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Identification of circulating microRNA-126-3p as a new biomarker for coronary artery calcification

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Abstract

Objective: Several circulating microRNAs, including microRNA-126-3p, have been identified as diagnostic and prognostic biomarker of cardiovascular disease. However, whether microRNA-126-3p is an independent risk predictor for coronary artery calcification is unclear.

Methods: In this prospective single-center study, we collected blood samples from coronary artery atherosclerosis patients ($n=54$), patients with coronary artery calcification ($n=33$) and controls ($n=56$). Total RNA was extracted from plasma and blood cells with TRIzol reagents. The microRNA-126-3p level was determined via quantitative real-time polymerase chain reaction (RT-PCR).

Results: MicroRNA-126-3p levels were significantly increased in patients with coronary artery calcification than in coronary artery atherosclerosis patients or controls. The highest expression of microRNA-126-3p was observed in patients with moderate calcification who were diagnosed with Grade 2 calcification by coronary angiography. Age, microRNA-126-3p expression in veins, hypertension and diabetes significantly influence the occurrence of coronary artery calcification, among which diabetes and venous microRNA-126-3p expression were found to be independent risk factors for coronary artery calcification.

Conclusions: Taken together, the data in this study suggest that circulating microRNA-126-3p may be a novel noninvasive biomarker for coronary artery calcification. Regulating microRNA-126-3p expression may be an effective and promising strategy for the diagnosis and treatment of cardiovascular diseases, especially coronary artery calcification.

Keywords

MicroRNA-126-3p, cardiovascular disease, coronary artery calcification, independent risk factor, biomarker

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Introduction

Vascular calcification is a complex process that includes active osteogenesis and passive calcium and phosphate ion deposition on collagen and elastin fibers.[1] Epidemiological studies have demonstrated that coronary artery calcification (CAC) is strongly correlated with coronary events and mortality.[2] According to the location, vascular calcification can be classified into two forms in which calcification is detected within the intima (intimal calcification) or in the tunica media. CAC can be detected by noninvasive or invasive imaging techniques, including computed tomography, optical coherence tomography, intravascular ultrasound and coronary angiography (CAG).[3,4] CAC is concomitant with the development of atherosclerosis. Many studies have proposed that spotty calcification is a predictor of unstable

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plaques and acute cardiovascular events, whereas extensive calcification is associated with stable plaques.[5–7] However, extensive calcification may cause failure of percutaneous coronary intervention, equipment damage, and stent malapposition.[8] Therefore, investigating of mechanisms of CAC, as well as biomarkers for CAC, is beneficial for the clinical treatment of patients with CAC.

Several biomarkers have been identified as biomarkers for CAC. For example, serum free fatty acid, chenodeoxycholic, deoxycholic, glycolithocholic acid, alkaline phosphatase, and matrix Gla protein have been identified as independent risk factors for CAC.[3,9–11] Recent studies have shown that microRNAs can be secreted into the blood, enter the circulatory system and exist stably in the blood, which makes them potential new molecular markers or targets for the diagnosis and treatment of cardiovascular diseases.[12–14] In calcified mouse vascular smooth muscle cells, 987 and 92 microRNAs were found to be significantly upregulated and downregulated, respectively, in exosomes among which mmu-let-7e-5p is downregulated and mmu-microRNA-324-3p is upregulated.[15] Plasma miR-6821-5p is associated with the presence of coronary artery calcium in familial hypercholesterolemia-patients.[16] The levels of microRNA-1, microRNA-133, microRNA-499, and microRNA-208 in the blood of patients with acute myocardial infarction were increased. MicroRNA-137 and microRNA-106b-5p were reported to be novel blood-based biomarkers for unstable angina and myocardial ischemia in coronary artery disease patients.[14,17,18] In patients with cardiovascular disease and diabetes, the level of microRNA-126-3p in the blood decreased significantly.[12,13]

MicroRNA-126-3p is highly expressed in endothelial cells (ECs) and regulates vascular development and angiogenesis.[19,20] Pre-microRNA-126 produces two mature microRNAs, microRNA-126-3p and microRNA-126-5p. Schober et al.[21] determined that microRNA-126-5p promotes EC regeneration, thereby preventing the formation of atherosclerotic lesions by inhibiting the Notch1 inhibitor delta-like 1 homolog. Moreover, microRNA-126-3p inhibits the development of atherosclerosis by promoting endothelium repair and inhibiting monocyte adhesion.[22–24] We previously reported that microRNA-126-3p reduces vascular calcification by inhibiting the canonical and noncanonical Wnt signaling pathways.[25] A recent study confirmed that microRNA-126-3p affects vascular calcium accumulation.[20] However, little is known about the relationship between circulating microRNA-126-3p and CAC.

Therefore, we hypothesized that circulating microRNA-126-3p may be a biomarker for CAC. We aimed to provide new research ideas for the diagnosis and treatment of cardiovascular diseases, especially CAC.

Materials and methods

Methods

This prospective single-center study was conducted between 1 October 2022 and 30 April 2023 at Tianjin Baodi Hospital. We collected blood samples from the control (with no known history of coronary heart disease or renal disease), atherosclerosis and calcification groups. The relationship between circulating miR-126-3p and CAC was analyzed. All studies were approved by the Ethical Committee of Tianjin Baodi Hospital.

