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Circular RNA encoded by *PPARG* in the peripheral blood and a lipopolysaccharide-induced cardiomyocyte inflammation model is identified as a marker of fulminant myocarditis

Shasha Huang[1†], Shumin He[2†], Fei Xiao[3], Yang Zhou[4,5*] and Su Lyu[6*]

Abstract

Background Fulminant myocarditis (FM), a critical cardiac disease, is characterised by atypical initial symptoms and rapid progression and tends to lead to cardiomyocyte degeneration or necrosis. Reliable biological markers for FM screening remain lacking. Circular RNAs (circRNAs) are highly stable in peripheral blood due to their special closed-loop structure, and reports have described their involvement in regulating inflammatory responses and cell injury in cardiomyocytes. This study attempted to identify the abnormal expression of circRNAs in the peripheral blood of patients with FM and to evaluate the potential diagnostic value.

Methods Peripheral blood was collected from 5 children with FM and 5 sex- and age-matched healthy controls; total RNA was extracted from each sample, and the extracted RNA from each group was pooled. After RNase R treatment, high-throughput sequencing was performed to screen for differentially expressed circRNAs in the peripheral blood of patients. Biological function classification and enrichment analysis were used to explore the main action pathways involving differentially expressed circRNAs. A lipopolysaccharide (LPS)-induced cardiomyocyte inflammation model was used to explore the effect of inflammation on the expression of these dysregulated circRNAs. Receiver operating characteristic (ROC) curves were used to evaluate the potential clinical value of FM-related circRNAs as biomarkers in a large sample of patients.

Results CircRNA expression profiling of peripheral blood samples from patients revealed 6,435 and 3,678 circRNAs with upregulated and downregulated expression, respectively, compared with healthy controls. The expression of 1,749 circRNAs did not significantly differ between the groups. GO and KEGG analysis revealed that the genes encoding the dysregulated circRNAs were associated with various biological functions related to the risk of FM development, including infectious diseases, the immune system, and signal transduction. The high expression of hsa_circ_0064338 (circ_PPARG) was confirmed in both the myocardial cell inflammation model and peripheral blood

[†]Shasha Huang and Shumin He should both be considered as first authors.

*Correspondence:
Yang Zhou
drzhou_xmu@163.com
Su Lyu
mime_milk@163.com
Full list of author information is available at the end of the article

from a large sample of FM patients. ROC curve analysis showed that the level of circ_PPARG in peripheral blood had a good ability to distinguish patients with FM from healthy controls.

Conclusions Large numbers of abnormally regulated circRNAs were identified in peripheral blood from patients with FM; among these, the highly expressed circ_PPARG could distinguish patients from healthy controls to a certain extent. It is expected to become a potential clinical biomarker of FM in the future.

Keywords Fulminant myocarditis, PPARG, CircRNAs, ROC curve, Biomarker

Introduction

Fulminant myocarditis (FM) is a cardiac disease that seriously affects patients' lives and health [1, 2]. Pathologically, FM manifests as acute diffuse inflammatory myocardial disease, which can lead to acute hemodynamic disorders and cause patients to quickly develop refractory shock or fatal arrhythmias [3]. In children, FM is mainly induced by viral infection. The initial symptoms are often atypical, but the disease progresses rapidly, leading to a dangerous condition with a poor prognosis [4, 5]. At present, the methods for diagnosing FM mainly include the observation of clinical manifestations, determination of serum biochemical indices, and electrocardiography; however, early disease-related biological indicators with stable and reliable performance remain lacking [6].

Previously, RNA was considered to be merely an intermediate carrier of information between DNA and protein, but recent discoveries have shown that the functions of RNA extend far beyond the simple transmission of genetic information. In particular, the discovery of circular RNA (circRNA) has profoundly changed our understanding of the biological role of RNA [7]. The ends of circRNA molecules form a closed ring structure from linear transcripts of the parent genes via a unique splicing mechanism. This unique ring structure gives circRNAs not only nonlinear characteristics but also a variety of unique biological functions [8]. CircRNAs not only act as regulatory factors in cells but also play important roles in regulating gene expression, cell differentiation, and disease occurrence by interacting with a variety of RNA-binding proteins (RBPs) and other RNAs. Research on the functions of circRNA has proved that these molecules are widely present in various organisms and play important roles in various molecular pathological processes in humans, such as cancers, cardiovascular diseases, and neuropsychiatric diseases [9–11]. Given their unique structure and functions, circRNAs have a wide range of potential applications in the medical field. CircRNAs exhibit great potential as biomarkers. With their relative intracellular stability, significant changes in expression associated with specific disease states, and diversity in molecular size, circRNAs can be used for the early diagnosis and prognostic assessment of diseases [12]. For example, the levels of specific circRNAs are significantly increased in the blood of some patients with cancer, providing new possibilities for non-invasive cancer diagnosis.

Recent studies have identified a new circRNA (hsa_circ_0071542) in the peripheral blood of human patients with myocarditis; this circRNA is upregulated in the acute phase of the disease, and dynamic changes in its expression are associated with disease progression [13]. Zhang et al. used circRNA microarray technology to explore the circRNA expression profiles in peripheral blood from children with FM [13]. The results showed that many abnormally expressed circRNAs could affect inflammation and immune function in myocardial tissues [13]. Studies of animal models of FM have confirmed that abnormally expressed circRNAs are strongly correlated with immune responses, and individuals with abnormal circRNA expression are more likely than others to develop cardiac dysfunction [14, 15]. In this study, we used advanced high-throughput sequencing technology to explore the circRNA expression profile in the peripheral blood of children with FM and screened for abnormally expressed circRNAs associated with the risk of disease, aiming to discover new biomarkers that are highly correlated with FM.

Methods

Subjects

Blood samples were collected from 50 patients with FM in the central laboratory of Zhongshan Hospital of Xiamen University. One millilitre of peripheral blood was collected from each patient within 30 min after diagnosis with FM in our hospital. All patients were younger than 18 years of age. All patients fulfilled the American Heart Association diagnostic criteria. The inclusion criteria for FM cases were as follows: a. first onset of FM; b. no family history of cardiac disease; and c. no other infectious diseases, tumours, or other serious physical diseases. The exclusion criteria were as follows: a. FM concomitant with other congenital cardiovascular diseases; b. blood system diseases; and c. abnormal liver and kidney function. All blood vessels were pretreated with EDTA-K2 as an anticoagulant. As a non-invasive human specimen, peripheral blood is particularly suitable for laboratory

clinical detection and research. However, RNA is a single-strand oligonucleotide and is extremely unstable, and RNase is widely distributed, ubiquitously degrades RNAs, and is difficult to inhibit; therefore, it is difficult to extract and preserve RNA from whole blood. The blood collection vessels used in this study were pre-loaded with a unique preservation solution containing a proprietary RNA stabiliser to rapidly inactivate RNases and inhibit RNA degradation (BioTeke, Wuxi, China). Standard venipuncture techniques were used to collect blood in EDTA-K2 anticoagulant vacuum collection vessels. Specifically, the blood collection vessel was connected to the blood RNA storage tube, and another blood collection needle was inserted outside the blood collection vessel cover. The negative pressure in the storage tube automatically transferred 1 mL of blood to the RNA storage tube. The stored blood can be used for the storage and transport of blood samples, the specific information is detailed in the product description. The storage solution could stabilise intracellular RNA in the blood, and through downstream experiments. RNA could be separated and purified from whole blood for RT-PCR, transcriptome analysis, and other molecular biological experiments and detection.

