Circulating miR-182-5p for protection of endothelial function from ADMA–induced injury inelderly coronary artery

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Research Article

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Abstract

Background

To investigate the correlation between the expression level of miR-182-5p and endothelial function in elderly individuals.

Methods

One thousand and fifty-eight patients > 60 years old living in the Pingguoyuan area in China were enrolled consecutively and were divided into four groups according to the results of reactive hyperemia index (RHI), and asymmetric dimethylarginine (ADMA). Gruop1 (n = 294):RHI ≥ 1.67 and ADMA ≤ 0.7umol/l (named the normal endotheltial function group); Group2 (n = 244):RHI ≥ 1.67 and ADMA > 0.7umol/l; Group3 (n = 242): RHI < 1.67 and ADMA ≤ 0.7umol/l; Group4 (n = 278):RHI < 1.67 and ADMA > 0.7umol/l(named the endotheltial dysfunction group). The association between miR-182-5p level, RHI and ADMA were analyzed using univariate correlation analysis and multiple regression analysis.

Results

RT-PCR results showed that the expression level of miR-182-5p in the endothelial dysfunction group was significantly lower than that in the normal endothelial function group. In addition,we found that the expression level of plasma miR-182-5p was negatively correlated with plasma ADMA.

Conclusion

MiR-182-5p had a protective effect on endothelial function and may be a potential therapeutic target for atherosclerosis in elderly individuals.

Introduction

Atherosclerosis (AS) is characterized by high morbidity and mortality, and is mainly characterized by chronic degeneration of arteries and gradual changes in the arterial wall[1]. The formation process of atherosclerosis is very complex, and the pathogenesis of atherosclerosis has not been fully elucidated yet[2, 3]. The dysfunction of endothelial cells has been regarded as the earliest phase in the progression of atherosclerosis, giving rise to atherosclerotic plaque formation and subsequent cardiovascular complications[4–6]. Previous studies have confirmed that asymmetric dimethylarginine (ADMA) can induce endothelial dysfunction by reducing nitric oxide utilization and increasing oxidative stress, and elevated ADMA can predict endothelial dysfunction[7, 8]. And, reactive hyperemia index (RHI) has been widely used to assess vascular endothelial function due to its advantages of convenient manual operation, good stability, and little error in multiple measurements[9, 10]. MicroRNAs (miRNAs) are a kind

of small, non-coding RNAs that miRNAs are involved in the regulation of metabolism and other important biological processes, and have potential applications in the treatment of atherosclerosis[11]. miRNAs regulate the progression of atherosclerosis through various targeting factors or signaling pathways, including regulation of endothelial function. Previous studies have revealed that miR-182-5p played significant roles in AS through inhibiting oxidative stress and apoptosis via inactivating TLR4 expression[12]. However, in elderly individuals with atherosclerosis, the relationship between endothelial function and miR-182-5p remains poorly understood. This study aimed to investigate the relationship between miR-182-5p level and endothelial dysfunction assessed by using reactive hyperemia index (RHI) and asymmetric dimethylarginine (ADMA) in individuals aged > 60 years.

Materials And Methods

Study population

We performed a community-based, cross-sectional study of 1058 elderly patients aged > 60 years from the Pingguoyuan area (Beijing, China), who underwent EndoPAT testing and plasma ADMA index testing. According to the results of ADMA and RHI, the subjects were categorized into four groups. Gruop1:RHI≥1.67 and ADMA≤0.7umol/l; Group2:RHI≥1.67 and ADMA>0.7umol/l; Group3: RHI<1.67 and ADMA≤0.7umol/l; Group4:RHI<1.67 and ADMA>0.7umol/l. Patients having the following conditions were excluded, such as bedridden status, mental illness, malignant tumors, systemic inflammatory reaction, major operation, and massive cerebral hemorrhage. This study was approved by the Ethics Committee of the People's Liberation Army General Hospital, and written informed consent was obtained from each patient. The study complied with the principles of the Declaration of Helsinki as well.

Clinical and biochemical data

Physical examinations were measured in the clinic following a standardized protocol. During the physical examination, data about age, complications, use of medications, highest blood pressure, waist circumference, hip circumference, height and weight were recorded. The levels of fasting glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, creatinine and uric acid were were measured at the clinic. The concentrations of serum homocysteine and ADMA were measured by enzyme-linked immunosorbent assay (ELISA) (Human ADMA/Hcy ELISA Kit,CUSABIO, Wuhan, China).

