:295.
5. Jones MI, Qureshi SA. Recent advances in transcatheter management of pulmonary regurgitation after surgical repair of tetralogy of Fallot. *F1000research.* 2018; 7: 679.
6. Athanasiadis DI, Mylonas KS, Kasparian K, Ziogas IA, Avgerinos DV. Surgical Outcomes in Syndromic Tetralogy of Fallot: A Systematic Review and Evidence Quality Assessment. Pediatric Cardiology. 2019;40(6):1105–12.
7. Wang B, Shi G, Zhu Z, Chen H, Fu Q. Sexual difference of small RNA expression in Tetralogy of Fallot. Sci Rep. 2018;8:12847.

8. Zaidi S, Brueckner M. Genetics and genomics of congenital heart disease. Circulation research. 2017;120:923–40.
9. Peng B, Han X, Peng C, Luo X, Deng L, Huang L. G9α-dependent histone H3K9me3 hypomethylation promotes overexpression of cardiomyogenesis-related genes in foetal mice. Journal of cellular molecular medicine. 2020;24:1036–45.
10. Yilbas A, Hamilton A, Wang Y, Mach H, Lacroix N, Davis DR, et al. Activation of GATA4 gene expression at the early stage of cardiac specification. Frontiers in chemistry. 2014;2:12.
11. Gasiūnienė M, Petkus G, Matuzevičius D, Navakauskas D, Navakauskienė R. Angiotensin II and TGF-β1 Induce Alterations in Human Amniotic Fluid-Derived Mesenchymal Stem Cells Leading to Cardiomyogenic Differentiation Initiation. International journal of stem cells. 2019;12:251.
12. Gao J, Xu W, Wang J, Wang K, Li P. The role and molecular mechanism of non-coding RNAs in pathological cardiac remodeling. International journal of molecular sciences. 2017;18:608.
13. Huang S, Li X, Zheng H, Si X, Li B, Wei G, et al. Loss of super-enhancer-regulated circRNA Nfix induces cardiac regeneration after myocardial infarction in adult mice. Circulation. 2019;139:2857–76.
14. Li S, Teng S, Xu J, Su G, Zhang Y, Zhao J, et al. Microarray is an efficient tool for circRNA profiling. Briefings in Bioinformatics. 2019;20(4):1420–33.
15. Patop IL, Wüst S, Kadener S. Past, present, and future of circRNAs. *The EMBO journal.* 2019; 38(16).
16. Han B, Chao J, Yao H. Circular RNA and its mechanisms in disease: From the bench to the clinic. Pharmacology Therapeutics. 2018;187:31–44.
17. Kristensen LS, Andersen MS, Stagsted LV, Ebbesen KK, Hansen TB, Kjems J. The biogenesis, biology and characterization of circular RNAs. Nature Reviews Genetics. 2019;20:675–91.
18. O'Brien JE, Kibiryeva N, Zhou XG, Marshall JA, Bittel DC. Noncoding RNA Expression in Myocardium From Infants With Tetralogy of Fallot. Circ Cardiovasc Genet. 2012;5:279–86.
19. Liao Z, Wang X, Zeng Y, Zou Q. Identification of DEP domain-containing proteins by a machine learning method and experimental analysis of their expression in human HCC tissues. Scientific reports. 2016;6:39655.
20. Wang X, Liao Z, Bai Z, He Y, Duan J, Wei L. MiR-93-5p promotes cell proliferation through down-regulating PPARGC1A in hepatocellular carcinoma cells by bioinformatics analysis and experimental verification. Genes. 2018;9:51.
21. Long J, Xiong J, Mao J, Zhang H, Bai Y, Lin J, et al. Construction and Investigation of a lncRNA-associated ceRNA Regulatory Network in Cholangiocarcinoma. Frontiers in oncology. 2019;9:649.
22. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic acids research. 2016;44:W90–7.
23. Davis AP, Grondin CJ, Johnson RJ, Sciaky D, King BL, McMorran R, et al. The comparative toxicogenomics database: update 2017. Nucleic acids research. 2017;45:D972–8.
24. Cunha SR, Hund TJ, Hashemi S, Voigt N, Li N, Wright P, et al. Defects in ankyrin-based membrane protein targeting pathways underlie atrial fibrillation. Circulation. 2011;124:1212–22.