Inclusion criteria

The detailed inclusion criteria for the patients were as follows: (1) stenosis of the left main stem, left anterior descending branch, left circumflex branch, right coronary artery and its large branches; if the patients' stenosis was $\geqslant$50%, then they were diagnosed with coronary heart disease and were included in the coronary artery atherosclerosis group; (2) patients with symptoms such as chest stuffiness or thoracalgia but no severe coronary stenosis observed by CAG, then they were included in the control group; (3) patients diagnosed with coronary heart disease and mild-to-severe calcification were included in the calcification group; (4) patients' age is $\geqslant$18 years; (5) ability to express their inner thoughts fluently; and (6) available complete patient data for clinical research. Sex was matched between the controls and patients with atherosclerosis or calcification via random sampling.

Exclusion criteria

The general exclusion criteria were as follows: (1) previous percutaneous coronary intervention; (2) severe congestive heart failure, severe liver insufficiency, infection or other diseases (malignant tumors, organ grafting); (3) left ventricular ejection fraction less than 35%; and (4) history of drug abuse. The radiographic exclusion criteria were as follows: (1) the presence of a thrombus or aortic dissection more than the National Heart, Lung and Blood Institute (NHBL) C grade as determined by CAG or intravascular ultrasound; (2) the presence of chronic complete occlusive lesions; (3) a lesion in stent restenosis; and (4) the existence of a coronary aneurysm or coronary artery fistula, which affects the imaging accuracy.

Coronary artery computed tomography angiography

Coronary artery computed tomography angiography (CTA) is a medical imaging test that is commonly used to evaluate coronary vascular lesions in the heart. This test uses a CT scanner and a special contrast agent to create fine three-dimensional images that show the structure and function of the heart and coronary arteries. The American Angiographic Association has classified CAC as mild, moderate or severe calcification on the basis of whether it can be visualized via CAG with the heart beating of the heart, (1) no calcification: no calcification shadows were found; (2) mild calcification: pale and faint calcification shadows were observed with the beating of the heart; (3) moderate calcification: clear calcification shadows were observed with the beating of the heart;

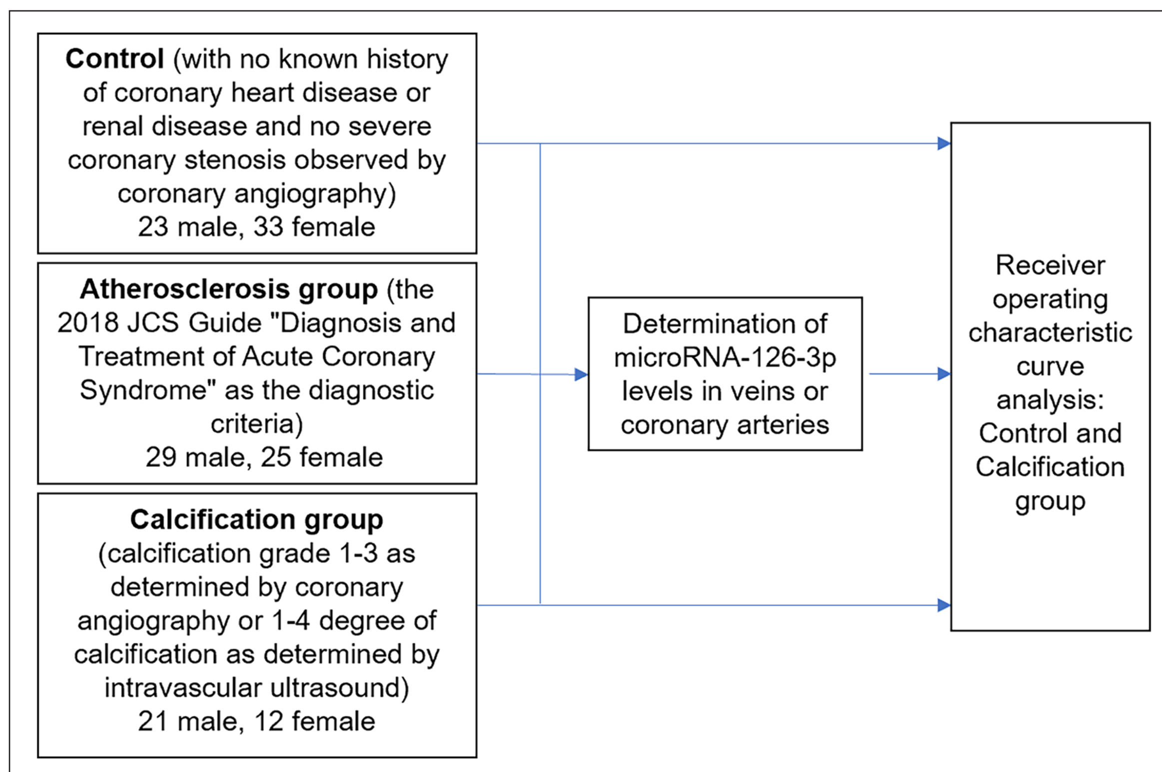

Figure 1. Flow diagram of the study protocol.

and (4) severe calcification: clear calcification shadows were observed with the heart both beating and at rest, and clear shadows of calcification were observed. The calcification was confirmed by two experienced interventional cardiologists independently.

