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Original Research

Effect of total flavones of *Clematis filamentosa Dunn* on the oxLDL-induced injury of vascular smooth muscle cells by regulating miR-455-5p

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Abstract: This experiment was conducted to investigate whether total flavones of *Clematis filamentosa Dunn* affect the inflammatory response and apoptosis of vascular smooth muscle cells induced by oxidized low-density lipoprotein (oxLDL) by regulating microRNA-455-5p (miR-455-5p). 50 mg/mL oxLDL was performed to stimulate the injury of vascular smooth muscle cells, and the total flavones of *Clematis filamentosa Dunn* were added at concentrations of 75, 150, and 300 μg/mL. The expressions of inflammatory factors IL-1β and TNF-α were analyzed by ELISA, the apoptosis was evaluated by flow cytometry, the expression of Bcl-2 and Bax was determined by western blot, and the real-time fluorescence quantitative PCR (qRT-PCR) was applied to detect miR-455-5p expression. MiR-455-5p mimic was transfected into vascular smooth muscle cells and then induced injury with oxLDL; miR-455-5p inhibitor was transfected into vascular smooth muscle cells and treated with oxLDL and 300 μg/mL total flavones of *Clematis filamentosa Dunn*. The above methods were employed to investigate the inflammatory response and apoptosis of cells. The total flavones of *Clematis filamentosa Dunn* significantly inhibited the expression of IL-1β, TNF-α, apoptosis rate, Bax protein expression of oxLDL induced vascular smooth muscle cells, and remarkably promoted the expression of Bcl-2 protein and miR-455-5p, which all showed concentration dependence ($p<0.05$). Overexpression of miR-455-5p reduced IL-1β, TNF-α expression, apoptosis rate, Bax protein expression, and greatly increased Bcl-2 protein expression in oxLDL injured vascular smooth muscle cells ($p<0.05$). After interfering with the expression of miR-455-5p, the inhibitory effect of total flavones of *Clematis filamentosa Dunn* on the expression of IL-1β, TNF-α, apoptosis, Bax protein expression of oxLDL-induced vascular smooth muscle cells was reversed, and its promotion effect on Bcl-2 protein expression was also reversed. Total flavones of *Clematis filamentosa Dunn* can reduce oxLDL-induced vascular smooth muscle cell inflammation and inhibit its apoptosis. The mechanism of action is related to the up-regulation of miR-455-5p expression.

Key words: Total flavones of *Clematis filamentosa Dunn*; Vascular smooth muscle cells; oxLDL; Inflammation; Apoptosis; miR-455-5p.

Introduction

Arteriosclerosis is a general term for thickening and hardening of the walls of the arteries; in other words, when the arteries and veins lose their elasticity and sometimes the diameter of the arteries changes. The disease occurs in large and medium-sized arteries. The most common type is atherosclerosis, or hardening of the arteries, which causes hardening of the walls of the arteries due to the presence of low-density fat and cholesterol plaques. High blood cholesterol, high blood pressure, genetic factors and smoking are probably the most important causes of atherosclerosis. Atherosclerosis is the most important cause of death in many countries due to myocardial infarction. Atherosclerosis is more common in men. Increased lipid peroxidation is one of the main causes of the onset and progression of arteriosclerosis. Atherosclerosis is a chronic arterial disease with a high mortality rate worldwide, which is a major cause of acute cardiovascular events (1-2). As an inflammatory disease, it is characterized by lipid accumulation, fibrous cap formation, and necrotic nucleogenesis. The formation of atherosclerotic lesions involves a variety of cell types, such as smooth muscle cells, endothelial cells, and macrophages (2). Previous studies have shown that abnormal proliferation and apoptosis of vascular smooth muscle cells have a close relation to the progression of atherosclerosis (3). Oxidized low-density lipoprotein (oxLDL) is a key risk factor in the progress of atherosclerosis, which can affect the vascular smooth muscle cell proliferation and apoptosis according to reports (4). Clematis filamentosa Dunn is Clematis Ranunculaceae leaves, which can calm the mind, reduce blood pressure and activate meridians to stop the pain. Studies have shown that total flavones of *Clematis filamentosa Dunn* (TFCD) protect rat myocardial cells with H/R injury, whose mechanism may be related to anti-lipid peroxidation and reduction of intracellular calcium overload (5).