High-throughput sequencing of circRNAs

Total RNA was extracted from the peripheral blood of 5 patients with FM and 5 healthy controls. The total RNA was analysed using an Agilent Bioanalyzer 2100 for quality control, and RNA samples that met the quality control requirements from each group were pooled to yield one peripheral blood total RNA sample each for the patient group and the healthy control group. The circRNA library was constructed on the Illumina standard platform via RNA sample de-linearisation, and high-throughput sequencing was performed using the BGI HiSeq4000 platform to ensure clean data ≥ 10G. RNase R was used to remove linear RNA. The relevant details can be found in a previous report [16]. The clinical disease characteristics of the samples used for circRNA sequencing are shown in Table 1.

Bioinformatics analysis

Raw data produced by high-throughput sequencing are called raw reads. In this project, SOAPnuke, a filtering software package independently developed by BGI, was used to filter the raw reads. The specific steps were as follows. First, reads containing joint contamination and reads with an unknown base N content > 5% were removed. Low-quality reads, defined as reads with a mass value of < 20 that accounted for > 20% of the total base number of the reads, were also removed. All remaining data were classified as clean reads. Two software packages, CIRI (https://sourceforge.net/projects/ciri/) and find_circ (https://github.com/marvin-jens/find_circ), were used to predict circRNAs in the above-filtered data, and the results produced by the two packages were integrated according to the circRNA start and end positions. In the actual analysis, circRNAs with start and end positions within the first and last 10 bases were combined into a class. According to the annotation results and official classification, the phyper function on the R platform was used to perform Gene Ontology (GO) functional analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) biological pathway classification enrichment analysis [17, 18]. The lncLocator online sequence analysis tool was used to identify the subcellular structural localisation of circRNAs (http://www.csbio.sjtu.edu.cn/bioinf/lncLocator/), [19, 20].

Establishment of a cell model of inflammatory response and the detection of circRNA expression

The AC16 human cardiomyocyte line can be used to study the expression and function of cardiac genes associated with pathological mechanisms at the cellular,

Table 1 Detailed clinical information about the patients with fulminant myocarditis (FM) and normal controls (N) in high-throughput sequencing analysis

Subject ID	Age (years)	Gender	Symptoms at onset	ECG
M1	5	F	Repeated syncope	VT, VF
M2	7	F	Headache, emesis	ST-T change
M3	7	M	Chest pain, poor spirit	III°AVB
M4	8	M	Fever, emesis	III°AVB
M5	9	F	Fever, headache, chest distress	Inverted T wave
N1	6	F	/	/
N2	7	M	/	/
N3	8	M	/	/
N4	9	F	/	/
N5	7	F	/	/

organelle, and molecular levels and during normal development. For this study, AC16 cells purchased from Wuhan Pricella Biotechnology Company and cultured in a standard cell culture incubator at 37 °C with 95% air + 5% carbon dioxide. The culture medium contained RPMI-1640 basal medium, 10% foetal bovine serum, and 1% penicillin–streptomycin. The inflammatory human cardiomyocyte model was induced using lipopolysaccharide (LPS) (Sigma, MO, USA) [21]. The cardiomyocytes were routinely cultured for 24 h and then starved in serum-free medium for 12 h. Subsequently, LPS was added to the culture medium at a concentration of 10 μg/mL to induce a cellular inflammatory response. The control group was treated with sterile phosphate-buffered saline instead of LPS. After 48 h of culture, total RNA was extracted from the cardiomyocytes and the expression of circRNAs was analysed using quantitative real-time PCR. For details, please refer to a previous report [16].

ROC curve analysis

The receiver operating characteristic (ROC) curve is a statistical tool used to evaluate the performance of a classifier by plotting the relationship between the true and false positive case rates [22]. The area under the curve (AUC) is the area under the ROC curve and ranges in value from 0.5 to 1. The larger the AUC value, the better the performance of the classifier. In this study, the expression level of circRNAs in each patient with FM was used as a sensory point in the model to draw a curve for evaluating the clinical value of peripheral blood circRNA levels for the assessment of FM risk.

Statistical analysis

Statistical Products and Service Solutions (SPSS17.0) software was used for statistical analysis in this study. The independent sample *T* test was used to compare the expression levels of circRNAs in peripheral blood from the FM group and healthy controls. A P value < 0.05 was considered statistically significant [23].

Results

Base content distribution in clean reads

The raw data obtained from the high-throughput sequencing of circRNAs contained many reads with low quality, contaminated joins, and high contents of unknown bases N. The filtered components of the raw data from the patient and control groups were statistically analysed (Fig. 1A, B, respectively). Reads that did not meet the quality requirements were removed before data analysis to ensure the reliability of the results. The base content distributions in the sequenced raw data from the peripheral blood total RNA of patients with FM and healthy controls after filtering are shown in Fig. 1C, D, respectively.

Prediction and distribution of circRNAs

The prediction results from CIRI and find_circ were integrated as described in the methods. Figure 2A, B shows Venn diagrams of circRNAs in the peripheral blood of healthy controls and patients with FM, respectively. The distribution of circRNAs across the genome was displayed using Circos software. Figure 2C, D shows the distribution of the origin positions of circRNAs in the peripheral blood of healthy controls and patients with FM, respectively.

Significant differences in circRNA expression in peripheral blood samples between patients with FM and healthy controls

In the high-throughput sequencing analysis, the numbers of junction reads at both ends of the circRNAs were compared to calculate the expression level. Volcano (Fig. 3A) and scatter plots (Fig. 3B) were used to demonstrate the differential circRNA distribution between patients with FM and healthy controls. A heat map of the expression of differentially expressed circRNAs in each group was generated (Fig. 3C). The number of circRNAs in peripheral blood samples from FM patients was significantly higher than that in samples from healthy controls, and the number of introgenic circRNAs was significantly higher than the number of intergenic circRNAs (Fig. 3D).

GO functional analysis and pathway functional analysis of genes encoding differentially expressed circRNAs

Based on the results of the above-described differential analysis, a GO functional classification analysis of the genes driving introgenic circRNAs was conducted. The GO functional classification results are shown in Fig. 4A, and the GO functional classification statistics of the source genes encoding the up- and downregulated circRNAs are shown in Fig. 4B. A KEGG biological pathway classification and enrichment analysis of genes driving introgenic circRNAs were also performed. The pathway classification results are shown in Fig. 5A, the enrichment results are shown in Fig. 5B, and the pathway statistics of genes encoding the up- and downregulated circRNAs are shown in Fig. 5C.