25. Wang X, Liao Z, Bai Z, He Y, Duan J, Wei L. MiR-93-5p Promotes Cell Proliferation through Down-Regulating PPARGC1A in Hepatocellular Carcinoma Cells by Bioinformatics Analysis and Experimental Verification. genes (basel). 2018; 9(1).
26. Moreau JL, Kesteven S, Martin EM, Lau KS, Yam MX, O'Reilly VC, et al. Gene-environment interaction impacts on heart development and embryo survival. Development. 2019;146:dev172957.
27. Herrer I, Roselló-Lletí E, Ortega A, Tarazón E, Molina-Navarro MM, Triviño JC, et al. Gene expression network analysis reveals new transcriptional regulators as novel factors in human ischemic cardiomyopathy. BMC medical genomics. 2015;8:14.
28. Bittel DC, Zhou X-G, Kibiryeva N, Fiedler S, O'Brien Jr JE, Marshall J, et al. Ultra high-resolution gene centric genomic structural analysis of a non-syndromic congenital heart defect, Tetralogy of Fallot. *PloS one.* 2014; *9*(*1*).
29. Domnina YA, Kerstein J, Johnson J, Sharma MS, Kazmerski TM, Chrysostomou C, et al. Tetralogy of Fallot. *Critical Care of Children with Heart Disease*. Springer. 2020, pp 191–197.
30. Kumarswamy R, Thum T. Non-coding RNAs in Cardiac Remodeling and Heart Failure. Circulation Research. 2013;113:676–89.
31. Altesha MA, Ni T, Khan A, Liu K, Zheng X. Circular RNA in cardiovascular disease. Journal of cellular physiology. 2019;234:5588–600.
32. Kreutzer FP, Fiedler J, Thum T. Non-coding RNAs: key players in cardiac disease. *The Journal of physiology(Lond.).* 2019: 31291008.
33. Li X, Yang L, Chen L-L. The biogenesis, functions, and challenges of circular RNAs. Molecular cell. 2018;71:428–42.
34. Menendez-Montes I, Escobar B, Palacios B, Gómez MJ, Izquierdo-Garcia JL, Flores L, et al. Myocardial VHL-HIF signaling controls an embryonic metabolic switch essential for cardiac maturation. Developmental cell. 2016;39:724–39.
35. Nonaka CKV, Cavalcante BRR, Alcântara ACd, Silva DN, Bezerra MdR, Caria ACl, et al. Circulating miRNAs as potential biomarkers associated with cardiac remodeling and fibrosis in chagas disease cardiomyopathy. International journal of molecular sciences. 2019;20:4064.
36. Li Z, Liu L, Hou N, Song Y, An X, Zhang Y, et al. miR-199-sponge transgenic mice develop physiological cardiac hypertrophy. Cardiovascular research. 2016;110:258–67.
37. Duygu B, Poels EM, Juni R, Bitsch N, Ottaviani L, Olieslagers S, et al. miR-199b-5p is a regulator of left ventricular remodeling following myocardial infarction. Non-coding RNA research. 2017;2:18–26.

Tables

Table 1: Primers used for qPCR analysis of circRNA and mRNA levels		
Target	Primer Sequence 5 → 3	
	Forward	Reverse
HIF1A	GTCTGCAACATGGAAGGTATTG	GCAGGTCATAGGTGGTTTCT
ACTB	GAGAAAATCTGGCACCACACC	GGATAGCACAGCCTGGATAGCAA
hsa_circ_0007798	CCTGGAAGAGATGGATCAGAAA	GCATGCACGGCAGAAATC