RNA extraction and qRT-PCR

Total RNA was extracted from plasma of patients using a Trizol reagent kit (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. RNA concentration and purity were detected using a Qubit Fluorometer (Thermo Fisher Scientific, Inc.). qPCR was performed using a SYBR Premix Ex Taq^{TM} Kit(Takara Bio Inc.). The expression of miR-182-5p was detected by a miRNA specific reverse transcription kit (D350,Takara, Tokyo, Japan) and then analyzed by qPCR with Power SYBR Green (DRR081 A, Takara, Tokyo, Japan).

Endothelial dysfunction measurements

Endothelial function was assessed via peripheral arterial tonometry, which is a non-invasive method, using the Endo-PAT 2000 device (Itamar Medical Ltd., Caesarea, Israel). Two flexible probes were placed on the index fingers of both hands along with inflatable cuffs and plethysmographic biosensors. The baseline data of the two sides were collected after resting for 30 min. Then, cuff occlusion in the left forearm lasted for 5 min, which resulted in transitory ischemia. All data of both fingers were recorded in the process of occlusion and post-occlusion. In the course, the parameter of RHI was calculated via an automated analysis. Thus, an RHI ≥ 1.67 indicated a normal vascular endothelial function, whereas an RHI < 1.67 represented an abnormal vascular endothelial function. PAT was used to assess endothelial function by measuring the pulse amplitude of one finger on each hand at rest and after induced reactive hyperemia (Endo-PAT2000, Itamar Medical, Caesarea, Israel). It includes three steps: baseline, occlusion, and hyperemia. First, it generates an inflation pressure on each of the fingers that was set to 10 mmHg below the subject's diastolic blood pressure or at least 70 mmHg. After baseline pulse amplitude was recorded from both fingers for 5 min and 45 s, the arterial flow was occluded for 5 min by a cuff placed on a proximal forearm with occlusion pressure higher than systolic blood pressure (SBP). Following cuff release, pulse amplitude was recorded for up to 5 min. During the process, the pulse amplitude was recorded from both fingers, and the pulse amplitude recordings were digitized and analyzed by an automated proprietary algorithm. The reactive hyperemia index (RHI) reflects microvascular endothelial function. In 2004, the Mayo Clinic found that PAT-RHI index of 1.67 for the diagnosis of endothelial dysfunction, with a sensitivity of 82% and a specificity of 77%[13].

Statistical methods

Statistical analysis of the data was performed using SPSS 26.0. Categorical variables are expressed as percentages and compared using χ^2 test.

Continuous variables are presented as mean±standard deviation (SD). One-way ANOVA was used for the comparison among the four groups, and SNK (Student-Newman-Keuls)q test was used for pairwise comparison of indicators with differences. Pearson correlation analysis was performed on ADMA, RHI and miR-18-5P respectively, simple linear regression analysis was performed on variables with relevant significance, and multiple linear regression analysis was performed in the case of considering age, BMI, fasting blood glucose, uric acid and other factors. A P-value < 0.05 was considered statistically significant.

Results

Clinical, and biochemical characteristics of the study population

The baseline characteristics of the study subjects were summarized in Table 1. A total of 1058 participants (377 men and 681 women) were included and the mean age was 66.72 ± 9.78 years. Among them, 294 (27.79%) had a normal endothelial function, which was defined as RHI ≥ 1.67 and ADMA ≤ 0.7umol/l (Group1), and 278 (26.28%) had endothelial dysfunction, which was defined as RHI < 1.67 and ADMA > 0.7umol/l (Group4). There were significant differences in age, smoking, DM, and hypertension across the four groups ($P < 0.05$). In addition, there was a significantly higher prevalence of smoking, hypertension, and DM in the endothelial dysfunction group compared to the normal endothelial function group($P < 0.05$), and the subjects were significantly older in the endothelial dysfunction group($P < 0.05$).

Table 1
The baseline characteristics of the study subjects categorized by ADMA and RHI level

