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Brief Report
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MiRNA Signaling in Viral Myocarditis Novel and Unique Pathological Features

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Background: Micro-RNAs (miRNAs) are small non-coding RNAs that modulate many target genes. Viral myocarditis is common cardiomyopathy, however, there is an absence of effective therapeutic strategies for viral myocarditis (VMC). The purpose of this research was to characterize changes in miRNAs expression in VMC mice.
Methods: Atrial myocytes were infected coxsackievirus B3 and miRNAs microarray was performed. miRNAs target predicted and the bioinformatics analysis was carried out by gene ontology (GO) and KEGG pathway analysis. To validate the results, Difference miRNAs were identified in heart of mice by real-time polymerase chain reaction (PCR).
Results: We identified 94 miRNAs that were differentially expressed (27 were up-regulated and 67 were down-regulated by at least 2.0-fold). Real time PCR analysis has confirmed that the expression levels of 7 miRNAs up-regulated, 18 miRNAs down-regulated. They were mainly involved in protein binding, small GTPase mediated signal transduction, protein phosphorylation by GO. Pathway analysis showed that a significant enrichment in several pathways related to cAMP signaling pathway, AMPK signaling pathway, RAS signaling pathway, Rap1 signaling pathway, ErbB signaling pathway, Oxytocin signaling pathway.
Conclusions: Our results provide a better understanding of the mechanisms of viral myocarditis pathophysiology.

Key Words: Cardiovascular disease • Gene

INTRODUCTION

Viral myocarditis (VMC) is caused by viral infection of myocardial parenchyma, interstitial limitations or disuse lesions.[1] Myocarditis-associated viruses mainly include intestinal and upper respiratory tract infection viruses, of which coxsackie B3 virus is the most common.[2] Many pathogenic mechanisms may contribute to myocardial cell loss, including cytokine production contributing to myocardium inflammation, viral invasion of vascular endothelium causing vascular spasms with reperfusion injury, and viral persistence which may produce an autoimmune response to cardiac myosin.[3] VMC is a cause of dilated cardiomyopathy, most commonly in children and young adults.[4] However, effective therapeutic strategies for VMC are lacking due to a limited understanding of the molecular mechanisms underlying this disease. Therefore, elucidating the molecular mechanisms of VMC is important to understand and successfully treat cardiomyopathy.

Micro-RNAs (miRNAs) are non-coding, small ribonucleic acid (RNA) with a length from 20-24 nucleotides. They can regulate gene expression at the post-transcriptional level by binding to the 3'-untranslated regions of their target mRNA.[5] It has been reported that about 30% of mRNAs are regulated by miRNAs. miRNAs have also been reported to play a significant role in cell death, differentiation, cell signaling and various disease

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states.[6-8] Therefore, the analysis of miRNA expression profiles is crucial to elucidate their roles in the regulation of gene expression in cardiomyopathy.

In recent years a number of studies have profiled the relationship between miRNAs and VMC. Xu et al. found that miRNA-20b suppressed the expression of ZFP-148 and miRNA-1 repressed Cx43 in viral myocarditis.[9,10] In addition, Zhang et al reported that miRNA-155 attenuated cardiac injury and dysfunction in viral myocarditis.[11] These observations led us to hypothesize that miRNAs may play an important role in VMC. However, no comprehensive overview of miRNAs in VMC is currently available. Therefore, in this study, we investigated the expression profiles of miRNAs in VMC using miRNA microarrays. In addition, we selected certain altered miRNAs associated with VMC. Functional annotation and signaling pathways of the differentially expressed miRNAs were analyzed using Gene Ontology (GO) and KEGG pathway analysis. The aim of this study was to identify potential factors related with VMC, and evaluate whether miRNAs could serve as potential diagnostic biomarkers.

METHODS

Ethics statement

All experiments in this study were conducted according to the approved guidelines of the Animal Care and Use Committee of Inner Mongolia University for the Nationalities. The use of all animals related to this research was approved by the Animal Care and Use Committee of Inner Mongolia University for the Nationalities, and all animals were treated humanely in accordance with the Ministry of Food and Drug Safety on the ethical use of animals.

Isolation and culture of atrial myocytes

Following cervical dislocation of six mice (three in the control group and three in the VMC group) 3 days after birth, the hearts were rapidly excised and extraneous tissue was removed. The hearts were then cut into pieces and digested with a digestion buffer containing collagenase II at 37 °C. The hearts were gently pipetted to disperse the cells in suspension. The atrial myocytes were then cultured with Dulbecco's Modified Eagle Medium supplemented with 10% FBS and 1% antibiotic-antimycotic solution at 37 °C in a humidified atmosphere containing 5% CO_2. The atrial myocytes were identified by immunohistochemistry. The process of the isolation and culture of atrial myocytes is illustrated in Figure 1.

Viral infection of the atrial myocytes/mice

Coxsackie virus B3 was provided by Jilin University. After generation of three passages, the virus was collected to infect atrial myocytes. The atrial myocytes were seeded in six-well plates and cultured for 24 hours in a 5% CO2 incubator at 37 °C. The monolayers were then infected (multiplicity of infection = 100) for 24 hours. After 24 hours, the infected media was removed; the cells were then rinsed twice with Phosphate-buffered saline followed by incubation with fresh complete medium.

Ten BALB/c mice were housed under standard conditions. After 1 week, the rats were divided into a control group (CON, n = 5) that was intraperitoneally injected with saline at the indicated time points, and a VMC group (n = 5) that was intraperitoneally injected with 0.1 mL 10-9 50TCIDCVB3. After 10 days, the mice were sacrificed.