Figures

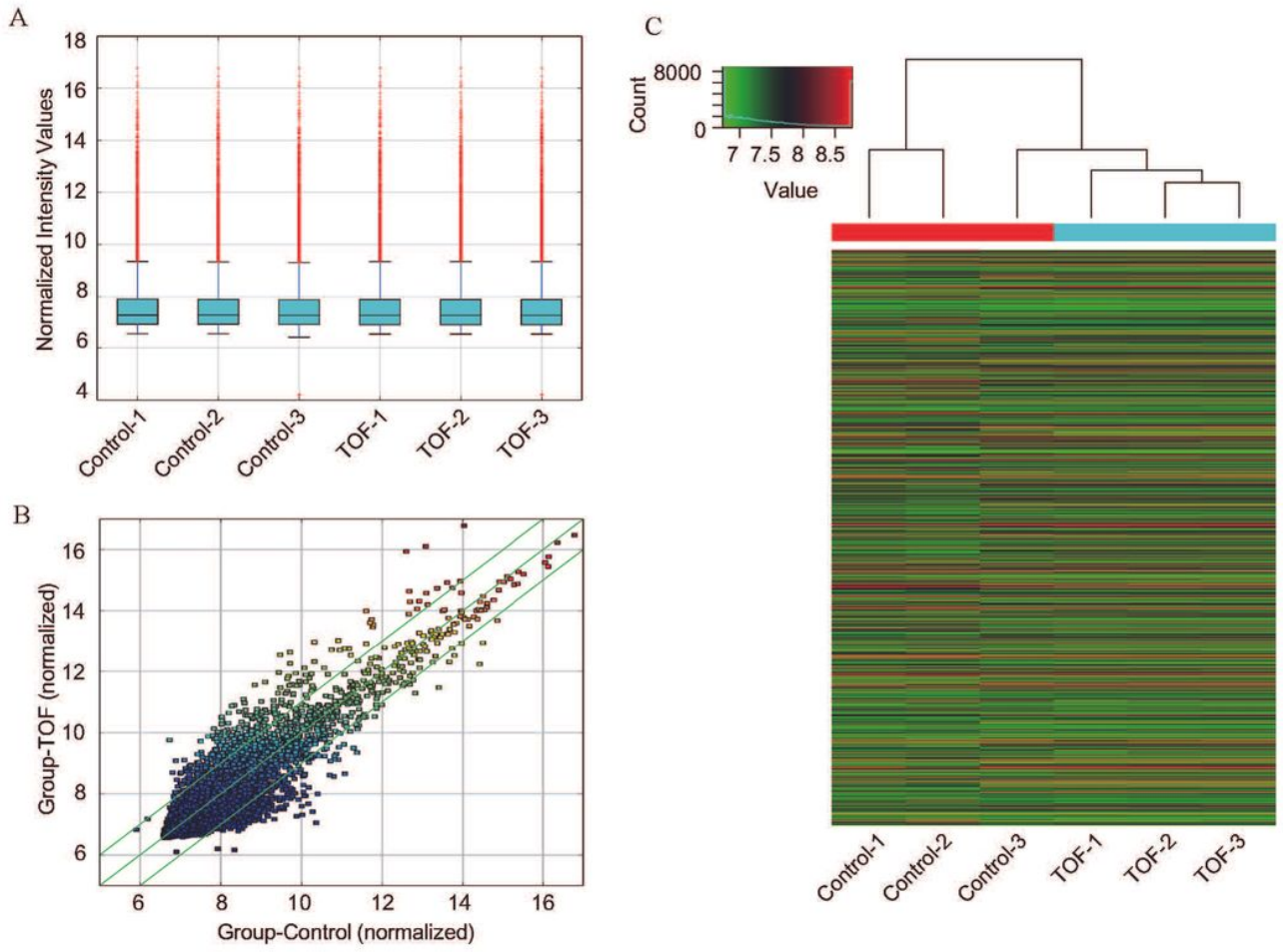

Figure 1

Overview of circRNAs expression profile. (A) Box plot is used to compare the distributions of circRNA expression values for TOF heart tissues and normal heart tissues (control) after normalization. (B) Scatter plot represented variation in circRNAs expression between TOF group and control group (log2

scaled). (C) Unsupervised hierarchical clustering of dysregulated circRNA expression among samples. The color scale shows the relative expression level of circRNA across different samples.

