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Original Research

Analysis of circRNA regulatory network in myocardial tissue of type 1 diabetic mice

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Abstract: Type 1 diabetes mellitus, also called insulin-dependent diabetes is associated with elevated blood glucose concentration arising from the inability of the pancreas to produce insulin. Diabetic cardiomyopathy is a major cause of death in diabetic patients. CircRNAs have been reported to participate in various human diseases, including diabetic cardiomyopathy. In this study, the regulation network of circRNA in type 1 diabetes mellitus was investigated. Streptozotocin treatment was implemented to induce type 1 diabetes mellitus in the mouse model, and echocardiography was implemented to detect the heart function of the type 1 diabetes mellitus mouse. Also, the qRT-PCR assay was used to identify the circRNA expression in type 1 diabetes mellitus mouse myocardial tissue. Findings showed that heart function of type 1 diabetes mellitus mouse was significantly damaged than control group mouse and cardiac hypertrophy in type 1 diabetes mellitus mouse, circRNAs were aberrantly regulated in type 1 diabetes mellitus mouse myocardial tissue. The following circRNAs were mmu_circ_0001560, mmu_circ_0001800, mmu_circ_0001801, mmu_circ_0002281 and mmu_circ_0000614 were expressed low in type 1 diabetes mellitus mouse myocardial tissue. In conclusion, type 1 diabetes mellitus caused alterations in the regulation network of circRNAs.

Key words: circRNA; Diabetes; Heart; Cardiac hypertrophy.

Introduction

Diabetic cardiomyopathy occurs in diabetic patients and its potential reasons are not connected to hypertension, atherosclerosis and valvopathy diseases, but because of diabetes mellitus (1-3). Heart failure has been the main danger of diabetic cardiomyopathy, which may cause sudden death (4). Diabetic cardiomyopathy can cause myocardial cells apoptosis and hypertrophy, and affect the normal function of myocardial cells (5). Non-coding RNAs (ncRNAs) have been identified as involved in the regulatory network of various human diseases, such as cancer and diabetes mellitus (6, 7). Circular RNA (circRNAs) was discovered as a new class of ncRNAs which was formed by a covalently closed loop (8, 9), and are stable in the cytoplasm of cells, playing a significant function in modulating the progression of human diseases (10-13). Considering this, we hypothesized that the circRNA regulatory network in diabetic cardiomyopathy could be specifically studied for developing better treatment for diabetic cardiomyopathy patients.

Materials and Methods

Establishment of type 1 diabetes mellitus mouse model

Twenty-five male C57 mice (8-week-old), weighing 23 to 25g, were available from the Shanghai Laboratory Animal Center of the Chinese Academy of Sciences (Shanghai, China). Mice were randomly divided into the diabetes mellitus group (n=15) and the control group (n=10). The animal study was implemented with approval from the Animal Research Ethics Committee of the Department of Medicine, Shandong Medical College, China. For the type 1 diabetes mellitus mouse model, mice were subjected to the single intraperitoneal injection of Streptozotocin (STZ, 150 mg/kg; Sigma Aldrich, St. Louis, MI). After 72 h, the random blood glucose in tail vein blood was detected by the One Touch blood glucose meter. A blood glucose level of more than 16.7 mmol/L was considered as the successful modeling. Mice in the control group were treated with a single intraperitoneal injection of equal saline solution.

Detection of heart function

8 weeks after modeling, echocardiography was performed on the mice. Mice were fixed on their backs with a slight tilt to the left, and M-mode ultrasound images of the parasternal short-axis images were obtained. The following indicators were measured: [1] left ventricular ejection fraction (LVEF) and left ventricular short-axis shortening rate (FS); [2] Left ventricular mass index (LVWI). Body weight and left ventricular mass of mice were measured before and after sacrifice, LVWI = left ventricular mass/body mass.

Histological examination of cardiomyocytes

Left ventricular myocardial tissue samples were acquired and fixed by 10% PBS neutral formaldehyde solution, then dehydrated. After washing, tissues were embedded in paraffin. Hematoxylin and eosin (H&E) staining was used for observing the morphological changes of cardiomyocytes, with light microscopy. Qu-

antitative analysis of cardiomyocytes cross-sectional area was achieved by QLAB image analysis software (Leica Microsystems, Wetzlar, Germany).

Quantitative real-time PCR (qRT-PCR)

Expression of circRNAs in myocardial tissue was measured by qRT-PCR. Using TRIzol reagent (Invitrogen, Carlsbad, CA), the total RNAs were extracted from the mice myocardial tissue, quantified by spectrophotometer (Bio-Rad, Hercules, CA). The RNAs from each group were subjected to cDNA synthesis for qPCR with SYBR green Supermix (Thermo Fisher, Waltham, MA). The change in circRNA expression was calculated employing the comparative change-in-cycle method (ΔΔCt), with GAPDH as the standardized gene.

Statistical analyses

Bio-triple repeats were used in all experiments, and the results were displayed with the standard deviation (S.D.). Statistical Product and Service Solutions (SPSS) 18.0 (SPSS Inc., Chicago, IL) was applied for comparison of groups with Student's t-test or one-way ANOVA. The threshold of statistical significance was set as $p < 0.05$.

Results

Type 1 diabetes mellitus mouse model succeeded

We searched the survival rate of type 1 diabetes mellitus mouse (Figure 1A). The survival rate of type 1 diabetes mellitus mouse was found significantly *($p<0.05$)* low compared to control group mouse. Meanwhile, echocardiography was implemented to detect the LVEF, LVWI and FS of type 1 diabetes mellitus mouse (Figure 1B). Results indicated that the heart function of the type 1 diabetes mellitus mouse was significantly *($p<0.05$)* damaged than the control group mouse. Hence, the type 1 diabetes mellitus mouse model was succeeded.