Group allocation

All the subjects were hospitalized at Tianjin Baodi Hospital (Tianjin, China) and clinically diagnosed via CAG with complete medical records, clinical parameters and follow-up data, including echocardiography and blood pressure data. On the basis of the results of CAG and intravascular ultrasound evaluation, the subjects were divided into controls (with no known history of coronary heart disease or renal disease, symptoms such as chest stuffiness or thoracalgia but no severe coronary stenosis observed by CAG), an atherosclerosis group (the 2018 JCS Guide "Diagnosis and Treatment of Acute Coronary Syndrome"[26] as the diagnostic criterion) and a calcification group (1–4 degree of intimal calcification as determined by intravascular ultrasound and severe coronary stenosis) (Figure 1).

Collection of human plasma

Venous blood was collected within 24 h of admission to the hospital, the patients fasted during venous blood collection, and blood was collected from the median elbow vein. During CAG in the enrolled patients, arterial blood was withdrawn from the anterior descending coronary artery, the left circumflex branch, and the right coronary artery into ethylenediaminetetraacetic acid (EDTA) tubes via a microcatheter. Venous and arterial blood was collected and centrifuged at 4°C for 20 min at 2000 rpm, and the collected plasma was stored at −80°C for measurement.

Isolation of white blood cells

Blood (5 ml) was obtained from participants in an EDTA-containing container. After centrifugation at 1000 rpm for 5 min, the precipitate was resuspended in 5 ml red blood cell lysis buffer to remove red blood cells. The remaining cells are defined as white blood cells. Total RNA was extracted with TRIzol reagent.

Determination of microRNA-126-3p levels

Total RNA was isolated from plasma or white blood cells with TRIzol reagent. The level of microRNA-126-3p in plasma and blood cells were analyzed via quantitative microRNA stem-loop RT-PCR technology (Ambion)[22] with the following primers: RT-primer, GTCGTATCCAGTGCA GGGTCCGAGGTATTCGCACTGGATACGACCGC ATT; microRNA-126-3p forward primer, GTGCAG GGTCCGAGGT; reverse primer, GCTGCATCGTACCGT GAGT; U6 forward primer, CTCGCTTCGGCAGCACA; reverse primer, AACGCTTCACGAATTTGCGT.

Table 1. Basal clinical characteristics of the study participants.

Clinical characteristics	Control ($n = 56$)	Atherosclerosis ($n = 54$)	Calcification ($n = 33$)
Age (years)	58.48 ± 10.01	60.9 ± 7.8	63.3 ± 9.17[a]
Sex M/F	23/33	29/25	21/12
Smoking	17 (30.4)	27 (50)	18 (54.5)
Hypertension	25 (44.6)	37 (68.5)	26 (78.8)
Diabetes	2 (3.6)	8 (14.8)	14 (42.4)[a,b]
UREA (mM)	5.48 ± 1.89	5.46 ± 1.43	5.93 ± 1.44
UA (μM)	305.19 ± 86.61	307.46 ± 119.81	354.01 ± 96.19
CREA (μM)	61.59 ± 15.64	65.65 ± 15.72	67.78 ± 15.18
Plasma TC (mM)	4.80 ± 1.20	4.79 ± 1.22	5.55 ± 1.53[a,b]
Plasma LDL-C (mM)	3.08 ± 0.90	3.14 ± 0.84	3.67 ± 1.11[a,b]
Plasma HDL-C (mM)	1.18 ± 0.22	1.12 ± 0.24	1.18 ± 0.33
Plasma TG (mM)	1.55 ± 0.80	1.64 ± 0.92	1.90 ± 0.89
Plasma apoA1 (g/L)	1.26 ± 0.20	1.12 ± 0.14[a]	1.09 ± 0.24[a]
EF (%)	62.66 ± 6.96	61.41 ± 7.94	57.07 ± 16.79
PR (s)	0.166 ± 0.027	0.161 ± 0.039	0.171 ± 0.028
QT (s)	0.397 ± 0.032	0.401 ± 0.032	0.401 ± 0.043
QTc (s)	0.429 ± 0.023	0.436 ± 0.029	0.434 ± 0.040
Calcification index	/	0.023 ± 0.034[a]	0.183 ± 0.083[a,b]
Lesion length (mm)	0.125 ± 0.429[a]	10.719 ± 6.601[a]	25.367 ± 13.437[a,b]
Plaque burden	0.025 ± 0.065	0.679 ± 0.163[a]	0.775 ± 0.080[a,b]

Data are shown as mean ± SD or n (%).
TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglyceride; EF: ejection fraction; PR: pulse rate.
[a]$p < 0.05$, control versus atherosclerosis or control versus calcification.
[b]$p < 0.05$, atherosclerosis versus calcification.

Data analysis

All patients' clinical characteristics are presented as the means ± standard deviations (SDs) or *n* (%). One-way analysis of variance (ANOVA) followed by the multiple comparisons Bonferroni correction test was used for statistical analysis. Binary logistic regression was used to analyze independent risk factors for CAC. Factors influencing plasma microRNA-126-3p expression were analyzed via multivariate linear regression. Receiver operating characteristic curve analysis was performed to assess the diagnostic accuracy (sensitivity and specificity) of the markers microRNA-126-3p and diabetes for CAC assessment. Statistical analyses were conducted via GraphPad Prism 8.0 (Boston, MA, USA) and IBM SPSS Statistics V22.0 software (Armonk, NY, USA). The threshold for statistical significance was set at $p < 0.05$.