Hsa_circ_0064338 was highly expressed in the peripheral blood of patients with FM and exhibited diagnostic value

The above-described abnormally expressed circRNAs were detected in the LPS-induced cardiomyocyte inflammation model, and five circRNAs were found to be highly expressed (Fig. 6A). Their expression profiles were further verified in clinical peripheral blood samples from

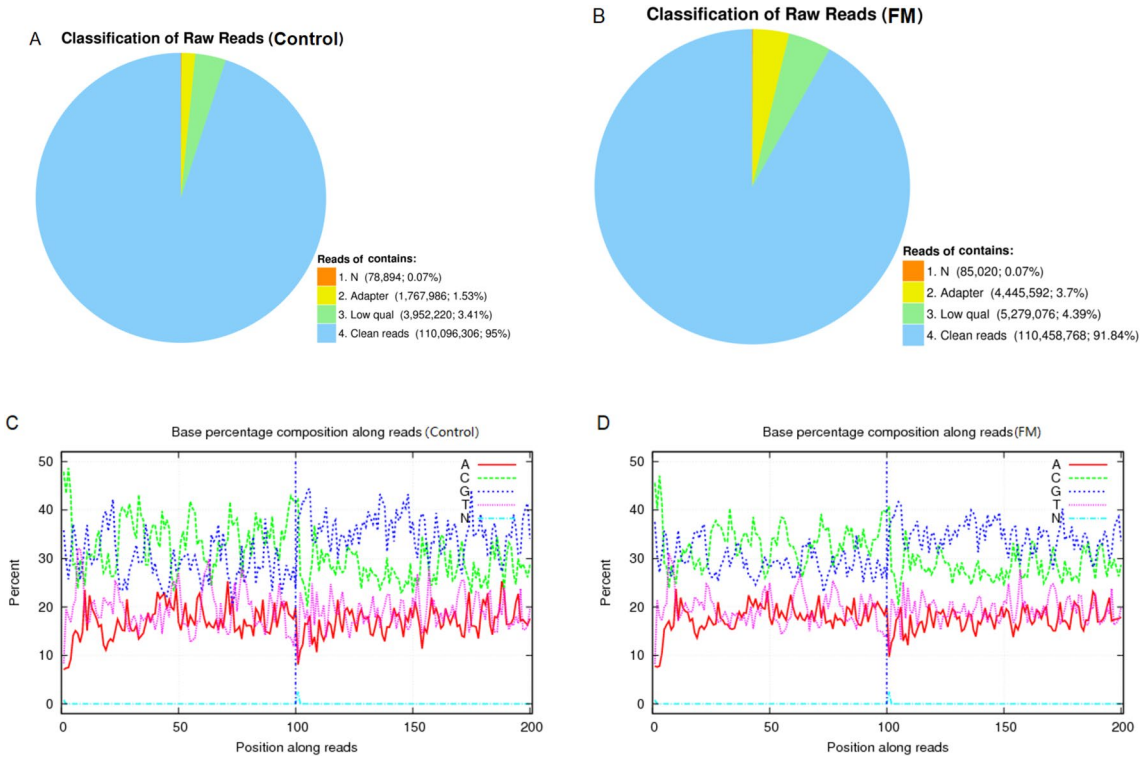


Fig. 1 Filtering of high-throughput sequencing data from peripheral blood circRNAs. Raw sequencing data contains reads with low quality, adapter contamination, and a high content of unknown base N. **A** Statistics of the raw data filtering components of peripheral blood circRNAs from healthy controls. **B** Statistics of the raw data filtering components of peripheral blood circRNAs from patients with FM. **C** Distribution of the base contents of clean reads in high-throughput sequencing data of peripheral blood circRNAs from healthy controls. **D** Distribution of the base contents of clean reads in high-throughput sequencing data of peripheral blood circRNAs from patients with FM. The *X*-axis indicates the position of the base in the read, and the *Y*-axis represents the content ratio of each base

patients with FM, and the results showed significantly high expression of hsa_circ_0064338 (Fig. 6B). Bioinformatics analysis showed that hsa_circ_0064338 was a linear transcript of the gene encoding peroxisome proliferator-activated receptor gamma (*PPARG*) that was further sheared and circularised, and it was mainly located in the cytoplasm in cells (Fig. 6C). ROC model analysis showed that the expression level of hsa_circ_0064338 (hsa_circ_PPARG) in peripheral blood has potential clinical value for distinguishing patients with FM from healthy controls (Fig. 6D).

Discussion

Given the continuous advancement of RNA molecular detection technology and functional research methods, it is anticipated that additional circular RNAs will be identified in cells and disease conditions, and these may be applicable in the clinical diagnosis and treatment of human diseases [9]. In this study, we used high-throughput sequencing technology to screen the expression profiles of differential circRNAs in the peripheral blood of 5 children with FM and explored the action pathways and functional classifications of genes driving the expression of these differential circRNAs (Figs. 1, 2, 3, 4, 5). Subsequently, we identified the upregulated expression of hsa_circ_0064338 in blood samples from these patients with FM, our myocardial cell inflammation model, and a large sample of clinical blood samples from patients with FM (Fig. 6). The ROC model showed that hsa_circ_0064338 may have clinical value in distinguishing between patients with FM and healthy controls (Fig. 6).

The low abundance of circRNAs and the similarity of these sequences to linear RNA sequences have made effective detection challenging. The only unique characteristic of circRNA is its circular structure, which makes it very difficult to distinguish and detect [9]. Conventional RNA sequencing methods often ignore circRNAs, because the construction of standard RNA libraries depends on the poly (A) tail of mRNA, which circRNA lacks. Currently, circRNA can be detected by removing linear RNA from the sample, thereby increasing the abundance of circRNA. RNase R is a commonly used nuclease that can degrade most linear RNAs while having little effect on circRNAs [24]. Pretreatment of total