Histopathological study

After a final echocardiographic examination, the mice were sacrificed under deep anesthesia and the hearts were immediately excised. The hearts were cut and processed for hematoxylin and eosin (HE) staining. For biochemical tests, the samples were fixed in 4% formalin and embedded in paraffin for histological analysis. HE staining was used to evaluate basic myocardial histology. Microscopy was used to analyze the results.

Chip hybridization

An Affymetrix miRNA chip with a corresponding GeneChip® Hybridization, Wash, and Stain Kit was hybridized in a scrolling hybridization oven at 48 °C for 16 hours according to the manufacturer's instructions. After hybridization, the chip was washed in a Fluidics Station 450 system following the procedures provided by Affymetrix.

miRNA target prediction and bioinformatics analysis

The results from the chip were scanned using a

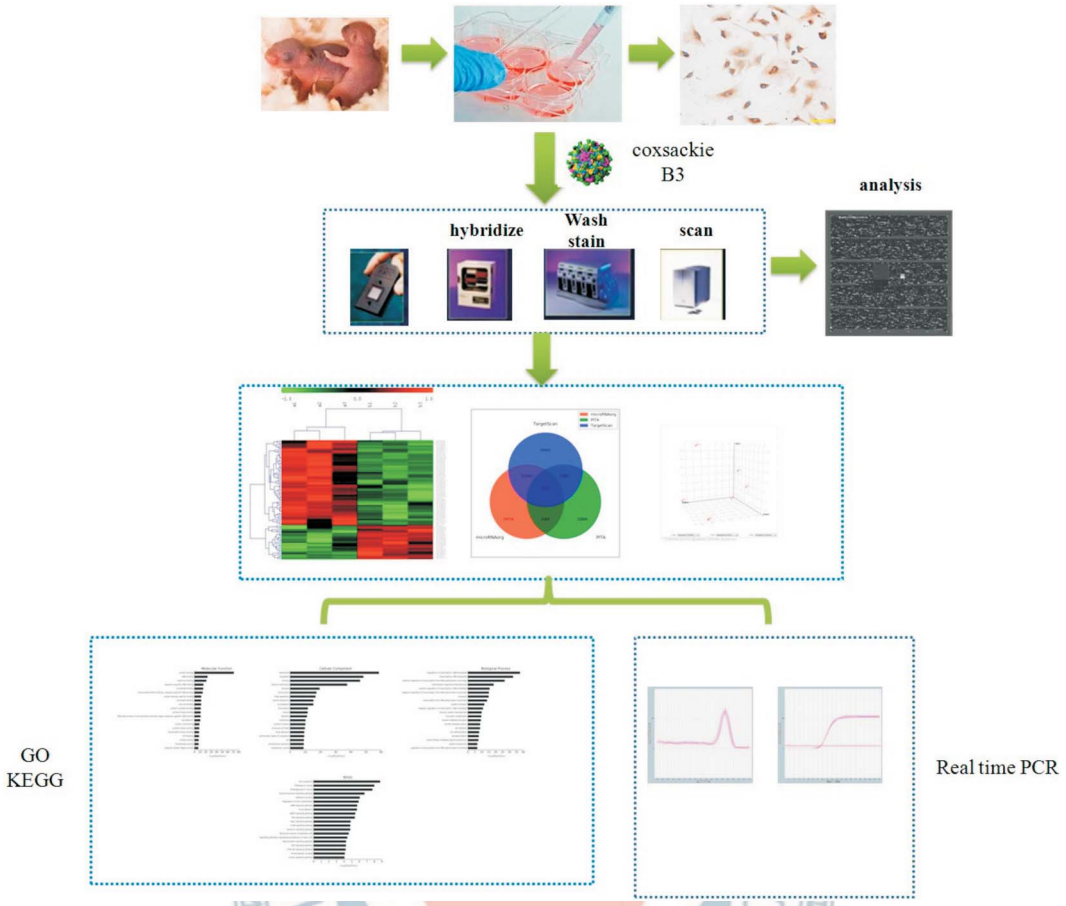

Figure 1. *The diagram of process.*

GeneChip Scanner 7G. Command Console Software 3.0 was used to load the raw data. Qualified data through quality testing were normalized using an Expression Console with the Robust Multichip Analysis and Detection above Background algorithm. TargetScan, miRDB, miRanda, miRBase, and miRWalk databases were used to predict the targets. The target genes were mapped to a gene ontology database (http://www.geneontology.org). The number of target genes was calculated, and a hypergeometric test was then used to find significantly enriched GO genes. GO enrichment included cell molecular function, molecular function and biological process. Based on KEGG pathway analysis, the biological functions of the genes were further identified. The target genes were mapped to the KEGG database (http://www.genome.jp/kegg), using the same methods as with GO enrichment. A P value ≥ 0.05 was taken to indicate that the target gene significantly enriched the GO function entry and the major KEGG signaling pathway.

Real time polymerase chain reaction (PCR)

Real time PCR was used to detect different miRNAs in each group. The primers of miRNA used for reverse transcriptase are shown in Supplementary Table 1. Each primer also had a 5' conserved region recognized by a universal reverse primer (5'-GTGCAGGGTCCGAGGT-3'). Amplification was performed in duplicate on an FTC-3000 Real-Time PCR system thermocycler using SYBR Green PCR Master Mix (TIANGEN, Beijing, China). The relative mRNA expression level of the miRNA was normalized to the level of U6 in the same sample. The average residual value was used as the final experimental retention data, and the $2^{-\Delta\Delta CT}$ method was used to analyze the data.