Results

Basic clinical characteristics of the study participants

Sex, smoking status, hypertension, plasma urea nitrogen (UREA), uric acid (UA), creatinine (CREA), plasma high-density lipoprotein cholesterol (HDL-C), plasma triglyceride (TG), ejection fraction (EF), pulse rate (PR) and Qt were not significantly different among the control, atherosclerosis and CAC groups (Table 1). However, the age and plasma apoA1 level were greater in CAC group than in the control group, whereas diabetes, lesion length, plaque burden and calcification index were highest in the calcification group (Table 1).

MicroRNA-126-3p levels were increased in patients with coronary artery calcification

The microRNA-126-3p level in veins was not significantly different between the control and atherosclerosis groups. However, the level of microRNA-126-3p was greater in CAC group (Figure 2(a)). To further determine whether microRNA-126-3p is a biomarker for patients with CAC, we also collected blood from coronary arteries. We found that the microRNA-126-3p level was elevated in the CAC group compared with the control and atherosclerosis groups (Figure 2(b)). Next, we compared the level of microRNA-126-3p in veins and coronary arteries in the calcification group. Compared with the level of microRNA-126-3p in plasma collected from coronary arteries, the microRNA-126-3p level tended to increase in the veins of most patients with CAC (Figure 3(a)). Moreover, the microRNA-126-3p level in circulating white blood cells isolated from patients with CAC was greater than that in patients with atherosclerosis (Figure 3(b)).

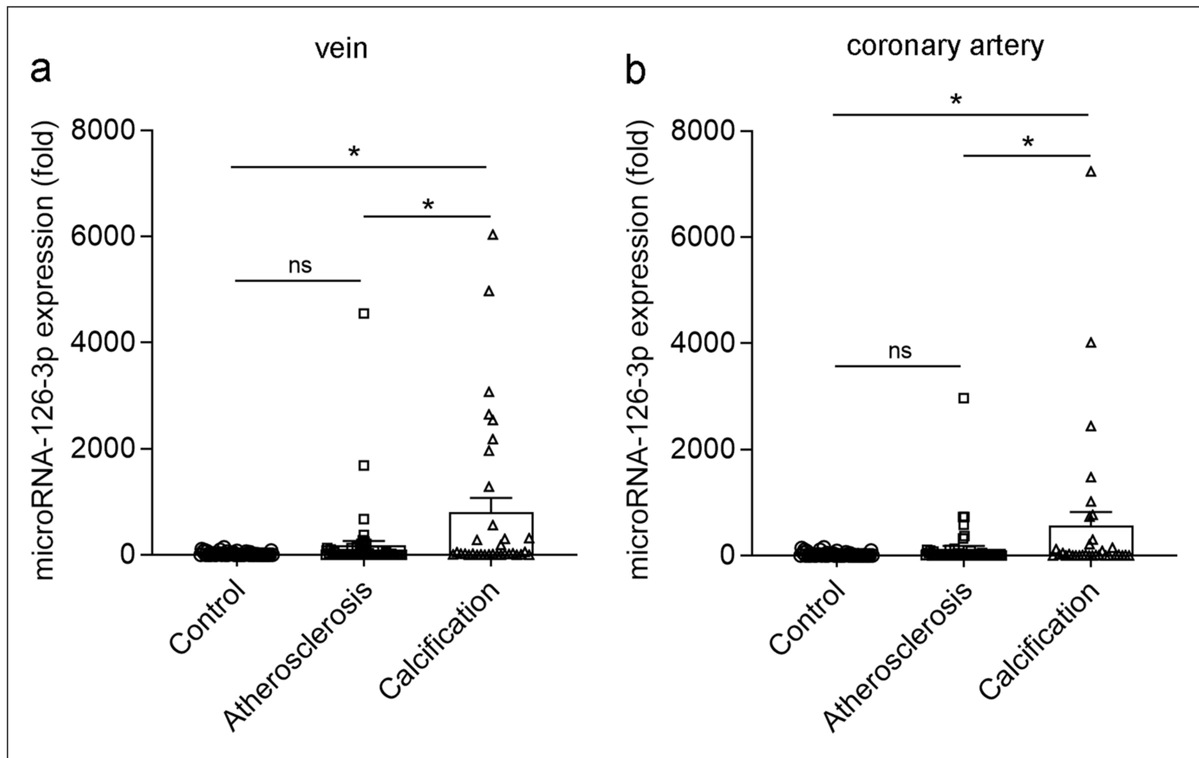

Figure 2. Circulating microRNA-126-3p was elevated in plasma of coronary artery calcification patients. Relative level of microRNA-126 in vein (a) and coronary artery (b) in samples from different groups.
ns: no significance.
$*p < 0.05$.