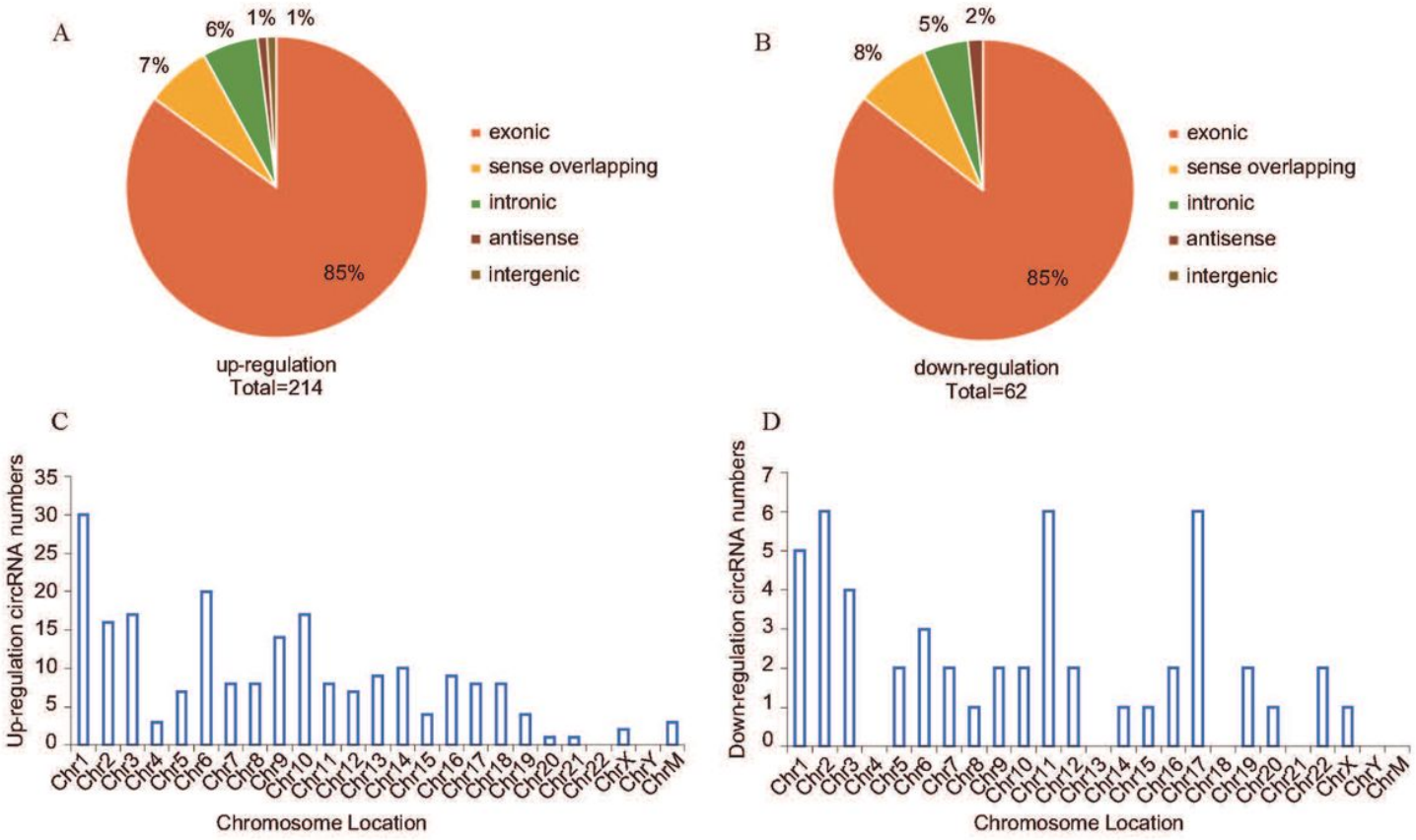

Figure 2

Annotation of differentially expressed circRNAs in TOF heart tissues. (A and B) Classification of dysregulated circRNAs. “Exonic” represents circRNA arising from the exons of the linear transcript; “Intronic” represents the circRNA arising from an introns of the linear transcript; “antisense” represents circRNA whose gene locus overlap with the linear RNA, but transcribed from the opposite strand; “sense overlapping” represents circRNA originated from same gene locus as the linear transcript. (C) The location of up-regulated circRNAs in human chromosomes. (D) The location of down-regulated circRNAs in human chromosomes.