Characteristics	All participants	RHI ≥ 1.67 and ADMA ≤ 0.7	RHI ≥ 1.67 and ADMA > 0.7	RHI < 1.67 and ADMA ≤ 0.7	RHI < 1.67 and ADMA > 0.7	P-value
N	1058	294	244	242	278	
Age (yrs)	66.72 ± 9.78	64.58 ± 9.63	66.84 ± 9.37	65.32 ± 9.23	68.51 ± 9.54*	< 0.001
Men (%)	377(35.7)	104(35.5)	80(32.8)	93(38.4)	100(36.0)	0.900
Smoking (%)	232(22.0)	37(12.6)	62(25.4)	44(18.2)	89(32.0)*	< 0.001
BMI (kg/m2)	25.86 ± 3.38	25.58 ± 3.68	25.74 ± 3.53	25.62 ± 3.04	26.08 ± 3.12	0.081
SBP (mmHg)	128.20 ± 20.09	129.58 ± 20.34	130.24 ± 18.82	126.33 ± 17.80	127.12 ± 19.95	0.145
DBP (mmHg)	74.11 ± 11.36	76.47 ± 11.22	74.34 ± 12.32	73.23 ± 13.22	75.45 ± 11.43	0.282
TC (mmol/l)	4.89 ± 0.94	4.84 ± 0.92	4.78 ± 0.88	4.96 ± 0.91	4.99 ± 0.96	0.703
TG (mmol/l)	1.53 ± 0.68	1.48 ± 0.69	1.57 ± 0.63	1.52 ± 0.58	1.59 ± 0.67	0.054
HDL-C (mmol/l)	1.44 ± 0.37	1.47 ± 0.39	1.43 ± 0.38	1.42 ± 0.32	1.41 ± 0.36	0.057
LDL-C (mmol/l)	3.17 ± 0.83	3.19 ± 0.82	3.16 ± 0.76	3.18 ± 0.80	3.15 ± 0.85	0.567
FBG (mmol/l)	5.96 ± 185	5.82 ± 1.61	5.92 ± 1.32	5.95 ± 1.20	6.07 ± 2.01	0.100
Hcy (mmol/l)	15.74 ± 5.99	15.60 ± 5.94	15.83 ± 5.88	15.76 ± 5.45	15.91 ± 6.04	0.536
Cr (umol/l)	72.67 ± 15.44	70.34 ± 15.37	71.14 ± 14.27	73.35 ± 15.24	69.25 ± 15.58	0.400
UA (umol/l)	287.52 ± 72.16	284.50 ± 73.05	288.33 ± 72.14	285.79 ± 71.08	290.49 ± 71.91	0.324
CVD (%)	460(43.5)	125(42.5)	104(42.6)	107(44.2)	124(44.6)	0.613
DM (%)	269(25.5)	38(13.0)	50(20.5)	73(30.2)	108(38.8)*	< 0.001

Characteristics	All participants	RHI ≥ 1.67 and ADMA ≤ 0.7	RHI ≥ 1.67 and ADMA > 0.7	RHI < 1.67 and ADMA ≤ 0.7	RHI < 1.67 and ADMA > 0.7	P-value
Hypertension (%)	397(37.6)	83(28.3)	87(35.7)	95(39.3)	132(47.5)*	< 0.001
Hyperlipidemia(%)	506(47.9)	138(47.2)	121(49.6)	111(45.9)	136(48.9)	0.695

Date expressed as mean ± standard deviation, or number(percentage).

RHI ≥ 1.67 and ADMA ≤ 0.7umol/l was named the normal endotheltial function group; RHI < 1.67 and ADMA > 0.7umol/l was named the endotheltial dysfunction group.

BMI: body mass index, TC: total cholesterol, TG: triglyceride, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, FBG: fasting blood glucose, Cr: creatinine, UA: uric acid, Hcy: homocysteine, SBP: systolic blood pressure, DBP: diastolic blood pressure, CVD: cardiovascular disease, DM: diabetes mellitus

*$P < 0.05$ (endotheltial dysfunction group vs. the normal endotheltial function group)

Comparison Of Mir-182-5p Between The Normal Endothelial Function Group And The Endotheltial Dysfunction Group

The expression level of miR-182-5p between the normal endothelial function group and the endothelial dysfunction group were shown in Fig. 1. RT-PCR results showed that the expression level of miR-182-5p in the endothelial dysfunction group (1.49 ± 0.23, N = 278) was significantly lower than that in the normal endothelial function group (1.61 ± 0.17, N = 294) ($P < 0.05$).

The Relation Of Adma And Mir-182-5p

Through scatter plot analysis, we can conclude that there is a linear trend between plasma miR-182-5p and ADMA, but there is no linear trend between plasma miR-182-5p and RHI (Fig. 2). The expression of plasma miR-182-5p in the elderly was negatively correlated with plasma ADMA, and the correlation coefficient was − 0.412 by Pearson correlation analysis. Adjust for age, gender, body mass index (BMI), fasting blood glucose (FBG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDLC), triglyceride (TG), etc. The multiple linear regression was performed with miR-182-5p as the independent variable and ADMA as the dependent variable. The regression equation was: $y=-0.703 + 0.021X1-0.552X2 + 0.158X3$ (X1: age, X2: miR-182-5p, X3 :diabetes mellitus, y: ADMA,$P < 0.05$, Table 2).