Statistical analysis

All values were expressed as mean ± SEM. Multigroup comparisons of the means were performed using a matched t-test with SPSS software version 17.0. The data matrix was analyzed using pattern recognition in

multivariate analysis. EZinfo software version 2.0 was used for principal component analysis (PCA).

RESULTS

Data analysis

The purity of the atrial myocytes was checked by α-SCA (Figure 2A). The RNA of the atrial myocytes was isolated, and the concentration, purity and integrity of the RNA samples in this study were found to be suitable for microarray experiments. After chip hybridization, the resulting signal strength picture was scanned (Figure 2B), and a darker background indicated a stronger hybridization signal.

PCA is a frequently used pattern recognition approach. In this study, PCA exhibited satisfactory classification, and both the control and VMC groups could clearly be differentiated in PCA score plots (Figure 2C).

Differential expression of miRNA in VMC as determined by a microarray

To explore whether the expressions of miRNAs were altered in response to VMC, we performed miRNA analysis using mice atrial myocytes or VMC. The results identified 94 miRNAs which were differentially expressed (Figure 3A, Supplementary Dataset 2) with a Q < 0.05. The expressions of 27 miRNAs were significantly increased in the mice atrial myocytes from the VMC group, whereas the expression of 67 miRNAs was significantly decreased (Figure 3B). These results demonstrated that the changes in the amount of miRNA occurred in response to VMC in the mice atrial myocytes.

Target gene prediction of known miRNAs

To improve the potential role in biosynthesis of the

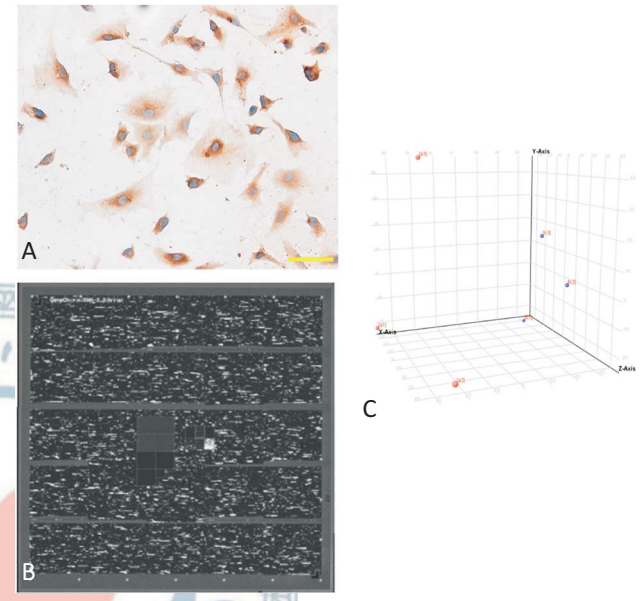

Figure 2. *Data analysis of miRNA profiling. (A) Atrial myocytes were identified by α-SCA. (B) Scan of chip hybridization. (C) PCA score plots of miRNA profiling in the control (a1-3) and VMC (b1-3) groups. miRNA, microRNAs; PCA, principal component analysis; VMC, Viral myocarditis.*

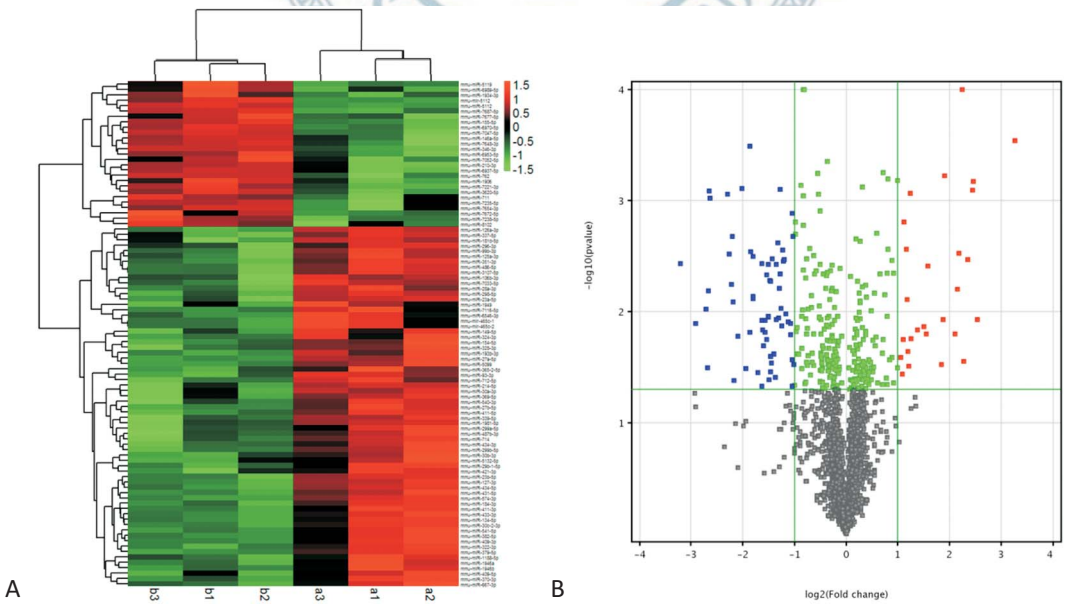

Figure 3. *Clustering of miRNAs differentially expressed in atrial myocytes, using affymetrix chip (A), and Volcano plot of miRNA (B).*

94 differentially-expressed miRNAs, we used GO and KEGG pathway analysis to predict and annotate the target genes. In GO, the putative miRNA targets were input for functional enrichment, and the main 61 GO terms are shown in Figure 4. These results demonstrated that the target genes of the 94 miRNAs were involved in a wide variety of pathophysiological processes after virus infection in the atrial myocytes.

