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Research Article

Analysis of miRNA Associated with Coronary Artery Calcification

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Cardiovascular diseases seriously endanger human physical and mental health and life safety, to investigate correlation between miR-let-7b and miR-29b and coronary artery calcification of various patients. At present, real-time fluorescence quantitative PCR (qRT-PCR) was used to detect the expression levels of plasma miR-let-7b and miR-29b in patients with coronary artery calcification and noncoronary artery calcification and to analyze whether the expression levels of miR-let-7b and miR-29b were different between the two groups. It was shown that there was no significant difference in the expression of miR-let-7d-3p between the two groups. But the expression of miR-29b in the observation group was significantly lower than that in the control group. Taken together, miR-29b might be a risk factor for coronary artery calcification and may be a marker for early diagnosis of coronary artery calcification.

1. Introduction

On a global scale, cardiovascular diseases seriously endanger human physical and mental health and life safety [1]. According to China Cardiovascular Disease Report 2015, cardiovascular disease is the main cause of death in China, accounting for 44.60% in rural areas and 42.51% in urban areas. Arterial calcification is one of the pathological manifestations of many cardiovascular diseases and is closely related to the occurrence of cardiovascular diseases. Clinical epidemiological studies have shown that vascular calcification occurs in 80% of vascular injury and 90% of coronary artery disease patients. A prospective study of the assessment of cardiovascular molecular calcification with 10-year follow-up found that the presence of thoracic aortic calcification or increased calcium load increased the risk of cardiovascular events by an average of 3.7 times [2]. In a cohort follow-up study of nearly 10,000 participants using abdominal aortic calcification as a predictor of long-term cardiovascular events, severe abdominal aortic calcification was found to be a strong predictor of cardiovascular-related acute events or death, including myocardial infarction, stroke, coronary heart disease, and intermittent claudication and significantly increased the incidence and mortality of cardiovascular diseases [3, 4].

Coronary artery calcification is a pathological mineralization of hydroxyapatite crystals deposited on the arterial wall due to the disorder of calcium and phosphate mineral metabolism, which is widely associated with chronic inflammatory diseases, such as chronic kidney disease, diabetes, atherosclerosis, and aging [5, 6]. However, recent studies have shown that coronary artery calcification is a multifactor process that is regulated by cells and can prevent reversible and physiologically similar active bone mineralization process [7, 8]. Although the occurrence of coronary artery calcification has been well understood, there is still a lack of effective treatment measures. With the advancement of molecularly targeted therapies in recent years, there has been a trend to study ncRNA in cardiovascular disease.

miRNAs are a class of endogenous, single-chain, noncoding RNA molecules with the size of 18-25 nucleotides. miRNAs can perform epigenetic modification on target

genes after gene transcription, promote the degradation of target genes, or inhibit the synthesis of target genes and thus participate in the regulation of the occurrence and development of diseases. Currently, studies on miRNAs in cardiovascular diseases have been widely reported, and it is believed that miRNAs are involved in the regulation of atherosclerosis, intimal restenosis after artery injury, myocardial infarction, arterial calcification, and other lesions [9]. Studies have found that miRNAs also regulate several aspects of arterial calcification and can inhibit and promote the formation of arterial calcification. MiRNAs can inhibit target gene osteogenic transcription factors and the acquisition of osteogenic phenotypes of VSMCs, thereby inhibiting calcification [10, 11]. In addition, miRNAs also affect the expression of VSMC calcium homologous proteins and intracellular calcium ion concentration [12], and some miRNAs can promote the formation of osteoclast cells to resist osteoblast cells and play a role in inhibiting calcification [13]. Multiple biological databases have shown that miR-let-7b and miR-29b-3p are closely related to coronary atherosclerosis. Therefore, this study explores the correlation between miR-let-7b and miR-29b-3p and coronary artery calcification.

In this manuscript, we have thoroughly investigated correlation between miR-let-7b and miR-29b and coronary artery calcification of various patients. For this purpose, real-time fluorescence quantitative PCR (QRT-PCR) was used to detect the expression levels of plasma miR-let-7b and miR-29b in patients with coronary artery calcification and non-coronary artery calcification and to analyze whether the expression levels of miR-let-7b and miR-29b were different between the two groups.