MicroRNAs (abbreviated miRNAs) are small, non-encoded RNA sequences (about 22 nucleotides) found in plants, animals, and some viruses that function in inhibiting RNA, post-transcription, and gene expression. While most miRNAs are located inside the cell, others, commonly called circulating or extracellular miRNAs, are also present in extracellular environments, such as various biological fluids and cell culture media. The miRNAs act based on a base pair relationship with their complementary sequences within mRNA molecules, and as a result, the mRNA molecule is inhibited during

one or more processes (6-7).

microRNA (miRNA) is a class of non-coding endogenous small RNAs with a length of about 18-22 nucleic acids. In atherosclerosis, many miRNAs have been proved as important regulators of pathological processes, such as endothelial dysfunction and lipid metabolism (6). According to reports, miR-455-5p is down-regulated in hypoxia-induced myocardial cell injury, interfering with lncRNA TTTY15 expression and reducing hypoxia-induced myocardial cell injury via negative regulation of miR-455-5p (7). miR-455-5p has a certain anti-inflammatory effect in the immune system, and its down-regulation advances the inflammatory pathway of recurrent multiple sclerosis disease in replacing period (8). However, it remains unknown regarding the effects of total flavones of *Clematis filamentosa Dunn* and miR-455-5p on oxLDL-induced vascular smooth muscle cell injury, and whether total flavones of *Clematis filamentosa Dunn* affect oxLDL-induced vascular smooth muscle cells by regulating miR-455-5p expression is unclear. This study aims to elucidate the protection of total flavones of *Clematis filamentosa Dunn* against oxLDL-induced vascular smooth muscle cell injury, and to explore the potential molecular mechanism based on miR-455-5p, thereby providing new clues for the treatment of atherosclerosis.

Materials and Methods

Materials

Total flavones of *Clematis filamentosa Dunn* (counted as 67% by rutin, Guangdong Chinese Medicine Research Institute), human aortic smooth muscle cells (HA-VSMC, American Type Culture Collection (ATCC)), SmGM -2 medium (Lonza, Switzerland), oxLDL (Solarbio, Beijing), Lipofectamine 2000 (Invitrogen, USA), miR-455-5p mimic, negative control miR-NC, miR-455-5p inhibitor (anti-miR-455-5p), negative control anti-miR-NC (Shanghai GenePharma), bicinchoninic acid (BCA) protein detection kit, phospholipid binding protein V-FITC(Annexin V-FITC)/ Propidium Iodide(PI), apoptosis detection kit (Jiangsu KeyGen Biotechnology Co., Ltd.), glyceraldehyde-3-phosphate dehydrogenase(GAPDH) antibody, B cell lymphoma/lewkmia-2 (Bcl-2) antibody, Bcl-2 Associated X Protein (Bax) antibody, HRP labeled sheep anti-rabbit IgG secondary antibody (Abcam, USA), interleukin-1β(Inter leukin-1β, IL-1β), tumor necrosis factor-α(TNF-α), Enzyme-Linked ImmunoSorbent Assay(ELISA) kit(Shanghai Mlbio Company), SuperScript First - Strand kit (Thermo Fisher, USA), QuantiTect SYBR Green kit (Qiagen, USA).

Cell culture

Vascular smooth muscle cells were cultured in SmGM-2 medium containing 5% fetal bovine serum and placed in a saturated and moist incubator at 37℃ with 5% CO_2.

Experiment grouping and treatment

Before transfection, vascular smooth muscle cells were divided into Con group (control group, normal saline with equal oxLDL was given for 24 h), oxLDL group (model group, 50 mg/mL oxLDL was given for 24 h(9)), oxLDL+ TFCD-l group (low concentration drug, 50 mg/mL oxLDL and 75 μg/mL total flavones of *Clematis filamentosa Dunn* were given for 24 h), oxLDL+ TFCD-M group (medium concentration drug, 50 mg/mL oxLDL and 150 μg/mL total flavones of *Clematis filamentosa Dunn* were given for 24 h), and oxLDL+ TFCD-H group(high concentration drug, 50 mg/mL oxLDL and 300 μg/mL total flavones of *Clematis filamentosa Dunn* were given for 24 h).