The 61 GO terms were then used to explore the possible mechanisms. The results showed that they were mainly involved in protein binding, small GTPase-mediated signal transduction, and protein phosphorylation (Figure 4A).

KEGG pathway analysis was used to evaluate biological pathways. The results showed that the main enriched pathways were the cAMP signaling pathway, AMPK signaling pathway, RAS signaling pathway, Rap1 signaling pathway, ErbB signaling pathway and oxytocin signaling pathway (Figure 4B).

Validation of results with real-time PCR measurements

The mice hearts were analyzed using HE staining, which showed that no pathological changes were present in the control group. However, the hearts from the VMC mice revealed the formation of cytoplasmic vacuoles and myofibrillar loss (Figure 5A).

The expressions of selected miRNAs were determined using RT-PCR in order to confirm the results of the array expressions. We chose 25 miRNAs with significant differences (Figure 5B, C, D, E, F). The VMC-treated mice exhibited decreased expressions of mmu-miR-23b-5p, mmu-miR-27b-5p, mmu-miR-99b-3p, mmu-miR-126a-3p, mmu-miR-127-3p, mmu-miR-184-3p, mmu-miR-298-5p, mmu-miR-23a-5p, mmu-miR-27a-5p, mmu-miR-93-3p, mmu-miR-339-5p, mmu-miR-351-3p, mmu-miR-28a-3p, mmu-miR-379-5p, mmu-miR-441-5p, mmu-miR-431-5p, mmu-miR-434-5p, and mmu-miR-486-5p, and increased expressions of mmu-miR-7047-5p, mmu-miR-7227-3p, mmu-miR-7238-5p, mmu-miR-3620-5p, mmu-miR-7687-5p, mmu-miR-346-3p, and mmu-miR-155-5p.

DISCUSSION

The pathophysiological role of VMC is currently unknown. In order to address this problem, the present study assessed the levels of myocyte miRNAs using gene microarray analysis to compare the expression levels of

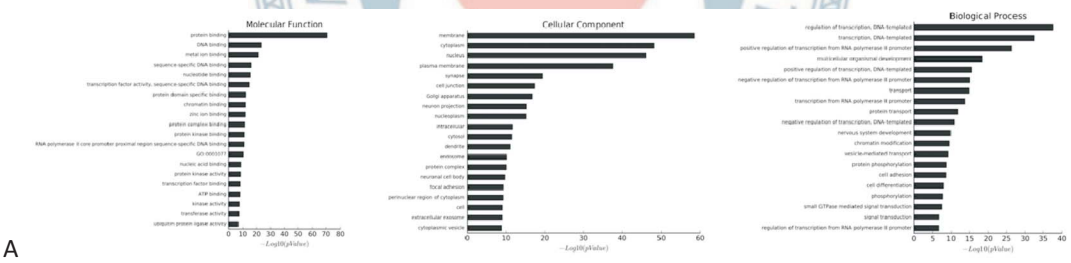

Figure 4. *The most enriched GO categories (A) and the most enriched pathway (B) of the differentially expressed target genes of the differentially expressed miRNAs. GO, gene ontology.*

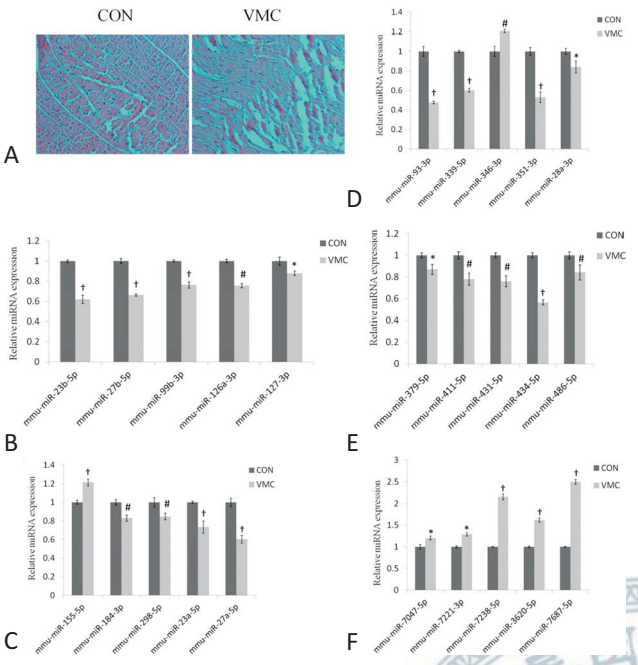

Figure 5. *Expressions of selected miRNAs were identified. (A) Histological features of the hearts from the mice in the control (CON) or VMC groups (H&E staining). (B/C/D/E/F) 25 miRNAs expressions in the mice hearts were analyzed using real-time PCR. All data are shown as mean ± SEM (n = 5). * p < 0.05, # p < 0.01, † p < 0.001. CON, control group; real time PCR, real-time polymerase chain reaction; SEM, structural equation modeling; VMC, viral myocarditis.*

miRNAs in the viral infection of atrial myocytes with controls. A total of 94 differentially expressed miRNAs were detected in this study, of which 27 were upregulated and 67 were downregulated. The 25 significantly different miRNAs were analyzed using real-time PCR, of which 18 were found to be downregulated and 7 upregulated. These results suggested that VMC is related to protein binding, small GTPase mediated signal transduction, protein phosphorylation factors, and the cAMP, AMPK, RAS, Rap1, ErbB, and oxytocin signaling pathways. Chip hybridization is a common method used to generate miRNA expression profiles. It has also been used to research the functional linkage between miRNAs and disease.[12] In this study, 94 miRNAs were analyzed, including mmu-miR-210-3p which targeted lnc-RI and PLK1 to effect heart function, and mmu-miR-155-5p which has been shown to be upregulated in an ApoE-deficient mouse model.[13] MiR-146a has been shown to protect against ischemic cardiac injuries,[14] and attenuated inhibition of mmu-miR-296-3p has been shown to promote the differentiation of embryonic stem cell into a cardiac lineage.[15] These observations indicate that the dysregulation of miRNA expressions may be involved in the mechanism of cardiomyopathy.