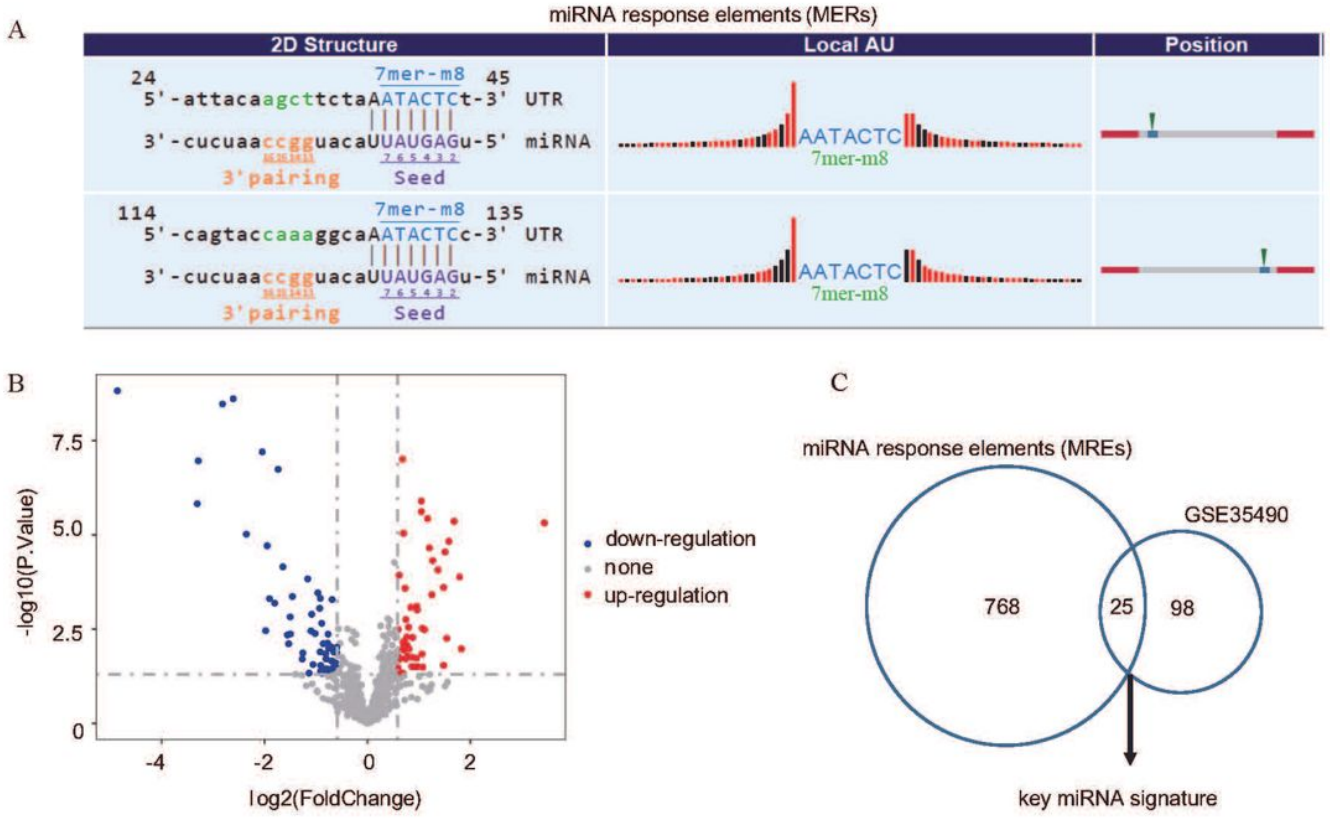

Figure 3

Identification of a 25 miRNA-based signature. (A) Results of target prediction and sequence analysis of microRNA response elements (MREs). The 2D Structure shows the MRE sequence and the corresponding target miRNA seed type. (B) Volcano plot indicated the differentially expressed miRNAs obtained from GSE35490. The vertical lines correspond to 