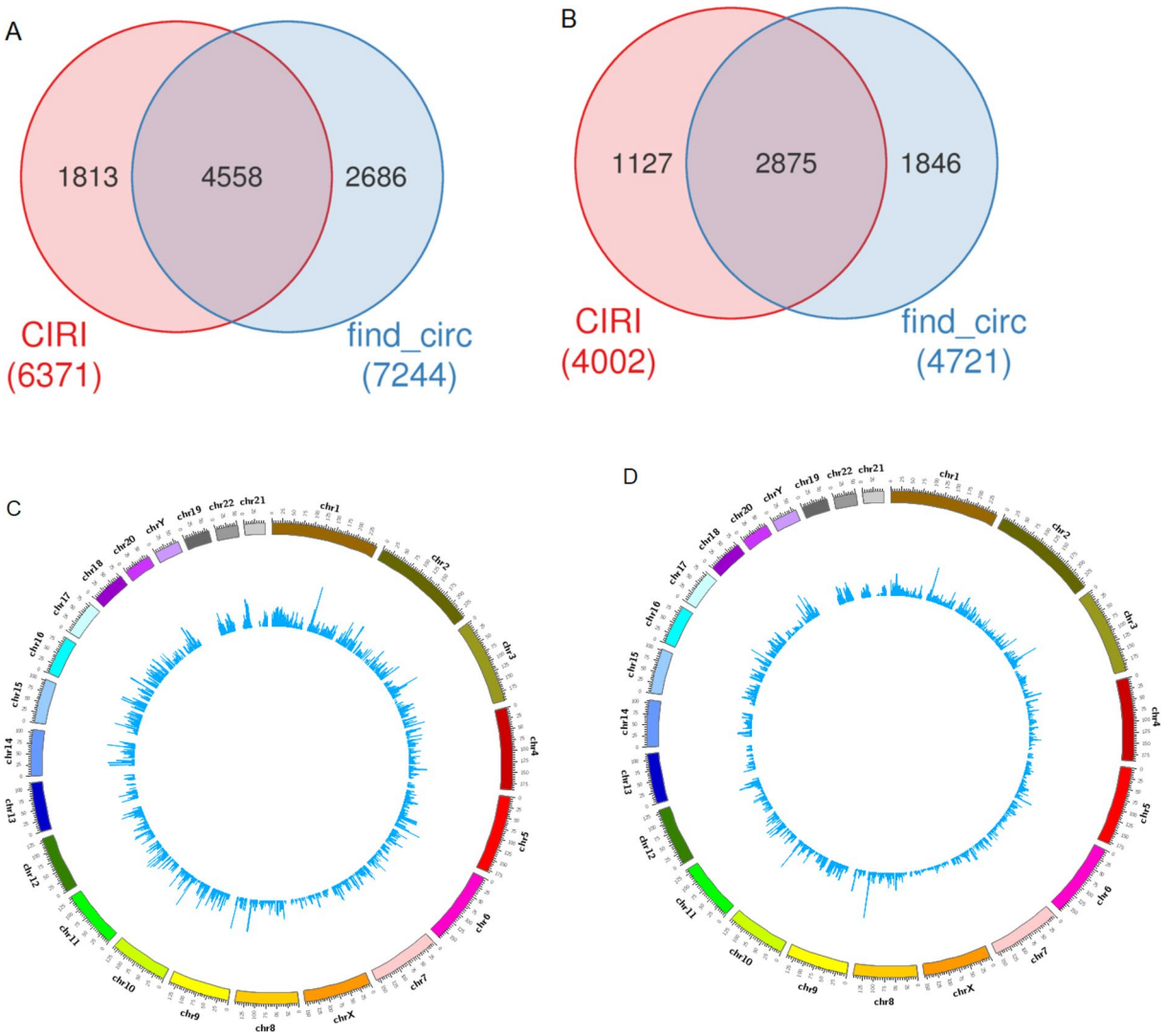


Fig. 2 Prediction and annotation of circRNAs via high-throughput sequencing. **A** Venn diagram of circRNAs in the peripheral blood of healthy controls as predicted by bioinformatics. **B** Venn diagram of circRNAs in the peripheral blood of patients with FM as predicted by bioinformatics. **C** Distribution of the number of circRNAs in the peripheral blood of healthy controls across the genome. **D** Distribution of the number of circRNAs in the peripheral blood of patients with FM across the genome

RNA samples with RNase R can remove most linear RNAs, thereby enriching circRNAs for subsequent analysis [24]. Combined with cutting-edge high-throughput sequencing technology, this approach has made it possible to fully identify and quantify circRNAs. In this study, the total RNA extracted from the peripheral blood of 5 patients with FM was mixed and digested with RNase R to meet the quality requirements of high-throughput sequencing (Fig. 1). Subsequent bioinformatics identification and analysis further clarified the chromosomal origins of these circRNAs (Fig. 2).

In this study, the expression profile of circRNAs in the peripheral blood of patients with FM was preliminarily identified using high-throughput sequencing. The results identified 6,435 upregulated and 3,678 downregulated circRNAs in patients compared with healthy controls. The expression levels of 1,749 circRNAs were not significantly changed (Fig. 3). The number of differentially expressed circRNAs was significantly higher than that reported by a previous study using a circRNA microarray analysis technique [13]. This difference may be due to a number of reasons. First, we enriched circRNAs by pretreating total RNA samples with RNase R, which enhanced the detection of circRNAs [24]. Second, the high-throughput sequencing technology used in this study can theoretically detect RNA molecules with

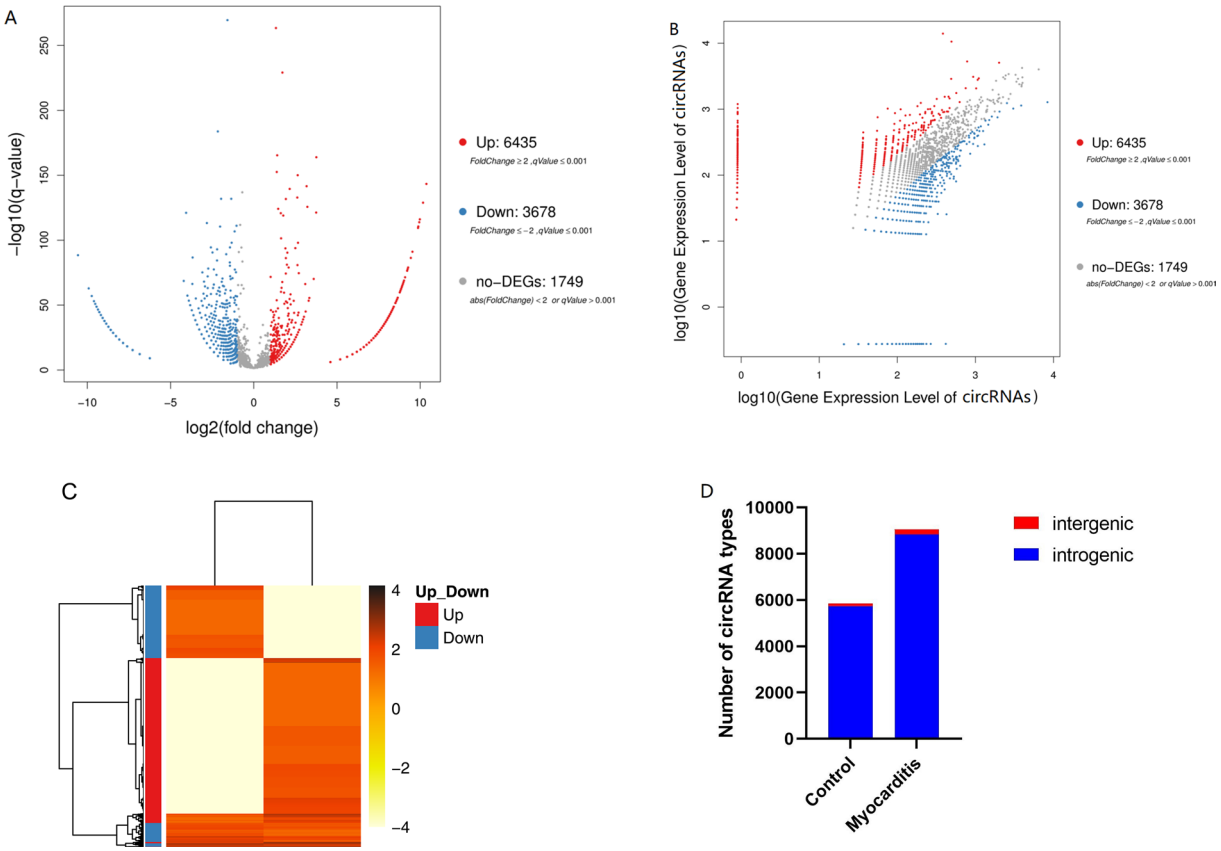


Fig. 3 Screening of circRNAs that are differentially expressed in the peripheral blood of patients with FM compared with healthy controls. **A** Volcano plot of distribution reveals differentially expressed circRNAs in peripheral blood from patients with FM. **B** Scatter plot of the distribution of differentially expressed circRNAs in peripheral blood from patients with FM. **C** Heat map of the expression levels of FM-related differentially expressed circRNAs. **D** Statistics regarding the origins of circRNAs

circRNA characteristics in almost all samples, whereas circRNA microarrays can detect only a limited number of known circRNAs [25–28]. Third, we mixed RNA samples from different sources before sequencing to avoid the loss of circRNAs with low abundance in individual samples. The above advantages in detection strategies have enabled us to provide additional information about the circRNA expression profile in the peripheral blood of patients with FM.