GO analysis has been used to assign functions to differentially expressed genes and their downstream target genes.[16] We used KEGG pathway analysis in this study, and found that the 94 differentially expressed miRNAs were closely associated with the cAMP, AMPK, RAS signaling pathway, Rap1, ErbB, and oxytocin signaling pathways.

As an inflammatory process of the myocardium, VMC results in injury to the cardiac muscle cells and the manifestations range from subclinical to sudden death.[17,18] In VMC, the ERK signaling pathway has been shown to play a key role.[19] The small GTP binding protein Ras has been shown to activate Raf/MEK/ERK cascade by binding Raf and anchoring it at the cell membrane, where it is phosphorylated and activated by other kinases.[20] Our results showed that small GTPase mediated signal transduction and activated the RAS signaling pathway, which then activated the ERK signaling pathway in VMC. The ErbB signaling pathway has been associated with both cardiac development and the maintenance of structural and functional integrity of the adult heart.[21] Downregulation of ErbB has been reported to result in a low incidence of cardiac dysfunction, and although ErbB-deficient conditional mutant mice were viable at birth, adult animals developed severely dilated cardiomyopathy.[22] The ErbB pathway is activated during VMC. Interestingly, we found that the oxytocin signaling pathway was involved in VMC, and we plan to study the association between oxytocin and VMC in future studies.

Limitations

The conclusion was made from previous experiments and related literature, and there were still many uncertainties. The different miRNAs were initially verified using real-time PCR, and further in depth studies are needed to verify the results.

CONCLUSIONS

VMC is a serious cardiomyopathy, and the identification of the mechanisms through which it regulates the

expressions of miRNAs will lead to a better understanding of this complex pathway. This work provides the first small RNA expression analysis in VMC. GO and KEGG pathway analyses revealed the predicted targets of the miRNAs expressed in a variety of pathways targets. These findings may lead to the development of advanced prognostic assays and therapeutic approaches for VMC.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Cooper LTJ. Myocarditis. *N Engl J Med* 2009;360:1526-38.
2. Lv S, Rong J, Ren S, et al. Epidemiology and diagnosis of viral myocarditis. *Hellenic J Cardiol* 2013;54:382-91.
3. Liu ZL, Liu ZJ, Liu JP, et al. Herbal medicines for viral myocarditis. *Cochrane Database Syst Rev* 2010;7:CD003711.
4. Fairweather D, Cooper LT Jr, Blauwet LA. Sex and gender differences in myocarditis and dilated cardiomyopathy. *Curr Probl Cardiol* 2013; 38:7-46.
5. Anna MB, Konrad JD, Katarzyna L. Alterations in miRNA levels in the dentate gyrus in epileptic rats. *PLoS One* 8:e76051.
6. Heinrich EM, Dimmeler S. MicroRNAs and stem cells: control of pluripotency, reprogramming, and lineage commitment. *Circ Res* 2012;110:1014-22.
7. Chen W, Shao D, Gu H, et al. Hsa-mir-499 rs3746444 t/c polymorphism is associated with increased risk of coronary artery disease in a chinese population. *Acta Cardiol Sin* 2017;33:34-40.
8. Mendell JT. MicroRNAs: critical regulators of development, cellular physiology and malignancy. *Cell Cycle* 2005;4:1179-84.
9. Xu HF, Ding YJ, Shen YW, et al. MicroRNA-1 represses Cx43 expression in viral myocarditis. *Mol Cell Biochem* 2012;362:141-8.
10. Xu HF, Gao XT, Lin JY, et al. MicroRNA-20b suppresses the expression of ZFP-148 in viral myocarditis. *Mol Cell Biochem* 2017; 429:199-210.
11. Zhang Y, Zhang M, Li X, et al. Silencing MicroRNA-155 attenuates cardiac injury and dysfunction in viral myocarditis via promotion of M2 phenotype polarization of macrophages. *Sci Rep* 2016; 6:22613.
12. Taro U, Takashi F. Label-free quantification of MicroRNAs using ligase-assisted sandwich hybridization on a DNA microarray. *PLoS One* 2014;9:e90920.
13. Jia C, Xiong M, Wang P, et al. Notoginsenoside R1 attenuates atherosclerotic lesions in ApoE deficient mouse model. *PLoS One* 2014;9:e99849.
14. Huang W, Tian SS, Hang PZ, et al. Combination of microRNA-21 and microRNA-146a attenuates cardiac dysfunction and apoptosis during acute myocardial infarction in mice. *Mol Ther Nucleic Acids* 2016;5:e296.
15. Sun M, Yan X, Bian Y, et al. Improving murine embryonic stem cell differentiation into cardiomyocytes with neuregulin-1: differential expression of microRNA. *Am J Physiol Cell Physiol* 2011;301: C21-30.
16. Ashburner M, Ball CA, Blake JA, et al. Gene ontology: tool for the unification of biology. *Nat Genet* 2000;25:25-9.
17. Zhou X, Xin Q, Wang Y, et al. Total flavonoids of astragalus plays a cardioprotective role in viral myocarditis. *Acta Cardiol Sin* 2016; 32:81-8.
18. Hung Y, Lin WH, Lin CS, et al. The prognostic role of QTC interval in acute myocarditis. *Acta Cardiol Sin* 2016;32:223-30.
19. Luo H, Yanagawa B, Zhang J, et al. Coxsackievirus B3 replication is reduced by inhibition of the extracellular signal-regulated kinase (ERK) signaling pathway. *J Virol* 2002;76:3365-73.
20. Marais R, Light Y, Paterson HF, Marshall CJ. Ras recruits Raf-1 to the plasma membrane for activation by tyrosine phosphorylation. *EMBO J* 1995;14:3136-45.
21. Liu X, Gu X, Li Z, et al. Neuregulin-1/erbB-activation improves cardiac function and survival in models of ischemic, dilated, and viral cardiomyopathy. *J Am Coll Cardiol* 2006;48:1438-47.
22. Crone SA, Zhao YY, Fan L, et al. ErbB2 is essential in the prevention of dilated cardiomyopathy. *Nat Med* 2002;8:459-65.