1.5-fold up and down, respectively, and the horizontal line across the screen represents P=0.05. (C) Venn diagram showed numbers of key miRNA signature.

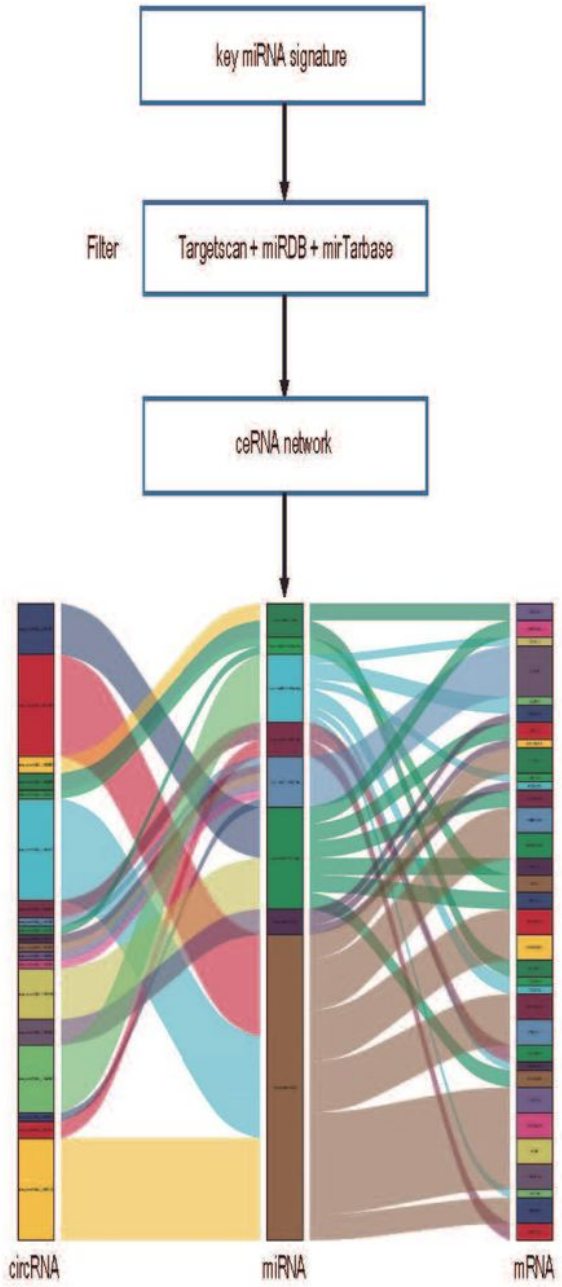

Figure 4

Sankey diagram showed the ceRNA network in TOF. Each rectangle represents a element (circRNA, miRNA, mRNA), and the connection degree of each element is indicated based on the size of the rectangle.