The rest of the manuscript is organized as given in the following paragraph.

In Section 2, the proposed method, i.e., correlation between miR-let-7b, miR-29b, and coronary artery calcification of various patients, is described in detailed along with detailed discussion on how these patients are selected. Various results of the experiments are presented in form of a comparative analysis in Section 3 of the manuscript which is followed by a detailed and comprehensive discussion section. Lastly, conclusion is provided along with possible and useful materials from literature which are utilized in the manuscript.

2. Proposed Method

2.1. Participants. A total of 64 hospitalized patients over 50 years old with chest tightness were selected, aged from 50 to 75 years old. Among them, 32 cases with coronary artery calcification were the observation group, and 32 cases without coronary artery calcification were the control group. They all have high risk factors of atherosclerosis, such as family history of coronary heart disease, hypercholesterolemia, hypertension, and obesity. Patients with established myocardial infarction, heart failure, or stroke were excluded.

2.2. Total miRNA Was Extracted from Serum. Total serum miRNA was extracted by TIANGEN miRcute miRNA extraction and separation kit (DP501) in strict accordance with the kit instructions.

2.3. Reverse Transcription of Total miRNA in Serum. Total serum miRNA reverse transcription was performed using the TIANGEN miRcute Enhanced miRNA cDNA First-strand Synthesis Kit (KR211) strictly according to the instructions of the kit.

2.4. The Purpose of MicroRNA PCR. Total miRNA PCR was detected by TIANGEN miRcute Enhanced miRNA Fluorescence quantitative assay Kit (FP411), and the instructions were strictly followed.

The following are the primer sequences:

(i) hsa-let-7d-3p: CTATACGACCTGCTGCCTTTCT

(ii) hsa-miR-29b-3p: TAGCACCATTTGAAATCAGT GTT

2.5. Statistical Analysis. SPSS17.0 statistical software was used for analysis, and data were expressed as the mean ± standard deviation. A t-test was performed for comparison between two groups, ANOVA was performed for comparison between multiple groups, and Spearman's correlation analysis was performed. $P < 0.05$ was considered statistically significant.

3. Experimental Results and Observations

3.1. The General Information. Through the analysis of the basic information of the two groups of patients, it can be seen that coronary artery calcification is not related to the patient's age, HDL, LDL, but gender, smoking, TC, and TG which may be the risk factors for coronary artery calcification. See Table 1 for details.

3.2. The Expression Analysis of miR-let-7d-3p and miR-29b. There was no significant difference in the expression of miR-let-7d-3p between the two groups. But the expression of miR-29b in the observation group was significantly lower than that in the control group. Therefore, the expression of miR-let-7d-3p is not associated with coronary artery calcification, while the expression of miR-29b-3p may be a coronary artery risk factor (Table 2 and Figure 1).

4. Discussion

Vascular calcification is the pathological deposition of calcium and phosphorus in cardiovascular tissues, as well as the common pathological changes of diabetes, hypertension, atherosclerotic vasculopathy, and other vascular injuries, and is one of the important factors contributing to the high incidence and mortality of cardiovascular and cerebrovascular diseases [1, 2]. It is even considered to be an accurate predictor of cardiovascular adverse events [3]. Vascular calcification has always been a difficulty in the field of clinical treatment of cardiovascular diseases, and the current research focus in this field is how to effectively control the progression of arterial calcification [5]. The key reason for the lack of effective treatment is that the mechanism of the

Table 1: The basic information of the two groups was compared.

Group	Observation group ($n = 32$)	Control group ($n = 32$)
Gender (F/M)	13/19	23/9
Age	66.09 ± 4.93	63.81 ± 6.42
Smoking (Y/N)	3/29	17/15
TC	4.10 ± 1.00	5.25 ± 1.19
TG	4.22 ± 0.65	5.86 ± 1.12
HDL	1.04 ± 0.25	1.11 ± 0.33
LDL	2.62 ± 0.90	2.79 ± 1.04

Table 2: The expression analysis of miR-let-7d-3p and miR-29b.