SUPPLEMENT

Supplementary Table 1

miRNA	prime
mmu-miR-23b-5p	GGGTTCCTGGCATGCTGATTT
mmu-miR-27b-5p	AGAGCTTAGCTGATTGGTGAAC
mmu-miR-99b-3p	CAAGCTCGTGTCTGTGGGTCCG
mmu-miR-126a-3p	TCGTACCGTGAGTAATAATGCG
mmu-miR-127-3p	TCGGATCCGTCTGAGCTTGGCT
mmu-miR-155-5p	TTAATGCTAATTGTGATAGGGGT
mmu-miR-184-3p	TGGACGGAGAACTGATAAGGGT
mmu-miR-298-5p	GGCAGAGGAGGGCTGTTCTTCCC
mmu-miR-23a-5p	GGGGTTCCTGGGGATGGGATTT
mmu-miR-27a-5p	AGGGCTTAGCTGCTTGTGAGCA
mmu-miR-93-3p	ACTGCTGAGCTAGCACTTCCCG
mmu-miR-339-5p	TCCCTGTCCTCCAGGAGCTCACG
mmu-miR-346-3p	AGGCAGGGGCTGGGCCTGCAGC
mmu-miR-351-3p	GGTCAAGAGGCGCCTGGGAAC
mmu-miR-28a-3p	CACTAGATTGTGAGCTGCTGGA
mmu-miR-379-5p	TGGTAGACTATGGAACGTAGG
mmu-miR-411-5p	TAGTAGACCGTATAGCGTACG
mmu-miR-431-5p	TGTCTTGCAGGCCGTCATGCA
mmu-miR-434-5p	GCTCGACTCATGGTTTGAACCA
mmu-miR-486-5p	TCCTGTACTGAGCTGCCCCGAG
mmu-miR-7047-5p	TGAGGGAGGAGGGCTGGGTCTGA
mmu-miR-7221-3p	TGACTGTGGGCTGGGGACTGG
mmu-miR-7238-5p	ATGCGGATGGGACGCGGC
mmu-miR-3620-5p	CTGTGGGCTGGGCTGGGAAGCA
mmu-miR-7687-5p	AGGCGGGGAACCTGAGGCGCAGGCT

Supplementary Dataset 2. The differently expressed miRNA between mice atrial myocytes or VMC

Probe Set ID	Transcript ID (Array Design)_1	a1	a2	a3	b1	b2	b3	a mean	b mean	t-test	
20500243	mmu-miR-23b-5p	7.058	7.11	6.519	4.50605	3.791367	4.491651	6.9	4.3	0.00	down
20500245	mmu-miR-27b-5p	7.235	6.772	6.081	4.5707	4.512128	4.218266	6.7	4.4	0.00	down
20500247	mmu-miR-29b-1-5p	4.623	4.633	3.516	2.58677	3.078969	2.901978	4.3	2.9	0.02	down
20500250	mmu-miR-30a-3p	6.428	6.689	6.565	5.8764	5.239662	5.063925	6.6	5.4	0.01	down
20500252	mmu-miR-30b-3p	3.551	4.266	3.135	1.78413	2.459478	1.87639	3.7	2.0	0.01	down
20500256	mmu-miR-99b-3p	6.7	6.308	6.136	4.9902	4.502249	5.113517	6.4	4.9	0.00	down
20500262	mmu-miR-125a-3p	6.565	6.003	5.713	4.34331	3.820297	4.727686	6.1	4.3	0.01	down
20500266	mmu-miR-126a-3p	7.800	7.531	7.554	5.54986	5.731235	6.021346	7.6	5.8	0.00	down
20500268	mmu-miR-127-3p	10.5	10.76	10.37	9.31393	9.104083	9.378083	10.5	9.3	0.00	down
20500279	mmu-miR-134-5p	8.560	8.626	7.307	5.99886	5.742778	6.20188	8.2	6.0	0.01	down
20500299	mmu-miR-146a-5p	7.148	5.868	7.801	9.60572	9.596407	9.245592	6.9	9.5	0.01	up
20500301	mmu-miR-149-5p	5.846	6.423	6.441	5.44127	5.03489	4.864625	6.2	5.1	0.01	down
20500311	mmu-miR-154-5p	5.526	6.51	5.649	4.40072	4.628177	4.147443	5.9	4.4	0.01	down
20500313	mmu-miR-155-5p	9.045	8.65	9.132	10.7967	11.09335	10.66839	8.9	10.9	0.00	up
20500370	mmu-miR-184-3p	7.674	7.734	7.142	6.45186	6.046933	6.242541	7.5	6.2	0.00	down
20500641	mmu-miR-296-3p	6.512	6.522	5.89	5.15804	4.104545	5.318274	6.3	4.9	0.03	down
20500643	mmu-miR-298-5p	6.973	6.814	6.806	6.0587	5.689951	5.72008	6.9	5.8	0.00	down
20500645	mmu-miR-299a-5p	7.336	7.508	6.694	6.34371	6.285244	5.767265	7.2	6.1	0.03	down
20500662	mmu-miR-106b-3p	6.879	6.989	7.169	6.15549	5.457778	6.186553	7.0	5.9	0.01	down
20500855	mmu-miR-30c-2-3p	5.558	5.623	4.832	3.97261	3.696945	3.925376	5.3	3.9	0.01	down
20500895	mmu-miR-23a-5p	6.957	7.056	6.82	5.58827	4.632391	5.064385	6.9	5.1	0.00	down
20500906	mmu-miR-27a-5p	4.808	5.507	4.955	3.17325	3.071717	2.984106	5.1	3.1	0.00	down
20500913	mmu-miR-93-3p	4.347	3.837	4.441	1.66804	2.491463	1.85826	4.2	2.0	0.00	down