Next, we used GO and KEGG enrichment analysis to predict the potential biological functions of differentially expressed circRNAs in the peripheral blood of patients with FM. We found the enrichment of multiple functional entries closely related to the onset of FM, such as infectious diseases, the immune system, and signal transduction (Figs. 4, 5). FM is a non-ischemic cardiac inflammatory disease usually caused by microbial infection. We constructed a human cardiomyocyte inflammation model to clarify the expression status of abnormally expressed circRNAs in myocardial inflammation that were identified via high-throughput sequencing. The results showed that the expression of multiple circRNAs was increased by 4 times in this model (Fig. 6A). To further clarify the clinical value of these circRNAs in FM, we verified their expression levels in the peripheral blood of 50 patients with FM and healthy controls. The results showed that hsa_circ_0064338 was significantly highly expressed in the peripheral blood of patients (Fig. 6B). After analysing its source through bioinformatics, we found that hsa_circ_0064338 is derived from the splicing of the linear transcript (NM_138711) of *PPARG*. Some circRNAs can exert biological functions similar to those of their parent genes, because they contain similar sequences of a certain length. PPARG has been shown to regulate macrophage differentiation, inflammatory activation, polarisation, lipid metabolism, and other key pathologic activities associated with macrophage function [29, 30]. Macrophage polarisation is closely related to the occurrence of myocarditis. M1 macrophages can significantly aggravate myocarditis, while M2 macrophages can effectively relieve myocardial inflammation [31].

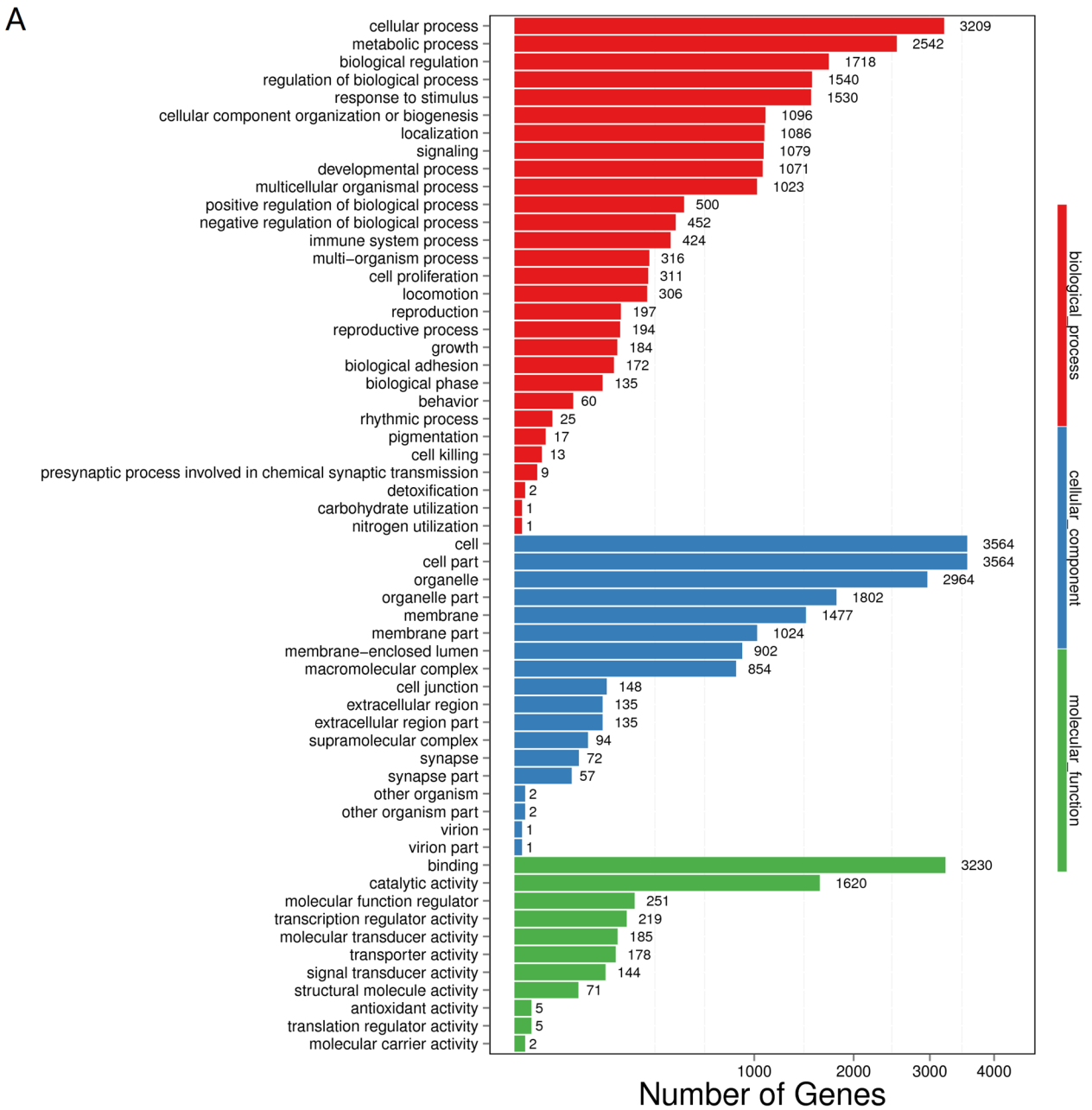


Fig. 4 GO functional analysis of genes driving differentially expressed circRNAs associated with FM. **A** GO functional classification of genes driving the expression of FM-related differentially expressed circRNAs. **B** Classification and statistics of GO function of source genes driving differentially upregulated or downregulated circRNAs

Studies have confirmed that M2 macrophages can promote the M2 polarisation of heart-infiltrating macrophages in mice with viral myocarditis by secreting specific non-coding RNAs, thereby reducing the degree of myocardial inflammation and playing a protective role [31]. In this study, we conducted a bioinformatics analysis of the intracellular distribution of hsa_circ_0064338 and found that it was mainly localised in the cytoplasm (Fig. 6C). This suggests that hsa_circ_0064338 can be secreted outside the cell and may play a regulatory role in the disease process. It also lays a foundation for exploring its potential value as a peripheral marker for FM. The ROC model confirmed that the level of hsa_circ_0064338 in peripheral blood