Table 2
The multiple linear regression of ADMA and miR-182-5p

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std.Error	Beta		
3	(Constant)	− .703	.326		-2.152	.033
	Age	.021	.004	.401	5.357	.000
	miR-182-5p	− .552	.186	− .220	-2.964	.004
	DM	.158	.077	.143	2.059	.041

Discussion

The endothelium is an active inner layer of the blood vessel and is the key regulator of vascular homeostasis. Endothelial dysfunction is the earliest measurable deterioration of the vessel wall in atherogenesis. However, to date, the specific mechanism of endothelial dysfunction has not been fully defined[14–16]. In this community-based, cross-sectional study, we analyzed the correlation between the expression level of miR-182-5p and endothelial function in elderly individuals in China. We found that the expression level of miR-182-5p in the endothelial dysfunction group was significantly lower, and the expression of plasma miR-182-5p was negatively correlated with plasma ADMA. These results suggested that miR-182-5p had a protective effect on endothelial function and may be a potential therapeutic target for atherosclerosis in elderly individuals.

Currently, several studies have been performed using different methods to assess vascular function, including coronary angiography, Doppler flow guidewire, blood Flow-mediated vasodilation (FMD), and reactive hyperemic peripheral arterial manometry (RH-PAT) [17–19]. In addition, the endogenous nitric oxide synthase inhibitor ADMA has also been suggested as a marker and mediator of endothelial dysfunction [20–21]. In this study, two methods of EndoPAT and plasma ADMA were used to evaluate endothelial function. The subjects who were RHI < 1.67 and plasma ADMA > 0.7 were included in the endothelial dysfunction group, and the subjects who were RHI ≥ 1.67 and ADMA ≤ 0.7 were included in the normal endothelial function group, to ensure the reliability of grouping as much as possible, and then provide the most solid foundation for detecting the expression levels of miR-182-5p in different groups in the next step.

Previous studies have shown that different microRNAs (miRNAs) may alter the expression program of endothelial cells, thereby affecting cell contact and stability, and ultimately promoting the occurrence and development of atherosclerosis [22–23]. For example, researchers demonstrated that miR-21, miR-221, miR-222, and miR-145 play regulatory roles in vascular smooth muscle cells proliferation, a key pathological process in atherosclerosis [22]. However, few studies have investigated the relationship between endothelial function and miR-182-5p. Previous studies have shown that the expression level of miR-182-5p is significantly decreased in various tumors, and upregulation of miR-182-5p could inhibit the

proliferation of tumor cells[24]. In addition, miR-182-5p could also inhibit inflammation, and miR-182-5p alleviated the nonalcoholic steatohepatitis induced by a high-fat diet in mice[25]. Recent in vitro experiments confirmed that miR-182-5p may participate in the process of atherosclerosis by regulating endothelial function[26].

In addition, Jin CL et al. found that the expression level of miR-182-5p in CAD was significantly lower than that in the normal population[27]. Our findings were consistent with recent studies. In this in vivo study, we found that the expression level of miR-182-5p in the endothelial dysfunction group was significantly lower, and the expression of plasma miR-182-5p was negatively correlated with plasma ADMA in the elderly. These results suggested that miR-182-5p had a protective effect on endothelial function and may be a potential therapeutic target for atherosclerosis in elderly individuals.

However, in this study, we found that the expression of plasma miR-182-5p was independent of RHI in the elderly. This result may be attributed to the following conditions. In this study, the number of subjects over the age of 80 made up 11.7% of the total. First, with increasing age, the sympathetic nervous system degenerated, and the ability to regulate the target organs decreased, so the fingertip artery-vein mixed flow was unstable. Second, the thickening of the fingertip cuticle in the elderly may also affect the results. This is why we chose plasma ADMA and RHI double positivity as the criterion for endothelial dysfunction at the beginning of the study. The current study had some limitations. First, it was performed on Chinese residents from two communities in Beijing. Thus, the results may not represent Chinese individuals from other areas. Second, the present study cannot identify causal relationships due to inherent problems with the cross-sectional design. Accordingly, our observations need to be confirmed in large-scale prospective studies.

Declarations

Ethics approval and consent to participate: The study protocol was approved by the Ethics Committee of the Chinese People's Liberation Army General Hospital. All patients provided written, informed consent before enrollment. The study complied with the principles of the Declaration of Helsinki.

Consent for publication: Not applicable.

Availability of data and materials: The datasets generated and/or analyzed during the current study are not publicly available due to privacy or ethical restrictions but are available from the corresponding author on reasonable request.

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Competing interests: The authors declare that they have no competing interests.