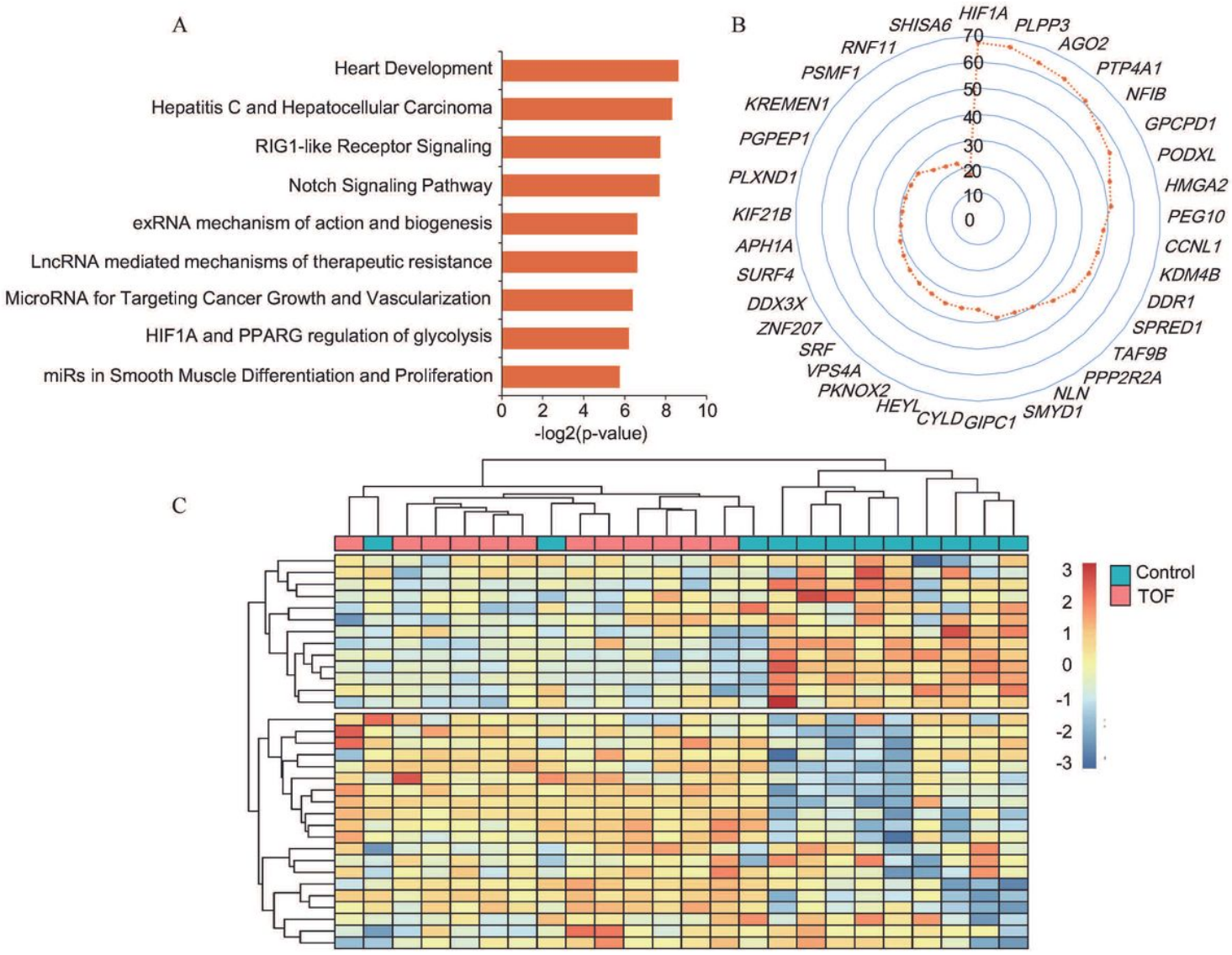

Figure 5

GO enrichment and pathway analysis for the key genes involved in the ceRNA network. (A) The top 9 significant enriched functional GO terms by using Enrichr. (B) Radar diagram depicting relationship of congenital heart defects related to the key genes based on the CTD database. (C) Unsupervised clustering of samples based on expression of the key genes.

