The molecular mechanism of serum microRNA124b induced coronary heart disease by inducing myocardial cell senescence


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Abstract. – OBJECTIVE: The incidence and mortality of coronary heart disease are rapidly increasing in recent years. Myocardial cell dysfunction and cell senescence may play a role in coronary heart disease. MicroRNA controls a variety of biological processes, but leaving its role in coronary heart disease has yet to be explored.

PATIENTS AND METHODS: Patients with coronary heart disease were regarded as subjects, and healthy volunteers as the control, on both of which microRNA124b level of serum was studied by Real-time PCR, and the heart function of patients was detected by using ultrasound. The relationship between serum microRNA124b level and cardiac function was analyzed along with the model of rat coronary artery disease; the level of aging proteins P21 and P53 in cardiac muscle cells was also tested.

RESULTS: MicroRNA124b in the serum of patients with coronary heart disease was increased, and the heart function of patients was decreased (p < 0.05). Serum level of microRNA124b in a rat model of coronary heart disease was increased, and the cardiac function was decreased (p < 0.05). When myocardial cell appeared ageing, the level of P21 and P53 was increased, and the level of microRNA124b was related with P53.

CONCLUSIONS: The level of microRNA124b in the serum of coronary heart disease patients and rat model may be related to the occurrence of coronary heart disease; microRNA124b may lead to the occurrence of coronary heart disease by causing cell senescence.

Key Words: microRNA124b level in serum, Color Doppler ultrasound, Cardiac function, P53.

Introduction

Coronary heart disease is one of the major public health concerns that affect human health worldwide. The incidence and mortality of coronary heart disease increased year by year. Thus, early detection and diagnosis of coronary heart disease is one of major challenges in the current research. Cell senescence may play a key role in the occurrence and development of cardiac muscle cell dysfunction and heart disease. Cell aging occurs with increasing levels of intracellular aging related proteins such as P21 and P53, both of which are important indicators of cell aging as shown in the staining of β-galactosidase. However, the specific molecular effects of cell senescence on coronary heart disease are not clear. MicroRNA regulates cell cycle, cell growth, cell death and cell senescence. Moreover, microRNA is closely related to many physiological and pathological processes in clinic. For example, microRNA126 is closely related to the repair of damaged nervous tissues, microRNA143 is associated with inflammation, and microRNA98 is related to many kinds of tumors, such as esophageal cancer and lung cancer. All these indicated that microRNA had a certain relationship with the occurrence and development of the disease and might be a molecular marker for the development of disease. Whether microRNA has an influence on coronary heart disease still needs research.

Color Doppler ultrasound technique is one of the important methods for noninvasive measurement of cardiac function in clinic.
paper, we study the cardiac function of patients with coronary heart disease by color Doppler ultrasound, and the relationship between serum microRNA124b and coronary heart disease along with potential possible mechanism, in order to provide theoretical basis for clinical practice.

Patients and Methods

Experiment Object

According to the inclusion criteria and exclusion criteria17,18, in the Affiliated Cardiovascular Hospital of Qingdao University, 100 patients with coronary heart disease and 100 healthy volunteers were regarded as the subjects and the control, respectively. The basic information of patients with coronary heart disease is as follows: the age was 28-60 years old (mean age 44.2 ± 8.5 years). This study has been approved by the Ethical Committee of the Affiliated Cardiovascular Hospital of Qingdao University. All subjects signed the consent forms before they were recruited in this study.

Blood Sample Collection

The morning fasting blood samples were collected from both patients and control groups. Serum was separated by the centrifugal of 200 g for 7 min.

Real-Time PCR

The level of microRNA 124b was detected by RT-qPCR kit according to the instruction (TaKaRa, Otsu, Shiga, Japan). Primers of microRNA124b and β-actin were synthesized by Shanghai Biological Engineering Co., Ltd. (Shanghai, China), the sequence were as follows: 5'-CTGCTGCAAAATGATGTGG-3' and 5'-AGCTGGAGAGGAAGAGTG-3', 5'-CTCAACTG GTTGGTGTGATTGAGCAGTGGAG-3' and 5'-CTCAACTG GTGTTGGGCGTGAAATTCGAGGATTC-3'.

Agarose Gel Electrophoresis Assay

According to the method reported in previous study21, agarose gel electrophoresis was performed to visually detect the products of RT-qPCR. Imaging system (Bio-Rad, Hercules, CA, USA) as well as the ImageJ analysis software (National Institutes of Health, Bethesda, MD, USA) were used for the analysis.

Establishment of Coronary Heart Disease Model in Rats

A rat model of coronary heart disease was established according to the method reported previously19,20. Standard specification was used for rat (provided by Qingdao University): rats were fed with high fat diet for 7 weeks, and then the ligation of heart coronary artery was carried out to cause myocardial ischemia.

Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of the affiliated Cardiovascular Hospital of Qingdao University.

Isolation of Rat Cardiac Myocytes

The rat with coronary heart disease was anaesthetized by 2% pentobarbital, cardiac muscle cells were surgically isolated with mechanical shear method and digested by trypsin according to the conventional method21,22.

Western Blot

Confluent cells were scraped and washed by phosphate buffered saline (PBS) for 3 times and lysed by 2 × SDS loading buffer at 95°C for 10 min. Proteins were separated by polyacrylamide gel electrophoresis at 100 V, 150 min and were transferred to the polyvinylidene fluoride (PVDF) membrane at 15 V for 20 min (Bio-Rad, Hercules, CA, USA). The membrane was blocked with 5% bovine serum albumin (BSA) at 4°C overnight and added with anti-aging protein P21 and P53 specific antibodies (2947 p21 Waf1/Cip1 (12D1), Rabbit mAb 1:500, 48818p53 (DO-7), Mouse mAb 1:500) for the incubation at 37°C for 1 h. After the membrane was washed with PBS for 3 times, it was added with second antibodies (dilution ratio 1:1000) successively (Abcam, Cambridge, MA, USA). Electrochemiluminescence (ECL) lighting system was used for the film developing and the result was observed with gel imaging system (Bio-Rad, Hercules, CA, USA).