Group	Observation group ($n = 32$)	Control group ($n = 32$)	F	P
miR-let-7d-3p	1.61 ± 0.14	1.34 ± 0.26	0.230	0.633
miR-29b	1.35 ± 0.16	2.04 ± 0.42	8.570	0.032

Note: $P < 0.05$, representing a statistically significant difference.

occurrence and development of arterial calcification is not fully understood. It is now believed that arterial calcification is actually an active, cell-mediated process similar to bone formation. The main process of arterial calcification is the transformation of vascular smooth muscle cells from contractile phenotype to osteoblast cells with secretory phenotype. This process includes three key steps: the loss of vascular smooth muscle contractile phenotype, the acquisition of osteoblast cells, and the deposition of intracellular calcium ion concentration. The main signal transduction pathways include phosphatidylinositol 3-kinase pathway (PI3K) and mitogen-activated protein kinase (MAPK). This transduction pathway plays an important role in the differentiation of vascular smooth muscle cells into osteoblasts. Vascular smooth muscle cells can synthesize and secrete alkaline phosphatase (ALP) and runt-related transcription factor 2 (α subunit) under the stimulation of various factors (Runx2) and type I collagen, and these osteogenic factors are also found to be expressed in arterial calcified plaques.

The basic pathological feature of coronary heart disease is the formation of atherosclerotic plaque. Calcification of coronary arteries is pathologically proven to be atherosclerotic plaques. Coronary artery calcification (CAC) refers to calcium deposits occurring at the site of coronary atherosclerosis and is an important marker of coronary atherosclerosis. The detection of coronary artery calcification indicates the presence of coronary atherosclerosis, and the degree of calcification is related to the size of atherosclerotic plaque. In general, the degree of coronary artery calcification is closely related to coronary artery disease events. CAC can produce serious clinical complications, including myocardial ischemia, angina pectoris, myocardial infarction, cardiac valvular insufficiency, arrhythmias, and decreased vascular wall elasticity. The more obvious the degree of calcification, the more serious the atherosclerosis, and the more extensive the lesion range. Therefore, it is of great clinical value to find risk factors for coronary artery calcification.

The presence of calcification is an important marker of coronary atherosclerosis, and studies suggest that the degree of calcification is directly related to the presence and severity of coronary atherosclerosis [14–18]. The formation of CHD in some patients is more insidious, especially in the young and middle-aged, and the symptoms are more typical when myocardial infarction occurs. Therefore, it is important to screen objective indicators at early stage. Calcification of atherosclerotic plaques is secondary to the development of coronary artery disease. Early detection of coronary calcification is an important marker for early diagnosis [19–24]. MicroRNA is a factor related to the formation of atherosclerosis that has been concerned in recent years. It can be widely found in normal cells and can regulate not only chromosomes and genetic factors but also oxidative stress-related factors. Some studies have also suggested that microRNAs play an important role in regulating lipid metabolism. For example, the MOVAS-1 cell calcification model downregulated mmu-let-7e-5p and upregulated mmu-miR-324-3p in exosomes [25]. In addition, miRNAs are involved in bone metabolism in coronary artery calcification [26, 27]. Similarly, vascular calcification but not arrhythmia in idiopathic atrial fibrillation was associated with sex differences in the miRNA profile of diabetic microvascular injury [28]. miRNA is an endogenous noncoding RNA with a length of 22 bp, which is an important transcription regulator [29–31]. miR-29b is a factor related to lipid metabolism that has been recognized in recent years and also a factor screened from gene banks that may be associated with coronary heart disease and atherosclerotic plaque formation. Some studies have suggested that miR-29b is involved in the formation of fatty liver and regulates macrophages in the epithelium and stroma by targeting PRDM2, resulting in the formation of lipid core [32, 33].