Supplementary Dataset 2. Continued

20500927	mmu-miR-322-3p	7.671	7.795	7.053	6.03592	6.317962	6.284594	7.5	6.2	0.01	down
20500937	mmu-miR-324-3p	3.711	5.449	5.159	3.2019	3.127244	2.859463	4.8	3.1	0.04	down
20500952	mmu-miR-328-3p	5.397	6.109	5.442	4.45432	4.590423	3.973314	5.6	4.3	0.01	down
20500972	mmu-miR-337-5p	6.894	6.741	6.432	4.96141	4.812746	5.797091	6.7	5.2	0.01	down
20500984	mmu-miR-339-5p	3.758	4.117	3.706	2.64078	2.468778	1.857463	3.9	2.3	0.00	down
20501008	mmu-miR-346-3p	5.332	5.183	5.676	6.49426	6.523997	6.5184	5.4	6.5	0.00	up
20501023	mmu-miR-351-3p	7.407	7.055	6	3.66386	3.059319	4.145864	6.8	3.6	0.00	down
20501096	mmu-miR-28a-3p	8.763	8.192	8.421	7.31941	6.811981	7.181855	8.5	7.1	0.00	down
20501106	mmu-miR-210-3p	4.108	4.134	5.791	6.5239	6.65056	6.408361	4.7	6.5	0.03	up
20501110	mmu-miR-214-5p	6.636	6.524	6.374	5.06794	4.103996	3.701334	6.5	4.3	0.01	down
20501262	mmu-miR-379-5p	9.616	9.704	9.107	8.24997	8.295544	8.176879	9.5	8.2	0.00	down
20501268	mmu-miR-382-5p	9.041	9.227	8.346	7.75449	7.751234	7.866969	8.9	7.8	0.02	down
20501786	mmu-miR-409-5p	5.177	5.6	4.332	4.21827	3.190034	3.223037	5.0	3.5	0.04	down
20501787	mmu-miR-409-3p	9.121	9.377	7.683	6.68749	6.525384	6.686067	8.7	6.6	0.02	down
20501792	mmu-miR-411-5p	6.163	6.059	5.662	4.66182	4.839479	4.428467	6.0	4.6	0.00	down
20501793	mmu-miR-411-3p	6.679	6.496	4.624	3.22885	2.672307	3.166369	5.9	3.0	0.01	down
20501797	mmu-miR-370-3p	5.547	5.651	3.277	2.72145	1.746371	1.980614	4.8	2.1	0.03	down
20502243	mmu-miR-431-5p	7.376	7.934	7.011	5.78803	5.477086	5.677668	7.4	5.6	0.00	down
20502246	mmu-miR-433-3p	8.734	9.179	7.53	5.86267	5.545388	6.037895	8.5	5.8	0.01	down
20502247	mmu-miR-434-5p	5.316	5.579	4.917	2.96437	2.687088	3.281727	5.3	3.0	0.00	down
20502248	mmu-miR-434-3p	8.293	8.809	7.706	6.82242	6.852587	6.243801	8.3	6.6	0.01	down
20502365	mmu-miR-365-2-5p	4.446	3.438	3.191	1.81541	2.458215	2.017236	3.7	2.1	0.02	down
20504161	mmu-miR-486-5p	7.728	7.547	7.268	6.47353	6.046932	6.448763	7.5	6.3	0.00	down
20504195	mmu-miR-540-3p	6.633	6.259	5.418	4.14372	3.46825	2.571287	6.1	3.4	0.01	down
20504200	mmu-miR-541-5p	10.46	10.71	9.704	9.06277	8.894844	8.874989	10.3	8.9	0.01	down
20504226	mmu-miR-487b-3p	6.696	7.133	6.208	5.47883	5.283536	4.549468	6.7	5.1	0.02	down
20504227	mmu-miR-369-5p	3.349	3.632	3.092	2.41917	1.614788	1.339837	3.4	1.8	0.01	down
20504629	mmu-miR-667-3p	5.835	6.561	4.218	3.52454	2.879233	3.701178	5.5	3.4	0.04	down
20504632	mmu-miR-762	7.945	8.367	8.541	9.44379	9.449389	9.448331	8.3	9.4	0.00	up
20504739	mmu-miR-711	1.852	4.038	3.195	5.20285	4.979016	5.753356	3.0	5.3	0.03	up
20504740	mmu-miR-712-5p	5.092	4.362	4.795	3.29265	3.286027	2.798494	4.7	3.1	0.00	down
20504743	mmu-miR-714	6.189	6.514	5.711	4.74894	4.448696	3.830518	6.1	4.3	0.01	down
20505648	mmu-miR-181d-5p	5.922	5.69	5.795	4.14222	4.435538	5.033362	5.8	4.5	0.01	down
20505695	mmu-miR-193b-3p	8.25	8.91	8.599	7.2627	7.404954	7.433349	8.6	7.4	0.00	down
20505702	mmu-miR-421-3p	6.488	6.306	5.547	4.13292	5.07823	4.520666	6.1	4.6	0.02	down
20505731	mmu-miR-574-3p	8.765	9.025	8.096	5.88157	5.881572	6.174248	8.6	6.0	0.00	down
20506735	mmu-miR-1188-5p	4.293	3.865	2.535	2.01886	1.513722	2.279879	3.6	1.9	0.05	down
20509215	mmu-miR-1906	2.184	2.516	3.46	5.36747	4.966049	4.140177	2.7	4.8	0.02	down
20510736	mmu-miR-1934-3p	2.64	3.202	3.02	5.07823	4.048916	4.232461	3.0	4.5	0.01	up
20510748	mmu-miR-1946a	5.632	5.02	4.161	3.33247	2.760436	3.151362	4.9	3.1	0.02	down
20510753	mmu-miR-1949	5.532	4.86	6.399	4.47534	3.324847	3.187769	5.6	3.7	0.03	down
20510783	mmu-miR-1946b	5.848	5.362	4.308	3.8949	3.677896	3.565872	5.2	3.7	0.03	down
20510805	mmu-miR-1981-5p	5.014	5.092	4.847	4.06661	3.986969	3.172978	5.0	3.7	0.01	down
20515484	mmu-miR-3107-5p	7.728	7.547	7.268	6.47353	6.046932	6.448763	7.5	6.3	0.00	down
20520354	mmu-miR-5099	8.275	8.644	8.185	7.30786	7.418662	7.304875	8.4	7.3	0.00	down
20520365	mmu-miR-5112	5.308	5.447	5.452	7.80913	7.564215	7.579542	5.4	7.7	0.00	up
20520370	mmu-miR-5119	1.586	1.508	1.275	3.70156	2.886644	2.419024	1.5	3.0	0.02	up
20520388	mmu-miR-5132-5p	3.026	3.502	2.442	1.7503	2.351868	1.740167	3.0	1.9	0.05	down
20521894	mmu-miR-299b-5p	5.893	6.385	5.609	4.66539	4.619392	4.232455	6.0	4.5	0.01	down
20525835	mmu-miR-6937-5p	5.264	5.397	6.592	7.73266	7.664437	7.484029	5.8	7.6	0.01	up