Detection of Cardiac Function Using Color Doppler Ultrasonography

According to the method reported previously23, the hearts of patients and rat model with coronary heart disease were detected by Color Doppler ultrasound diagnostic system (Kejian, Guangzhou, Guangdong, China), and ultrasonic software was used to detect the cardiac function, including the capacity of cardiac diastolic and contractile.
**Statistical Analysis**
All data were analyzed by using SPSS 20.0 (IBM SPSS, Armonk, NY, USA) software as previously reported. Results were represented by Mean ± standard error. \( p < 0.05 \) was regarded as significant statistical significance.

**Results**

*Serum microRNA124b Expression in Patients with Coronary Heart Disease by Real-time PCR Detection*

Compared with healthy volunteers, the level of microRNA124b in serum of patients with coronary heart disease was increased \( (p < 0.05) \). After the surgery, serum levels of microRNA124b in coronary heart disease patients were decreased, suggesting that the serum microRNA124b might be related to the occurrence and prognosis of coronary heart disease (Figure 1).

*Ultrasound Observation for Cardiac Function in Patients with Coronary Heart Disease*

As shown in Figure 2, ultrasound examination revealed a significant impairment of cardiac function in patients with coronary heart disease compared with the heart function in healthy individuals \( (p < 0.05) \) (Table I).

*Serum microRNA124b Expression in Rats with Coronary Heart Disease by Real-time PCR Detection*

Compared with the sham group, the levels of microRNA124b in the serum of rat model with coronary heart disease were elevated \( (p < 0.05) \) (Figure 3).

*Evaluation of Cardiac Function in Rats with Coronary Heart Disease*

Data indicated that the cardiac function of the rat model with coronary heart disease was statistically adversely affected compared to the sham group, which was similar to the result from patients with coronary heart disease \( (p < 0.05) \) (Figure 4).

*Western Blot Detection of Aging of Myocardial Cells in Rat Model of Coronary Heart Disease*

Cell senescence appeared accompanied by the changes of aging proteins, in particular, aging and...
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Correlation Analysis Between the Levels of microRNA124b and Aging Protein P53

As shown in Figure 6, Pearson correlation analysis revealed that the decreasing level of microRNA124b in cardiac muscle cells of the rat model with coronary heart disease was closely related to the rising expression of P53.

Discussion

Coronary heart disease poses a serious threat to the public health, and early and sensitive diagnosis of coronary heart disease is thus of great necessity in the field of medicine and scientific community[25]. At present, the wide-accepted biomarkers in the diagnosis of coronary heart disease include cardiac troponin I (cTnI) and CK-MB. MicroRNA-dependent mechanisms play significant role in DNA methylation, histone modifications, and various physiological and pathological processes in clinic[12,26]. Accumulative evidence showed that microRNA126, microRNA143, and microRNA98 contributed to certain functions to the occurrence and development of the diseases[13-15].

In this study, we found that the level of microRNA124b in serum of patients with coronary heart disease was increased, and ultrasound detection showed a significant decrease in LVEF and FS in patients compared to normal group. Concordantly, the expression of microRNA124b

<table>
<thead>
<tr>
<th>Cardiac index</th>
<th>Control group</th>
<th>Patient group</th>
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<tbody>
<tr>
<td>LVEF (%)</td>
<td>618 ± 0.3</td>
<td>47.2 ± 0.7*</td>
</tr>
<tr>
<td>FS (%)</td>
<td>31.6 ± 0.5</td>
<td>25.9 ± 0.8*</td>
</tr>
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*Compared with the control group, the difference was significant (p < 0.05).

Figure 3. Serum MicroRNA124b expression with Real-time PCR in rats with coronary heart disease. **Compared with the control group, the difference was significant (p < 0.01).

Figure 4. Ultrasound findings of cardiac function in rats with coronary heart disease.

Table I. Cardiac ultrasound results.

![Graph showing microRNA124b levels with control and model groups.](image)

![Control and Model ultrasound images](image)

in the serum of rat model with coronary heart disease was elevated, along with significant weakening of cardiac function ($p < 0.05$). In addition, the aging of myocardial cells were observed in the rat model, in which, the level of microRNA-124b was correlated with the expression of P53 protein, as consistent with previous results\textsuperscript{27}. Certain weakness and limitations, however, exist in the current study: the amount of patients in this study was relatively small. Our further investigation will focus on the correlation between serum microRNA124b level and cardiac function with larger amount of patients and animal model, and also, advanced detection techniques, such as Color Doppler ultrasound, are looked forward to be utilized for Real-time detection of cardiac function in patients and animal model with coronary heart disease\textsuperscript{28,29}.

**Conclusions**

We showed that the level of microRNA124b in serum of coronary heart disease patients and model rats was reduced and its level was negatively correlated with the expression of p53 in aging myocardial cells. MicroRNA124b played a critical role in the occurrence of coronary heart disease.

**Conflict of Interest**

The Authors declare that they have no conflict of interests.

**References**


![Figure 5. Western blot detection of aging of myocardial cells in rat model of coronary heart disease.](image)

![Figure 6. The expression level of microRNA124b and aging protein P53 of rat model of coronary heart disease myocardial cells.](image)
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