Positive correlation between plasma microRNA-126-3p levels and the calcification index in patients with calcification

We further analyzed the expression of microRNA-126-3p among different calcification grades determined via CAG. The results revealed that microRNA-126-3p content was highest in the Grade 2 calcification group, as determined by CAG, in the plasma isolated from both veins and coronary arteries (Figure 4), indicating that the level of plasma microRNA-126-3p was a characteristic signature for moderate calcification. The plasma microRNA-126-3p level was positively correlated with the calcification index determined by intravascular ultrasound (Figure 5). Moreover, we performed multivariate linear regression analysis of microRNA-126-3p levels in veins and coronary arteries. The results revealed that age ($p = 0.800$), sex ($p = 0.667$), diabetes ($p = 0.213$), hypertension ($p = 0.756$) and smoking ($p = 0.059$) did not significantly affect the expression of microRNA-126-3p in veins, whereas the calcification index ($p = 0.049$; $\beta = 0.244$, 95% CI = 13.112–5003.673) significantly affected the content of microRNA-126-3p. However, microRNA-126-3p expression in coronary arteries was not significantly affected by age ($p = 0.573$), sex ($p = 0.641$), diabetes ($p = 0.444$), hypertension ($p = 0.565$), smoking ($p = 0.544$) or the calcification index ($p = 0.143$).

Venous microRNA-126-3p levels as diagnostic biomarkers for coronary artery calcification

We subsequently used binary logistic regression to analyze independent risk factors for CAC. Univariate screening with binary logistic regression revealed that age ($p = 0.03$), microRNA-126-3p expression in veins ($p = 0.03$), hypertension ($p = 0.002$) and diabetes ($p = 0.000191$) all significantly influenced the occurrence of coronary calcification, whereas the amount of microRNA-126-3p in coronary arteries ($p = 0.077$) had no significant effect on coronary calcification. We next included four factors, age, venous microRNA-126-3p content, hypertension and diabetes, in the multivariate binary logistic regression analysis, and the results revealed that diabetes and venous microRNA-126-3p expression were independent risk factors for coronary calcification (Table 2). The receiver operating characteristic curve and the area under the receiver operating characteristic curve were used to visualize and assess the prediction ability of the logistic regression model. The area under the ROC curve for diabetes patients was 0.694 (standard error = 0.062; 95% CI = 0.572–0.816; $p = 0.002$) (Figure 6(a)), whereas the area under the ROC curve for venous plasma

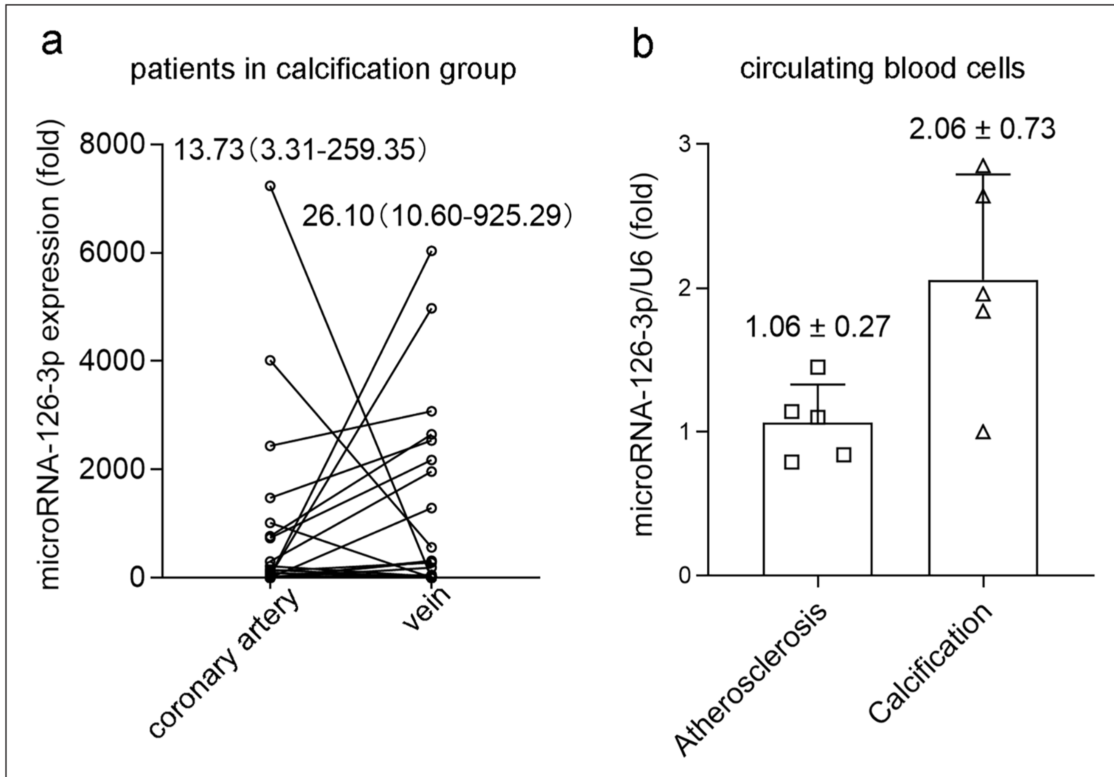

Figure 3. MicroRNA-126-3p level was greater in plasma and white blood cells of patients with coronary artery calcification than atherosclerosis. The expression level of microRNA-126-3p in vein and coronary artery in calcification group (a). The expression level of microRNA-126-3p in white blood cells in atherosclerosis and calcification group (b).