After transfection, the vascular smooth muscle cells were divided into oxLDL+ miR-NC group (after transfection with miR-NC for 24 h, 50 mg/mLoxLDL was given for 24h), oxLDL+ miR-455-5p group (after transfection with miR-455-5p mimic for 24 h, 50 mg/mLoxLDL was given for 24h), oxLDL+TFCD+ anti-miR-NC group(after transfection with anti-miR-NC for 24h, 50 mg/mL oxLDL and 300 μg/mL total flavones of *Clematis filamentosa Dunn* were given for 24h), oxLDL+TFCD+ anti-miR-455-5p group (after transfection with miR-455-5p inhibitor for 24 h, 50 mg/mL oxLDL and 300 μg/mL total flavones of *Clematis filamentosa Dunn* were given for 24h).

When transfection with vascular smooth muscle cells, the cells were seeded in a 6-well plate with a density of 1×10^5 cells/well. When the cell confluence was observed at 60%-70%, miR-455-5p mimic, miR-NC, miR-455-5p inhibitor, anti-miR-NC were transfected into the cells using Lipofectamine 2000 reagent according to the manufacturer's instructions, followed by grouping. Where quantitative real-time PCR (qRT-PCR) was used to evaluate miR-455-5p overexpression or interference with miR-455-5p expression efficiency.

Analysis of IL-1β, TNF-α expression by ELISA

In accordance with the manufacturer's guidelines, the supernatant of differently-treated vascular smooth muscle cells was collected to detect IL-1β, TNF-α expression by ELISA.

Apoptosis assessment by flow cytometry

According to the manufacturer's instructions, differently-treated vascular smooth muscle cells were collected, washed with phosphate buffer, suspended in binding buffer. 5 μL Annexin V-FITC and 5 μL PI staining solution were added into 1×10^5 cells, followed by reaction in the dark for 30 min. Cell apoptosis was analyzed using flow cytometry, and cell apoptosis rate equals to the sum of early apoptosis cells (Annexin V^+/PI^-) and late apoptosis cells (Annexin V^+/PI^+).

Detection of Bcl-2, Bax protein expressions by western blot

Protein was extracted from differently-treated vascular smooth muscle cells using RIPA lysate, followed by protein quantification using the BCA kit. 50 μg protein samples were extracted and subject to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and membrane transfer. The separated protein was sealed in 5% skim milk powder, and then fully washed with Tris-buffered saline with Tween (TBST). 1:1000 diluted Bcl-2, Bax and 1:2000 diluted control GAPDH primary antibody was added and incubated overnight at 4℃. On the next day, the 1:500 diluted HRP-labeled sheep anti-rabbit IgG secondary antibody was added and incubated

at room temperature for 1 h. After fully washed with TBST, the protein bands were visualized by enhanced chemiluminescence liquid, and the protein expression levels of Bcl-2 and Bax were analyzed by Quantity One software.

Detection of miR-455-5pexpression by qRT-PCR

Total RNA was isolated from vascular smooth muscle cells using Trizol reagent and then reverse transcribed using SuperScript First-Strand according to manufacturer's instructions. Real-time PCR was performed using the QuantiTect SYBR Green PCR kit. The primer sequence of miR-455-5p is: forward: 5'-CGAGCTTCCTTCTGCAGGT-3', reverse:5'-CACCACTGCCATCCCACA-3'. The primer sequence of internal reference U6 is: forward:5'-TGCGGGTGCTCGCTTCGGCAGC-3', reverse: 5'-GTGCAGGGTCCGAGGT-3'. miR-455-5p expression was analyzed using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

SPSS22.0 software was used for statistical processing of the data. Each independent experiment was repeated three times, and the results were expressed as mean ±standard deviation (x±s). t-test was used for data comparison between two groups, one-way analysis of variance was used for data comparison between multiple groups, and the SNK-q test was used for pairwise comparison between groups. $p<0.05$ indicates a significant difference.

Table 1. Effect of total flavones of *Clematis filamentosa Dunn* on the expression of oxLDL-induced inflammatory cytokines in vascular smooth muscle cells (x±s, n=9).

Group	IL-1β(pg/mL)	TNF-α(pg/mL)
Con	17.91±1.59	31.19±3.91
oxLDL	65.93±5.19[a]	93.52±7.23[a]
oxLDL+TFCD-L	53.79±4.79[b]	79.83±6.78[b]
oxLDL+TFCD-M	41.64±3.53[bc]	61.93±5.22[bc]
oxLDL+TFCD-H	27.97±2.30[bcd]	44.78±4.07[bcd]
F	239.290	182.818
p	0.000	0.000

Note: compared with Con group, [a]$p<0.05$; compared with oxLDL group, [b]$p<0.05$; compared with oxLDL+TFCD-L group, [c]$p<0.05$; compared with oxLDL+TFCD-M group, [d]$p<0.05$.