Supplementary Dataset 2. Continued

20525867	mmu-miR-6953-5p	3.228	2.999	3.654	4.35168	4.749096	4.305473	3.3	4.5	0.01	up
20525903	mmu-miR-6970-5p	4.661	3.927	4.449	7.0419	6.821381	6.500749	4.3	6.8	0.00	up
20525941	mmu-miR-6989-5p	2.638	2.059	2.013	3.93004	3.370574	3.010185	2.2	3.4	0.02	up
20526031	mmu-miR-7033-5p	6.735	6.619	7.051	5.46022	5.003038	5.642908	6.8	5.4	0.00	down
20526059	mmu-miR-7047-5p	6.202	5.8	6.211	7.37539	7.292303	7.270115	6.1	7.3	0.00	up
20526069	mmu-miR-7052-5p	2.037	2.388	2.798	3.51336	3.950972	3.005311	2.4	3.5	0.04	up
20526190	mmu-miR-7116-5p	2.683	1.649	2.324	1.15466	1.276717	1.20887	2.2	1.2	0.03	down
20527059	mmu-miR-7221-3p	5.292	5.319	5.97	7.40055	6.938006	7.001113	5.5	7.1	0.00	up
20527086	mmu-miR-7235-5p	3.053	4.165	3.522	4.79955	4.893777	4.824598	3.6	4.8	0.02	up
20527092	mmu-miR-7238-5p	2.289	2.257	1.565	4.14192	4.058033	4.989943	2.0	4.4	0.00	up
20528505	mmu-miR-6546-3p	3.162	2.023	3.47	1.42874	1.380593	1.734128	2.9	1.5	0.04	down
20528511	mmu-miR-7648-3p	5.518	4.857	6.245	7.72187	7.85415	7.522595	5.5	7.7	0.01	up
20528527	mmu-miR-7654-3p	1.441	2.554	1.924	3.45412	3.452432	3.157987	2.0	3.4	0.01	up
20528562	mmu-miR-7672-5p	1.112	1.263	1.026	1.69031	2.386	2.943945	1.1	2.3	0.03	up
20528580	mmu-miR-7677-5p	1.353	0.801	1.245	2.28302	2.618806	1.819741	1.1	2.2	0.02	up
20528590	mmu-miR-3620-5p	3.073	3.509	4.053	6.04242	5.764999	5.393424	3.5	5.7	0.00	up
20528614	mmu-miR-7687-5p	1.85	2.661	2.032	5.42645	5.672873	5.24463	2.2	5.4	0.00	up
20529965	mmu-miR-8102	2.328	1.872	1.353	2.87742	2.72175	3.137844	1.9	2.9	0.03	up
20535609	mmu-mir-465c-1	2.911	1.821	3.132	1.2703	0.955456	1.26992	2.6	1.2	0.03	down
20535610	mmu-mir-465c-2	2.911	1.821	3.132	1.2703	0.955456	1.26992	2.6	1.2	0.03	down
20537102	mmu-mir-5112	1.516	1.342	1.434	4.07634	4.217277	3.395331	1.4	3.9	0.00	up