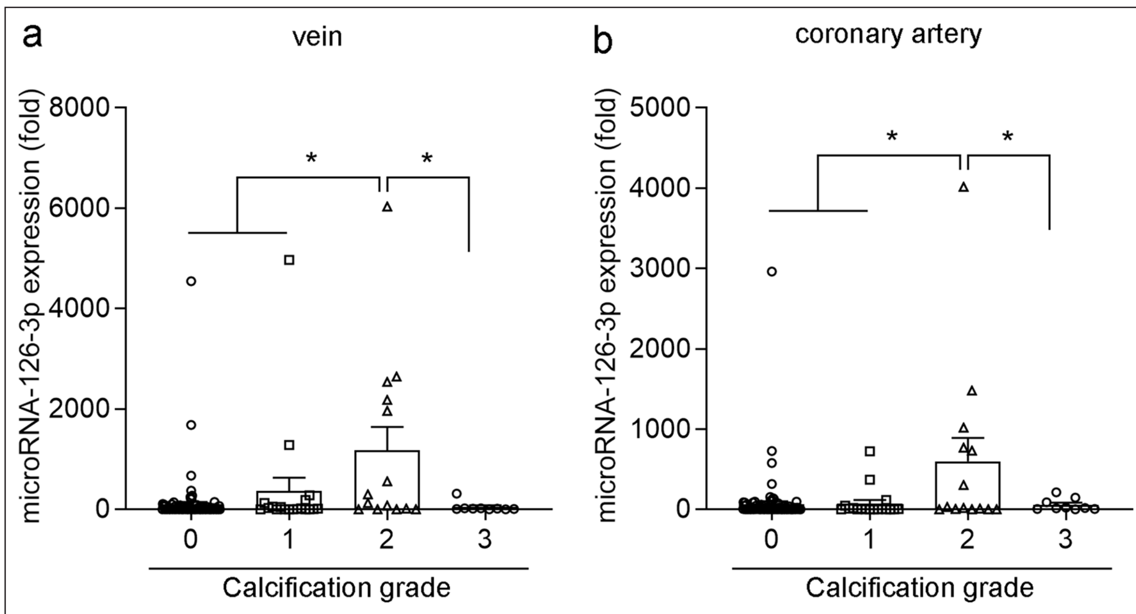

Figure 4. MicroRNA-126-3p content was highest in the Grade 2 calcification group determined by coronary angiography. The expression level of microRNA-126-3p in vein (a) and coronary artery (b) among different calcification grades.
$^{*}p < 0.05$.

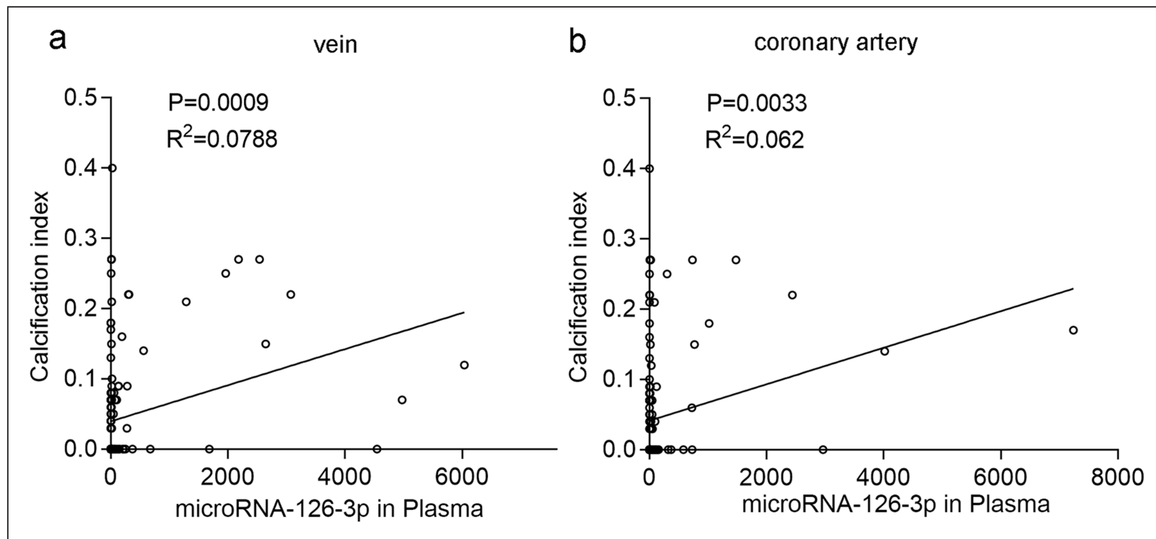

Figure 5. Positive correlation between microRNA-126-3p levels and calcification index. Relationship between plasma microRNA-126-3p level and calcification index in vein (a) and coronary artery (b) in patients with calcification was analyzed by Pearson's method.

Table 2. Risk factors for coronary artery calcification.

Risk factors	*B*	OR (95% CI)	*p*-Value
Age	0.056	1.057 (0.987–1.132)	0.013
Venous microRNA-126-3p	0.010	1.010 (1.000–1.021)	0.045
Hypertension	1.076	2.934 (0.806–10.678)	0.102
Diabetes	3.168	23.756 (4.089–138.005)	0.000417

microRNA-126-3p was 0.751 (standard error = 0.052; 95% CI = 0.649–0.852; p = 0.000084; cut-off value for prediction is 10.2627 judged by the Yoden index) (Figure 6(b)). These results suggest that circulating microRNA-126-3p may be a new biomarker for patients with CAC, which is increased in plasma and circulating blood cells.