Results

Effect of total flavones of *Clematis filamentosa Dunn* on the expression of oxLDL-induced inflammatory cytokines in vascular smooth muscle cells

The detection data of inflammatory cytokines are shown in Table 1. Compared with the Con group, oxLDL group has significantly increased expression levels of inflammatory cytokines IL-1β, TNF-α in vascular smooth muscle cells ($p<0.05$). After treatment with total flavones of *Clematis filamentosa Dunn*, compared with oxLDL group, oxLDL+TFCD-L group, oxLDL+TFCD-M group and the oxLDL+TFCD-H group have significantly decreased expression levels of IL-1β, TNF-α in vascular smooth muscle cells in a concentration-dependent manner($p<0.05$).

Effect of total flavones of *Clematis filamentosa Dunn* on oxLDL-induced apoptosis of vascular smooth muscle cells

The apoptosis detection results are shown in Table 2 and Figure 1B. Compared with the Con group, the oxLDL group has significantly increased the apoptosis rate of vascular smooth muscle cells ($P<0.05$). Compared with the oxLDL group, oxLDL+TFCD-L, oxLDL+TFCD-M and oxLDL+TFCD-H groups have

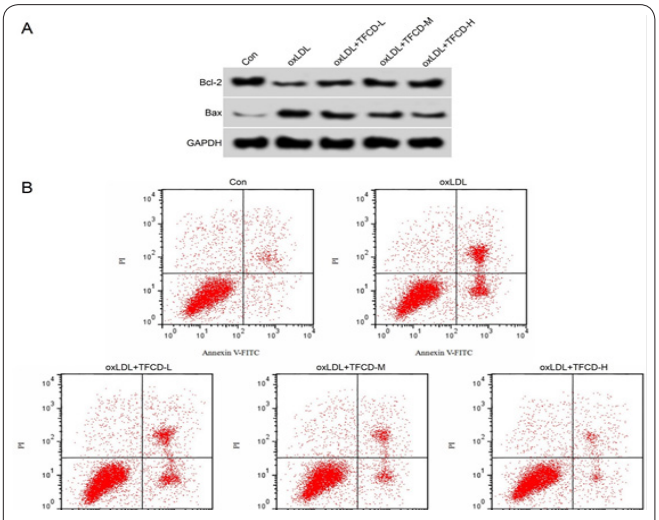

Figure 1. Effect of total flavones of *Clematis filamentosa Dunn* on oxLDL-induced apoptosis of vascular smooth muscle cells. A: Apoptosis-related protein expression; B: Flow cytometry of apoptosis.

Table 2. Effect of total flavones of *Clematis filamentosa Dunn* on oxLDL-induced apoptosis of vascular smooth muscle cells (x±s, n=9).

Group	Apoptosis rate(%)	Bcl-2 protein	Bax protein
Con	8.23±0.57	0.76±0.05	0.16±0.02
oxLDL	32.81±2.78[a]	0.28±0.03[a]	0.57±0.04[a]
oxLDL+TFCD-L	24.47±2.07[b]	0.41±0.04[b]	0.46±0.04[b]
oxLDL+TFCD-M	17.42±1.50[bc]	0.53±0.05[bc]	0.34±0.03[bc]
oxLDL+TFCD-H	11.53±1.16[bcd]	0.65±0.06[bcd]	0.22±0.02[bcd]
F	278.796	146.068	260.816
p	0.000	0.000	0.000

Note: Compared with Con group, [a]$p<0.05$; compared with oxLDL group, [b]$p<0.05$; compared with oxLDL+TFCD-L group, [c]$p<0.05$; compared with oxLDL+TFCD-M group, [d]$p<0.05$.

significantly reduced the apoptosis rate of vascular smooth muscle cells in a concentration-dependent manner ($p<0.05$). The expression detection results of apoptosis-related proteins Bcl-2 and Bax are shown in Table 2 and Figure 1A. Compared with the Con group, oxLDL group has significantly reduced Bcl-2 protein expression level and significantly increased Bax protein expression level in vascular smooth muscle cells ($p<0.05$). Compared with the oxLDL group, oxLDL+TFCD-L, oxLDL+TFCD-M and oxLDL+TFCD-H groups have significantly increased Bcl-2 protein expression levels and significantly decreased Bax protein expression level in vascular smooth muscle cells, both in a concentration-dependent manner ($p<0.05$).