Authors' contributions: All authors contributed to the study conception and design. Material preparation and data collection were performed by JZ, HY, XW, QS, YZ, YD, and PY. Data analysis was performed by

JZ, HY,YD, and PY. The first draft of the manuscript was written by JZ and HY. In addition, all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

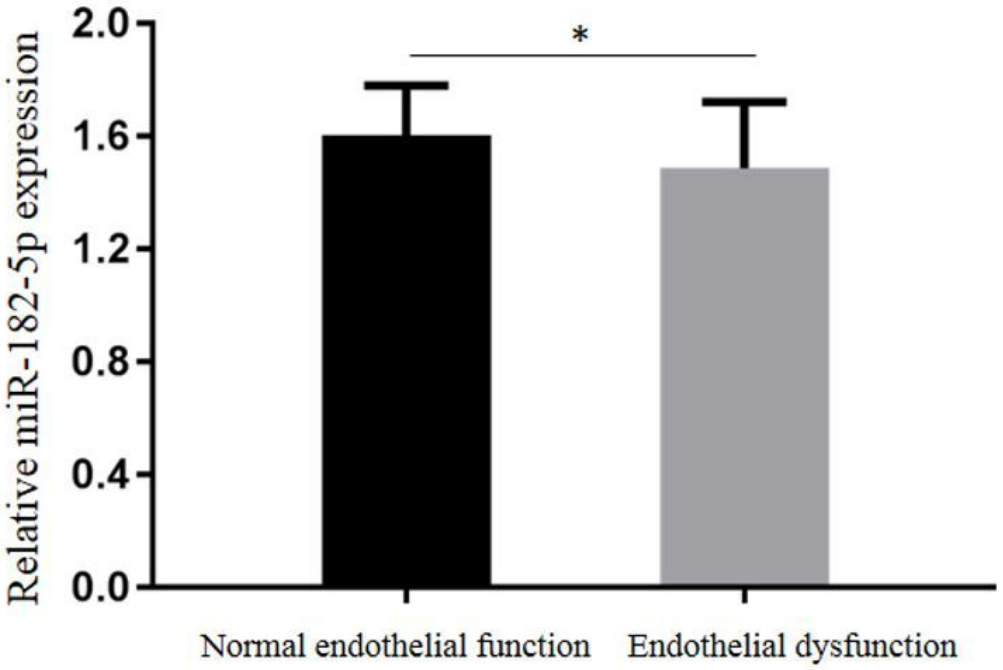

Figure 1

Comparison of miR-182-5p levels in normal endothelial function group and endothelial dysfunction group.

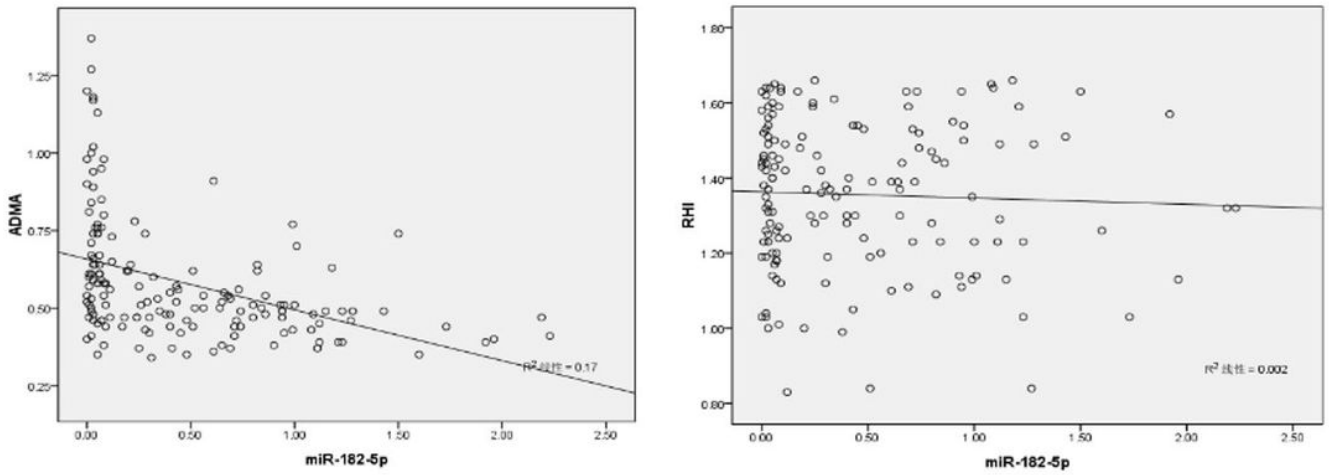

Figure 2

Scatter plots between MIR and ADMA and RHI, respectively.