Discussion

Cardiovascular disease is a serious threat to human health and the leading cause of death worldwide. Arterial calcification, like bone formation, is a complex and active process and is one of the manifestations of atherosclerosis. The age-standardized rate of cardiovascular disease mortality has declined in recent decades[27] because of the identification and modification of cardiovascular disease risk factors, as well as improvements in the arsenal of tools available to diagnose and treat cardiovascular disease.[28] Despite improved risk prediction and modern treatment capabilities, the incidence of cardiovascular disease events remains high. Therefore, the exploration of novel approaches for risk prediction and prevention of cardiovascular diseases is highly necessary.

The important role of microRNAs in cardiovascular diseases has been extensively studied in recent years. The stability of microRNAs in the circulation makes them suitable as diagnostic and prognostic markers for various cardiovascular diseases. Studies have shown that microRNAs are involved in a variety of cell biological processes, including development, cycle regulation, proliferation, differentiation, apoptosis and angiogenesis, as well as the responses of the immune and cardiovascular systems to pathogens and cancers.[29–31] With the advent of enabling technologies, whole-genome scans has been used to identify regulated microRNAs with greater efficacy, and many studies have focused on their role as predictive and prognostic biomarkers.[32] Liu et al.[33] reported that acute myocardial infarction patients had significantly higher levels of plasma microRNA-21 than healthy controls did. MicroRNA-21 was shown to be a novel biomarker that was predictive of left ventricular remodeling after acute myocardial infarction. In the serum or plasma of humans, some microRNAs, such as microRNA-130a, microRNA-210, microRNA-150, microRNA-191, microRNA-23b, microRNA-1246 and microRNA-451, are highly expressed and are defined as biomarkers for the early and precise diagnosis of hypertension.[34] A study by Rhodes et al.[35] demonstrated an important correlation between a decreased level of microRNA-150 and poor prognosis in patients with pulmonary arterial hypertension. Yao et al.[36] suggested that highly expressed microRNA-499 is a reliable biomarker that has higher sensitivity and specificity than troponin for the early diagnosis of acute myocardial infarction. Ovchinnikova et al.[37] reported that decreased levels of microRNA-18a,

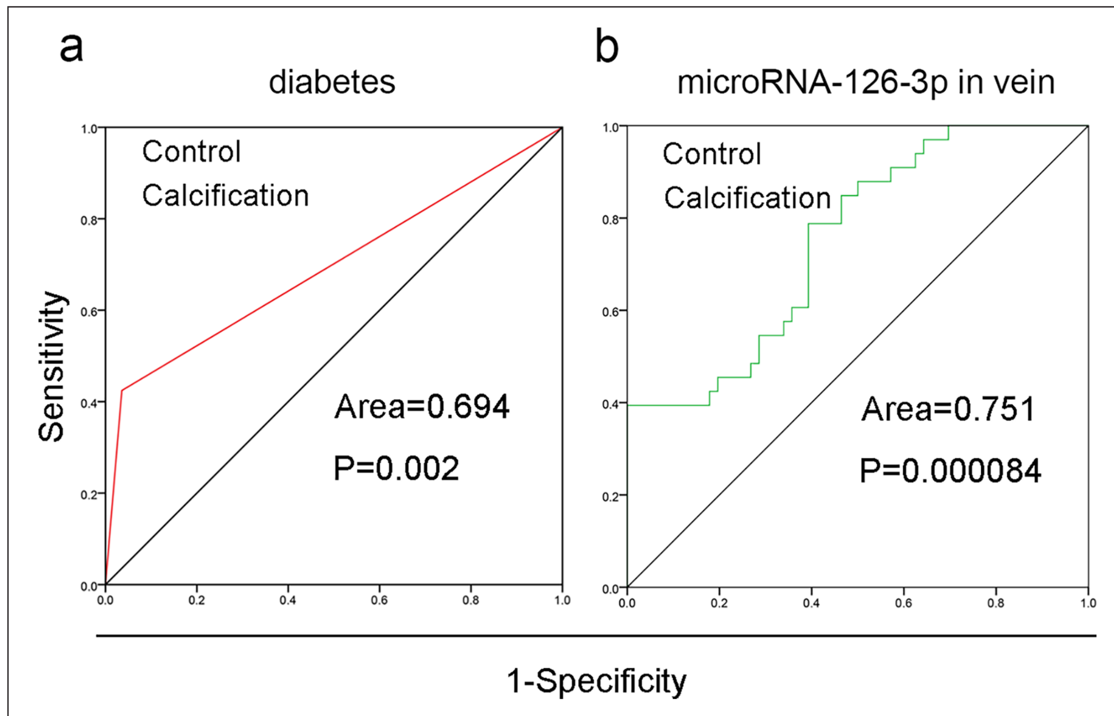

Figure 6. Venous microRNA-126-3p levels as diagnostic biomarkers for coronary artery calcification. Receiver operating characteristic curve of diabetes (a) and relative levels of microRNA-126-3p in veins (b).

microRNA-652, microRNA-18b, microRNA-301a, let-7i, microRNA-223 and microRNA-423 were significant independent biomarkers of 6-month mortality 48 h after admission for heart failure. According to some studies, the levels of microRNA-92a, microRNA-126 and microRNA-222 are reduced, whereas the levels of microRNA-21, microRNA-122, microRNA-130a, microRNA-150 and microRNA-211 are elevated in the blood of patients with atherosclerosis.[38–40] Escate et al.[16] reported up-regulated levels of miR-6821-5p in the plasma of asymptomatic familial hypercholesterolemia patients with coronary calcified atherosclerotic plaques.