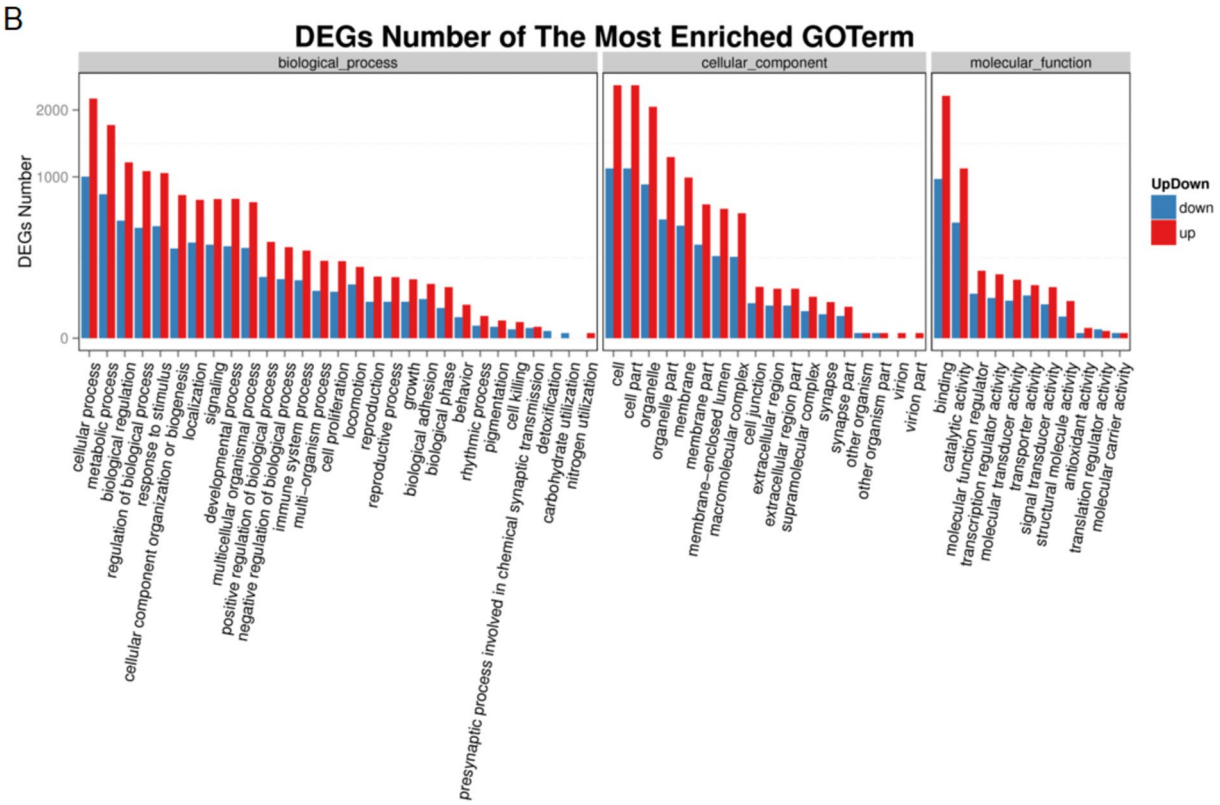


Fig. 4 continued

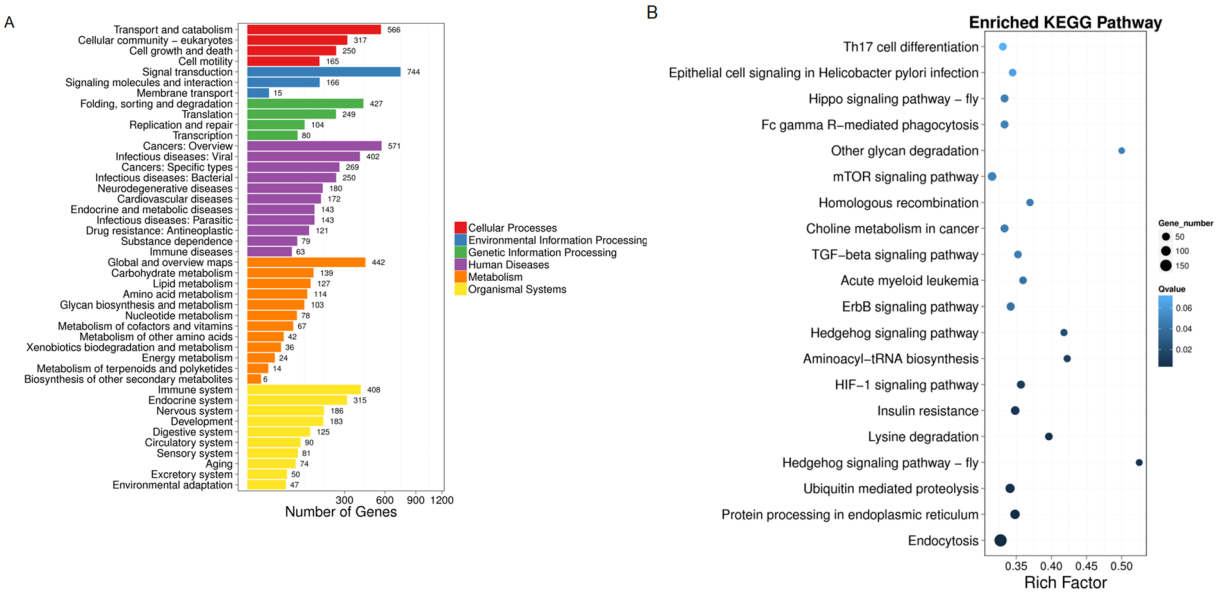


Fig. 5 Pathway functional analysis of genes driving differentially expressed circRNAs associated with FM. **A** Pathway classification of genes driving FM-related differentially expressed circRNAs. **B** Pathway enrichment analysis of genes driving FM-related differentially expressed circRNAs. **C** Enrichment pathway statistics of source genes driving differentially upregulated and downregulated circRNAs associated with FM