Cardiac hypertrophy was found in type 1 diabetes mellitus mouse

Next, we studied the heart of type 1 diabetes mellitus mouse. Quantitative analysis of the cross-sectional area of cardiomyocytes was implemented (Figure 2). Results showed that cardiac hypertrophy was obvious in type 1 diabetes mellitus mouse.

Some circRNAs were aberrantly up-regulated in type 1 diabetes mellitus mouse myocardial tissue

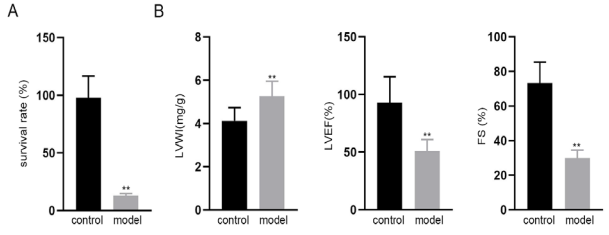

Figure 1. The type 1 diabetes mellitus mouse model was succeeded. A: survival rate of type 1 diabetes mellitus mouse was detected. B: echocardiography was implemented to detect the LVEF, LVWI and FS of type 1 diabetes mellitus mouse.

We investigated the expression of circRNAs in type 1 diabetes mellitus mouse myocardial tissue via qRT-PCR assay (Figure 3). We found several circRNAs were up-regulated in type 1 diabetes mellitus mouse myocardial tissue, and they were mmu_circ_0002083, mmu_circ_0000450, mmu_circ_0002554 and mmu_circ_0001460.

Some circRNAs were aberrantly down-regulated in type 1 diabetes mellitus mouse myocardial tissue

Also, we searched circRNAs that were lowly expressed in type 1 diabetes mellitus mouse myocardial tissue via qRT-PCR assay (Figure 4). And they were mmu_circ_0001560, mmu_circ_0001800, mmu_circ_0001801, mmu_circ_0002281 and mmu_circ_0000614.

Discussion

Diabetes mellitus can cause diabetic cardiomyopathy (14). In this study, we detected the left ventricular ejection fraction (LVEF), left ventricular short-axis shortening rate (FS) and left ventricular mass index (LVWI) of the mouse model showed changes following treatment of streptozotocin. Also, the mouse survival rate was reduced in the diabetic group. The heart function was significantly inhibited by streptozotocin treatment, which proved that the type 1 diabetes mellitus mouse model

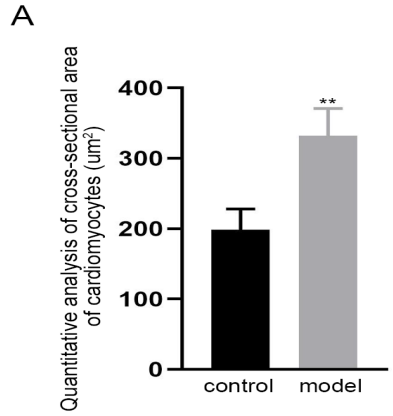

Figure 2. Cardiac hypertrophy was found in type 1 diabetes mellitus mouse. Quantitative analysis of the cross-sectional area of cardiomyocytes was presented.

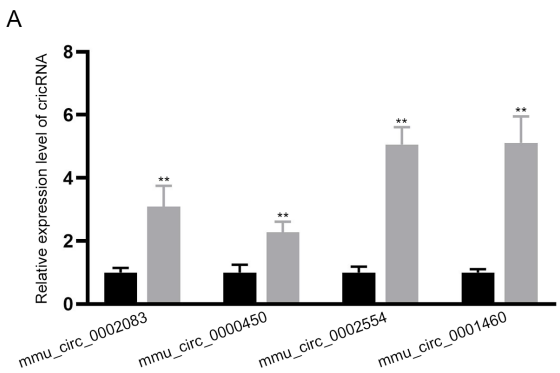

Figure 3. Some circRNAs were aberrantly up-regulated in type 1 diabetes mellitus mouse myocardial tissue. circRNA expressions were detected in type 1 diabetes mellitus mouse myocardial tissue via qRT-PCR assay.

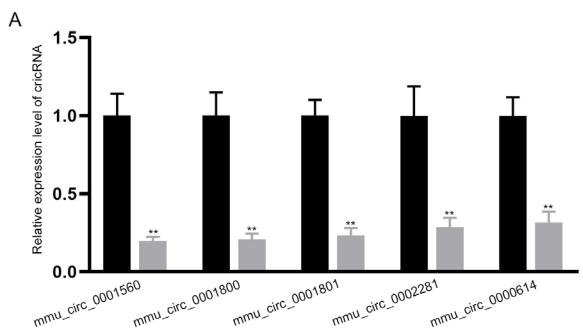

Figure 4. Some circRNAs were aberrantly down-regulated in type 1 diabetes mellitus mouse myocardial tissue. circRNA expressions were detected in type 1 diabetes mellitus mouse myocardial tissue via qRT-PCR assay.

was succeeded.

The previous study has been identified that circRNAs are playing regulatory functions in diabetes mellitus. For instance, circRNA_0054633 is connected to gestational diabetes mellitus (Wu *et al.,* 2019) and circ_0054633 can function as a biomarker of type 2 diabetes mellitus (15). In this study, we searched the expression of circRNAs in mouse myocardial tissue, and detected some circRNAs were dysregulated in mouse myocardial tissue via qRT-PCR.

In our study, we observed aberrant expressed circRNAs in type 1 diabetes mellitus mouse model myocardial tissue, which can give much improvement in study the circRNA regulatory network in diabetic cardiomyopathy.

Acknowledgements
None.

Conflict of interests
None.

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