In this study, we examined the expression of microRNA-126-3p in plasma from veins and coronary arteries in the control, atherosclerosis and calcification groups. The results revealed no significant changes in the amount of microRNA-126-3p in either the venous or arterial plasma of the atherosclerotic group compared with the control group. However, the plasma levels of microRNA-126-3p in the CAC group were significantly greater than those in the other two groups. In addition, we found that microRNA-126-3p was more abundant in the veins than in the arteries of patients with CAC, indicating that microRNA-126-3p may be retained in the veins, preventing its effect on blood vessels. Multivariate linear regression analysis suggested that the microRNA-126-3p level in coronary arteries did not significantly affect the calcification index ($p=0.143$). These inconsistent results may be caused by the lower level of microRNA-126-3p in the plasma isolated from coronary artery blood. The limited number of patients may be another reason for the inconsistent results. However, as a biomarker, the microRNA-126-3p level in plasma isolated from veins, which is higher in CAC patients, has clinical value. Moreover, the microRNA-126-3p level in circulating blood cells isolated from patients with CAC was greater than that in patients with atherosclerosis. A significant positive correlation was detected between plasma microRNA-126-3p levels and calcification index determined by intravascular ultrasound in patients with CAC via correlation analysis. Analysis of the receiver operating characteristic curve suggested that the combination of microRNA-126-3p and diabetes might be a new diagnostic biomarker for CAC.

We previously demonstrated that microRNA-126-3p increased DKK1 and LRP6 expression, thereby reducing vascular calcification by inhibiting the canonical and noncanonical Wnt signaling pathways.[25] However, we do not know the direct target gene of microRNA-126-3p in the Wnt signaling pathway. Single-cell transcriptome analysis revealed that hepatic and mesenchymal transitions, and pulmonary ECs were disorganized in microRNA-126 knockout mice. The mice exhibited excessive intussusceptive angiogenesis, reduced glucose and pyruvate tolerance and excessive accumulation of calcium.[20] Herein, we determined that the level of circulating microRNA-126-3p was highest in patients with moderate calcification but was reduced in those with severe calcification. However, the microRNA-126-3p level

was positively correlated with the calcification index determined by intravascular ultrasound. An increase in microRNA-126-3p when micro-calcification progresses may be protective. However, when calcification is severe, the secretion of microRNA-126-3p is reduced since dense calcification stabilizes the plaques, which reduces EC apoptosis.[41] Another possible explanation for this phenotype may be the small sample size in the Grade 3 calcification group. We will enroll more participants in the future to determine the underlying mechanism of reduced microRNA-126-3p levels in patients with severe calcification.

In this prospective single-center study, we found that the level of circulating microRNA-126-3p was elevated in patients with coronary artery atherosclerosis who experienced concurrent intimal calcification. Moreover, the microRNA-126-3p level may serve as an independent predictor of CAC. However, we determined that the microRNA-126-3p level was highest in Grade 2 calcification patients. Histologically, the types of arterial calcification are divided into the following categories: microcalcifications, punctate, patchy, lamellar and nodular calcifications. Lamellar calcification is very common in stable plaques, whereas microcalcifications, punctate and patchy calcifications are more common in unstable plaques. Thus, the presence of small calcifications may be a better predictor of unstable plaques, whereas more fused, dense calcifications may be a better predictor of stable plaques. In this study, the moderate calcification (grade 2) may be represented for the unstable plaques, which pose a greater danger for acute cardiovascular events. Thus, miRNA-126-3p may predict the severity of unstable plaques.

Limitations

The sample size of the study was relatively small, which may affect the conclusions. Therefore, the conclusion should be verified in the future by involving more participants via a power calculation to justify the sample size. Moreover, a previous study demonstrated that microRNA-126-3p was reduced in atherosclerosis patients. However, we determined that there were no significant differences between the control and atherosclerosis groups. We subjected the participants to the control group who have no atherosclerosis in the coronary artery. These participants may have atherosclerosis in the aorta, which should be determined in the future. We also determined the microRNA-126-3p level in white blood cells without apparently justification, which may cause bias in the findings. Finally, a familial history of cardiovascular disease may also affect the level of microRNA-126-3p, which should be determined in the future.

Conclusion

The data revealed that the levels of microRNA-126-3p in coronary arteries and veins were significantly greater in the CAC group than in the control group or in the atherosclerosis group, and that the microRNA-126-3p content increased with the calcification index. Regression analysis and receiver operating characteristic curve analysis suggested that the level of microRNA-126-3p in the vein may serve as an independent predictor of CAC, and the plasma microRNA-126-3p level may be a novel noninvasive biomarker for CAC patients.

Authors' contributions

XZ, MZ, and PZ carried out the experiments; XZ and MZ analyzed data; MG, HZ, SD, HH, and YL provided valuable suggestions and revised the manuscript. YC and HC designed the experiments and wrote the article. All authors read and approved the final manuscript.

Data availability

The data that support of the findings in this study are available from the corresponding authors on reasonable request.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethics approval

All studies with human blood samples were approved by the Ethical Committee of Tianjin Baodi Hospital and adhered strictly to the Declaration of Helsinki Principle 2008 (202002).

Informed consent

At the time of recruitment, all the participants were fully informed and signed consent statement.

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