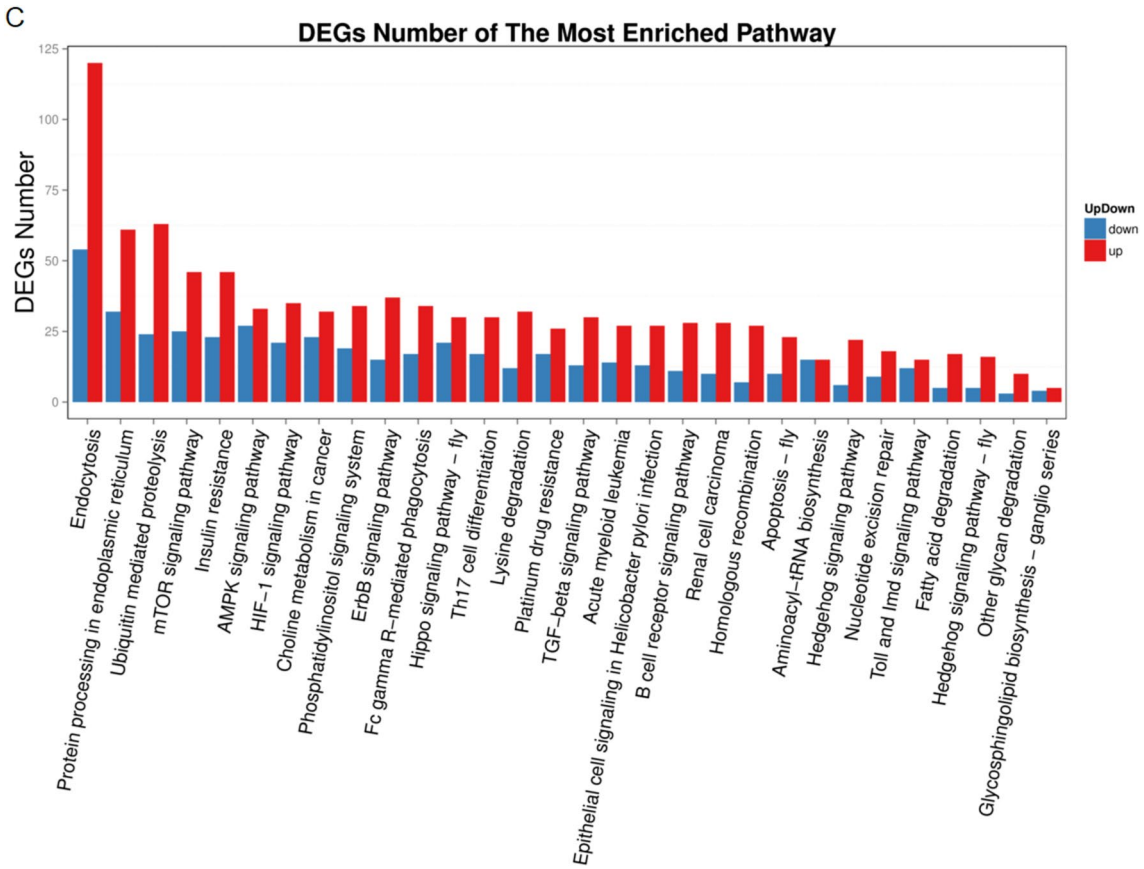
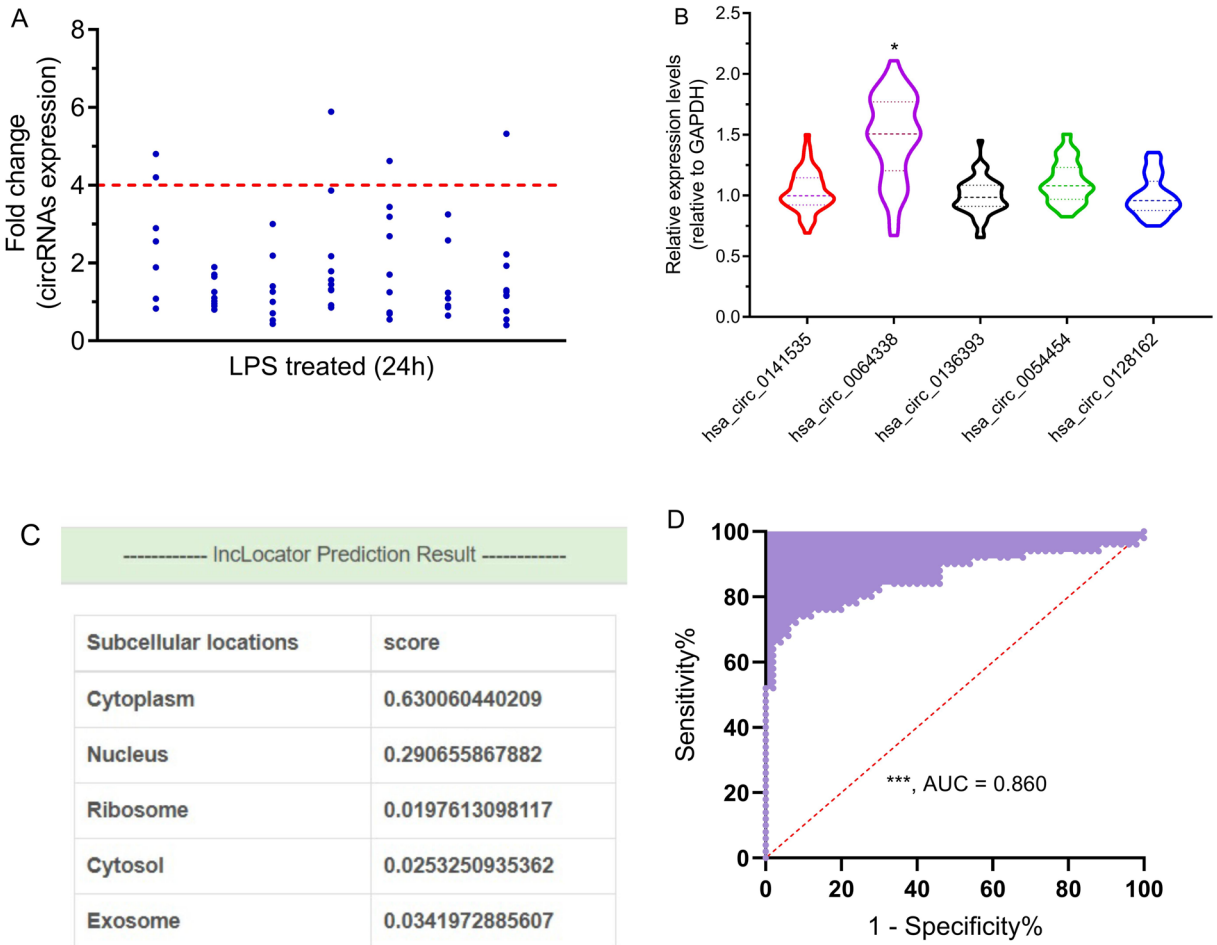


Fig. 5 continued

has a good clinical reference value for distinguishing FM patients from healthy controls (Fig. 6D).

Conclusion

In summary, we have demonstrated the feasibility of using high-throughput sequencing technology to screen differentially expressed circRNAs in the peripheral blood of patients with FM. An inflammatory state could trigger the release of circ_PPARG in our cardiomyocyte inflammation model. The expression of circ_PPARG in peripheral blood has potential clinical value as a biomarker for distinguishing patients with FM from healthy controls.



Subcellular locations	score
Cytoplasm	0.630060440209
Nucleus	0.290655867882
Ribosome	0.0197613098117
Cytosol	0.0253250935362
Exosome	0.0341972885607

Fig. 6 Identification and clinical value analysis of differentially expressed circRNAs in the peripheral blood of patients with FM. **A** Five circRNAs were expressed at high levels in LPS-treated human myocardial cells. **B** hsa_circ_0064338 was at significantly high levels in the peripheral blood of patients with FM before treatment. **C** Distribution of the subcellular localisation of hsa_circ_0064338 according to the sequence information. **D** ROC curve analysis demonstrating that the hsa_circ_0064338 level in peripheral blood could be used to distinguish individuals at risk of FM. *$P < 0.05$, **$P < 0.01$

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Author contributions
Shasha Huang, Su Lyu and Yang Zhou. Performed the study: Shasha Huang, Shumin He and Fei Xiao. Wrote the paper: Shasha Huang and Shumin He. All authors read and approved the final manuscript.

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Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All procedures performed in this study were in accordance with the Declaration of Helsinki of 1964 (2013 revision). Informed consent or an acceptable substitute was obtained from all individuals included in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Cardiology, Shenzhen People's Hospital The Second Clinical Medical College, Jinan University; The First Affiliated Hospital, Southern University of Science and Technology, Shenzhen 518020, Guangdong, China. [2]Department of Infectious Diseases, Zibo Ninth People's Hospital, Zibo 256400, Shangdong, China. [3]Department of Cardiology, Zibo Central Hospital, Zibo 255024, China. [4]Department of Pain Management, Zhongshan City People's Hospital, Zhongshan 528403, Guangdong, China. [5]Department of Central Laboratory, School of Medicine, Zhongshan Hospital of Xiamen University, Xiamen University, No. 201-209, Hubinnan Road, Siming District, Xiamen 361004, Fujian, China. [6]Department of Critical Medicine, The Friendship Hospital of Ily Kazak Autonomous Prefecture, No. 92 Stalin Street, Ili Kazakh 835000, Xinjiang, China.