miR-29b is a member of miR-29s family (miR.29a, miR.29B, and miR.29c). The three members have a common highly conserved recognition sequence with a length of 2.7 nucleotides, which is called seed sequence, so that they have a variety of common target proteins. The precursor of miR-29b can produce two mature miRNAs, miR-29b.3p and miR-29b-5p, which are processed by the arms at the 3′ and 5′ ends of the precursor, respectively. In recent years, many studies have found that the miR-29 family is closely related to the regulation of cardiovascular diseases. The research [34] found that the expression of three members of miR.29 family decreased in myocardial tissue close to the infarcted area compared with normal myocardial tissue in a mouse myocardial infarction model and human myocardial infarction cases. It was predicted by bioinformatics software and experiments in vivo and in vitro that miR.29s could target downregulate the expression of cellular fibrosis-related proteins COLIA1, COLIA2, COL3A1, FBNL, and ELNL and the expression of miR.29s in myocardial infarction tissue was negatively correlated with these proteins, suggesting that miR-29s can inhibit myocardial fibrosis in a myocardial damaged area. We studied the mouse model and found that the expression of miR-29s in the thoracic aorta of mature

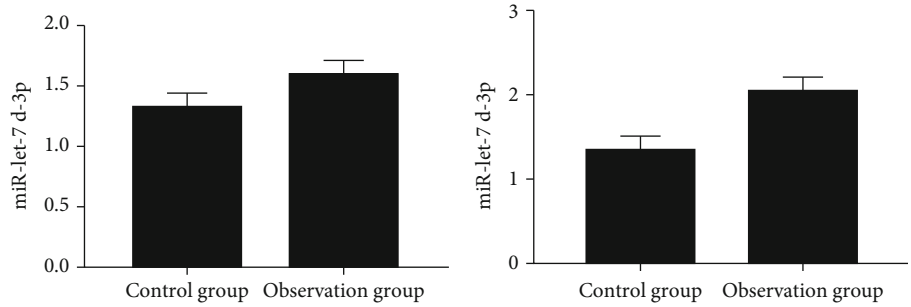

Figure 1: The expression analysis of miR-let-7d-3p and miR-29b.

mice was higher than that of newborn mice [35]. miR-29s promoted the maturation of arterial wall by downregulating the expression of elastin and matrix proteins COLIA1, COLIA2, and ELN. Other studies have found that miR-29b can prevent angiotensin II-induced cardiac fibrosis and cardiac dysfunction, revealing that miR-29b can be used as a new method for the treatment of chronic cardiovascular disease. These studies show that miR-29b has a protective effect on cardiovascular disease to a certain extent.

Long-term smoking inhibits the body's antioxidant function, promotes inflammation, accelerates the hyperoxidation process of low-density lipoprotein, changes the composition of blood lipids, and leads to the decline of antioxidant capacity of vascular endothelial dysfunction and promotes the formation and development of atherosclerosis. As our study found, smoking is a risk factor for coronary artery calcification. Starting from the HDL metabolic pathway, we preliminarily verified the function of miR-29b. Atherosclerotic calcification is an important pathogenesis of coronary heart disease, and dyslipidemia is an important risk factor for atherosclerotic calcification. However, reducing the level of plasma LDL through treatment cannot prevent the process of atherosclerosis. The data show that about 1/2 of patients with coronary heart disease are accompanied by low plasma HDL. This study found that HDL, LDL, and miR-29b were indeed risk factors of coronary artery calcification, and miR-29b was expected to become an early diagnostic marker of coronary artery calcification.

5. Conclusion

In this manuscript, we have thoroughly investigated correlation between miR-let-7b and miR-29b and coronary artery calcification of various patients. For this purpose, real-time fluorescence quantitative PCR (QRT-PCR) was used to detect the expression levels of plasma miR-let-7b and miR-29b in patients with coronary artery calcification and non-coronary artery calcification and to analyze whether the expression levels of miR-let-7b and miR-29b were different between the two groups. The data show that about 1/2 of patients with coronary heart disease are accompanied by low plasma HDL. This study found that HDL, LDL, and miR-29b were indeed risk factors of coronary artery calcification and miR-29b was expected to become an early diagnostic marker of coronary artery calcification.

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

Ning Yang put forward the idea of the paper, and all authors participated in the preparation and review of the paper.

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