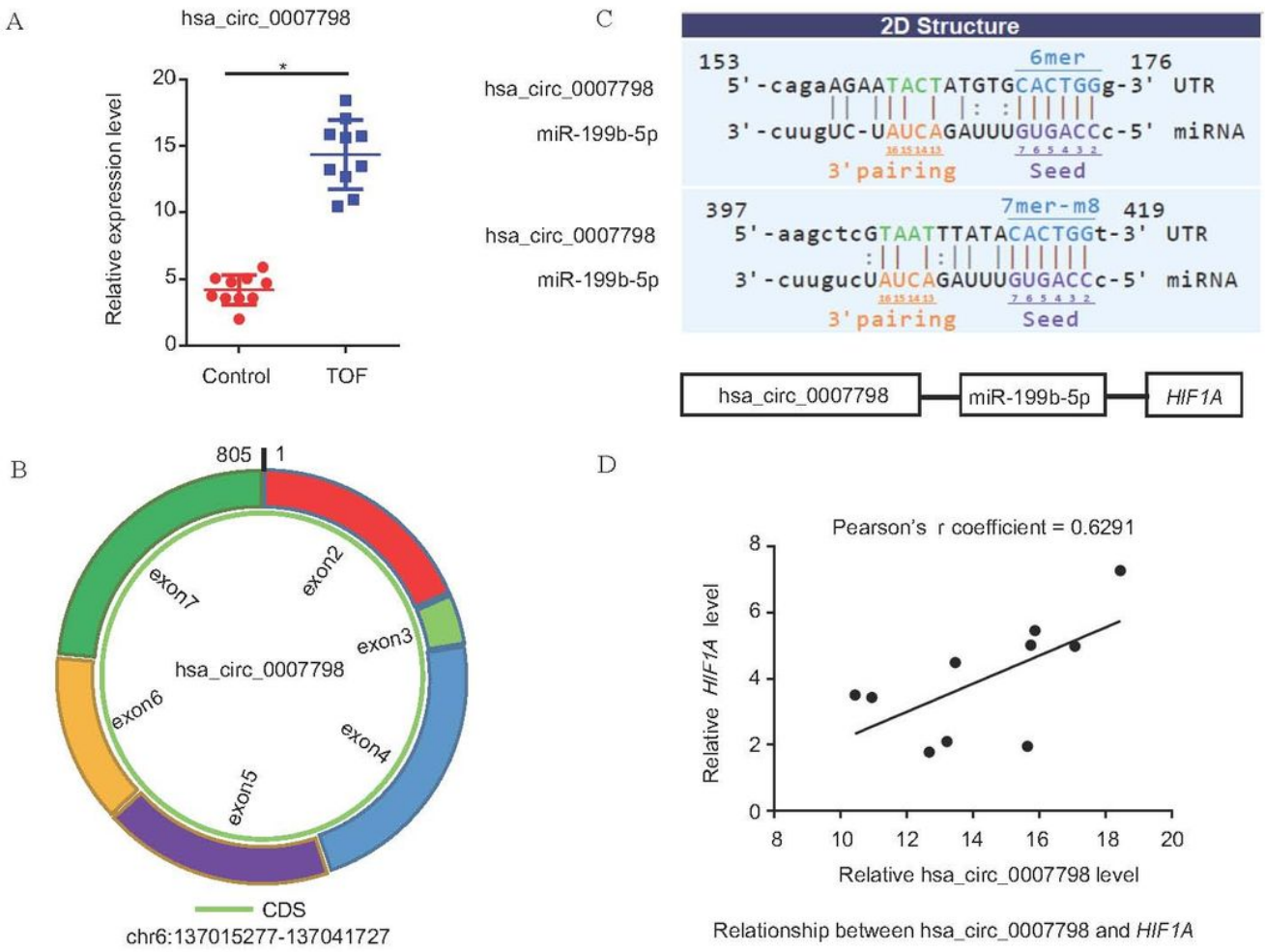

Figure 6

Functional hsa_circ_0007798/miR-199b-5p/HIF1A regulatory module. (A) Validation of the differential expression of hsa_circ_0007798. The expression level was analysed by using qPCR (TOF: n=10, Control: n=10). (B and C) Predicted miRNAs targeting to hsa_circ_0007798 based on Arraystar`s home-made software. (D) Validation of the relationship between hsa_circ_0007798 and HIF1A. The horizontal axis represents the expression of the mRNA, and the vertical axis indicates the expression of the circRNA.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable2.xls
- SupplementaryTable1.xls