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References

1. Sharma AN, Stultz JR, Bellamkonda N, Amsterdam EA. Fulminant myocarditis: epidemiology, pathogenesis, diagnosis, and management. Am J Cardiol. 2019;124:1954–60.
2. Kanaoka K, Onoue K, Terasaki S, Nakano T, Nakai M, Sumita Y, et al. Features and outcomes of histologically proven myocarditis with fulminant presentation. Circulation. 2022;146:1425–33.
3. Ginsberg F, Parrillo JE. Fulminant myocarditis. Crit Care Clin. 2013;29:465–83.
4. Gupta S, Markham DW, Drazner MH, Mammen pp. Fulminant myocarditis. Nat Clin Pract Cardiovasc Med. 2008;5:693–706.
5. Sansoe G, Aragno M. New viral diseases and new possible remedies by means of the pharmacology of the renin-angiotensin system. J Renin Angiotensin Aldosterone Syst. 2023;2023:3362391.
6. Hang W, Chen C, Seubert JM, Wang DW. Fulminant myocarditis: a comprehensive review from etiology to treatments and outcomes. Signal Transduct Target Ther. 2020;5:287.
7. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, et al. Natural RNA circles function as efficient microRNA sponges. Nature. 2013;495:384–8.
8. Cheng J, Zhuo H, Xu M, Wang L, Xu H, Peng J, et al. Regulatory network of circRNA-miRNA-mRNA contributes to the histological classification and disease progression in gastric cancer. J Transl Med. 2018;16:216.
9. Dance A. Circular logic: understanding RNA's strangest form yet. Nature. 2024;635:511–3.
10. Lu Y, Cheng J, Cai W, Zhuo H, Wu G, Cai J. Inhibition of circRNA circVPS33B reduces warburg effect and tumor growth through regulating the miR-873-5p/HNRNPK axis in infiltrative gastric cancer. Onco Targets Ther. 2021;14:3095–108.
11. Yang L, Fu J, Han X, Zhang C, Xia L, Zhu R, et al. Hsa_circ_0004287 inhibits macrophage-mediated inflammation in an N(6)-methyladenosine-dependent manner in atopic dermatitis and psoriasis. J Allergy Clin Immunol. 2022;149:2021–33.
12. Jeck WR, Sharpless NE. Detecting and characterizing circular RNAs. Nat Biotechnol. 2014;32:453–61.
13. Zhang L, Han B, Wang J, Liu Q, Kong Y, Jiang D, et al. Differential expression profiles and functional analysis of circular RNAs in children with fulminant myocarditis. Epigenomics. 2019;11:1129–41.
14. Nie X, Fan J, Li H, Wang J, Xie R, Chen C, et al. Identification of cardiac CircRNAs in Mice With CVB3-induced myocarditis. Front Cell Dev Biol. 2022;10: 760509.
15. Zhang L, Han B, Liu H, Wang J, Feng X, Sun W, et al. Circular RNA circACSL1 aggravated myocardial inflammation and myocardial injury by sponging miR-8055 and regulating MAPK14 expression. Cell Death Dis. 2021;12:487.
16. Huang S, Wu Z, Zhou Y. Hypoxia-induced circRNAs encoded by PPARA are highly expressed in human cardiomyocytes and are potential clinical biomarkers of acute myocardial infarction. Eur J Med Res. 2024;29:159.
17. Cheng J, Zhuo H, Wang L, Zheng W, Chen X, Hou J, et al. Identification of the combinatorial effect of miRNA family regulatory network in different growth patterns of GC. Mol Ther Oncolytics. 2020;17:531–46.
18. Huang L, Xiong S, Liu H, Li M, Zhang R, Liu Y, et al. Bioinformatics analysis of the inflammation-associated lncRNA-mRNA coexpression network in type 2 diabetes. J Renin Angiotensin Aldosterone Syst. 2023;2023:6072438.
19. Cao Z, Pan X, Yang Y, Huang Y, Shen HB. The lncLocator: a subcellular localization predictor for long non-coding RNAs based on a stacked ensemble classifier. Bioinformatics. 2018;34:2185–94.
20. Lin Y, Pan X, Shen HB. lncLocator 2.0: a cell-line-specific subcellular localization predictor for long non-coding RNAs with interpretable deep learning. Bioinformatics. 2021;37:2308–16.
21. Chong Y, Wu X-y, Wang Y-q, Gu Q-k, Zhang J-n, Meng X-l, et al. USP18 reduces the inflammatory response of LPS-induced SA-AKI by inhibiting the PI3K-AKT-NF-κB pathway and regulate apoptosis of cells. J Renin-Angiotensin-Aldosterone Syst. 2024. https://doi.org/10.1177/14703203241265218.
22. Gao S, Cheng J, Li G, Sun T, Xu Y, Wang Y, et al. Catechol-O-methyltransferase gene promoter methylation as a peripheral biomarker in male schizophrenia. Eur Psychiatry. 2017;44:39–46.
23. Ramirez-Hernandez D, Lezama-Martinez D, Velazco-Bejarano B, Valencia-Hernandez I, Lopez-Sanchez P, Fonseca-Coronado S, et al. The beneficial effects of swimming training preconditioning on reducing vascular reactivity in chronic myocardial infarction: Independent of NO production. J Renin-Angiotensin-Aldosterone Syst. 2024. https://doi.org/10.1177/14703203241294029.
24. Xiao MS, Wilusz JE. An improved method for circular RNA purification using RNase R that efficiently removes linear RNAs containing G-quadruplexes or structured 3′ ends. Nucleic Acids Res. 2019;47:8755–69.
25. Mantione KJ, Kream RM, Kuzelova H, Ptacek R, Raboch J, Samuel JM, et al. Comparing bioinformatic gene expression profiling methods: microarray and RNA-Seq. Med Sci Monit Basic Res. 2014;20:138–42.
26. Roh SW, Abell GC, Kim KH, Nam YD, Bae JW. Comparing microarrays and next-generation sequencing technologies for microbial ecology research. Trends Biotechnol. 2010;28:291–9.
27. Lee JY. The principles and applications of high-throughput sequencing technologies. Dev Reprod. 2023;27:9–24.
28. Git A, Dvinge H, Salmon-Divon M, Osborne M, Kutter C, Hadfield J, et al. Systematic comparison of microarray profiling, real-time PCR, and next-generation sequencing technologies for measuring differential microRNA expression. RNA. 2010;16:991–1006.
29. Yu L, Gao Y, Aaron N, Qiang L. A glimpse of the connection between PPARgamma and macrophage. Front Pharmacol. 2023;14:1254317.
30. Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM. PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell. 1998;93:241–52.
31. Zhang Y, Zhu L, Li X, Ge C, Pei W, Zhang M, et al. M2 macrophage exosome-derived lncRNA AK083884 protects mice from CVB3-induced viral myocarditis through regulating PKM2/HIF-1alpha axis mediated metabolic reprogramming of macrophages. Redox Biol. 2024;69: 103016.

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