Effect of total flavones of *Clematis filamentosa Dunn* on oxLDL-induced miR-455-5p expression in vascular smooth muscle cells

The measured miR-455-5p expression is shown in Table 3. Compared with the Con group, the oxLDL group has significantly reduced miR-455-5p expression in vascular smooth muscle cells ($p<0.05$). Compared with the oxLDL group, oxLDL+TFCD-L, oxLDL+TFCD-M and oxLDL+TFCD-H groups have gradually increased miR-455-5p expression level in vascular smooth muscle cells, showing concentration dependence ($p<0.05$).

Table 3. Effect of total flavones of *Clematis filamentosa Dunn* on oxLDL-induced miR-455-5p expression in vascular smooth muscle cells($\bar{x}\pm s$, n=9).

Group	miR-455-5p
Con	1.00±0.05
oxLDL	0.37±0.04[a]
oxLDL+TFCD-L	0.53±0.04[b]
oxLDL+TFCD-M	0.66±0.05[bc]
oxLDL+TFCD-H	0.82±0.07[bcd]
F	206.897
p	0.000

Note: Compared with Con group, [a]$p<0.05$; compared with oxLDL group, [b]$p<0.05$; compared with oxLDL+TFCD-L group, [c]$p<0.05$; compared with oxLDL+TFCD-M group, [d]$p<0.05$.

Effect of miR-455-5p overexpression on the expression of oxLDL-induced inflammatory cytokines in vascular smooth muscle cells

The detection results of miR-455-5p expression are shown in Table 4. Compared with the oxLDL+miR-NC group, the oxLDL+miR-455-5p group has significantly increased miR-455-5p expression level in vascular smooth muscle cells ($p<0.05$). The detection data of inflammatory cytokines IL-1β, TNF-α are shown in Table 4. Compared with the oxLDL+miR-NC group, the oxLDL+miR-455-5p group has significantly reduced expression levels of IL-1β, TNF-α ($p<0.05$).

Effect of miR-455-5p overexpression on oxLDL-induced apoptosis of vascular smooth muscle cells

The apoptosis detection results are shown in Table 5 and Figure 2B. Compared with the oxLDL+miR-NC group, the oxLDL+miR-455-5p group has significantly reduced the apoptosis rate of vascular smooth muscle cells ($p<0.05$). The detection results of Bcl-2 and Bax protein expression are shown in Table 5 and Figure 2A. Compared with the oxLDL+miR-NC group, the oxLDL+miR-455-5p group has significantly increased Bcl-2 protein expression level and significantly reduced Bax protein expression level ($p<0.05$).

Interference with miR-455-5p expression reverses the effect of total flavones of *Clematis filamentosa Dunn* (300μg/mL) on oxLDL-induced vascular smooth muscle cell injury

miR-455-5p expression and damage of vascular

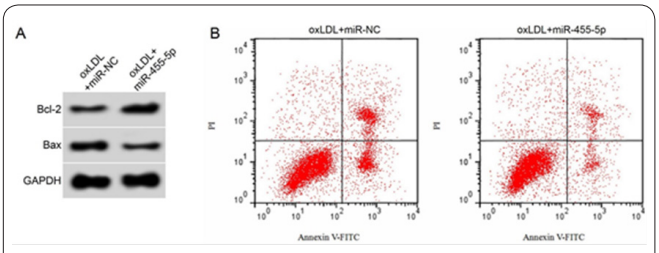

Figure 2. Effect of miR-455-5p overexpression on oxLDL-induced apoptosis of vascular smooth muscle cells. A: Apoptosis-related protein expression; B: Flow cytometry of apoptosis.

Table 4. Effect of miR-455-5p overexpression on the expression of oxLDL-induced inflammatory cytokines in vascular smooth muscle cells ($\bar{x}\pm s$, n=9).

Group	miR-455-5p	IL-1β(pg/mL)	TNF-α(pg/mL)
oxLDL+miR-NC	1.00±0.06	67.17±6.56	98.29±9.40
oxLDL+miR-455-5p	2.91±0.24[a]	39.72±3.65[a]	53.67±4.27[a]
t	23.162	10.970	12.965
p	0.000	0.000	0.000

Note: Compared with the oxLDL+miR-NC group, [a]$p<0.05$.

Table 5. Effect of miR-455-5p overexpression on oxLDL-induced apoptosis of vascular smooth muscle cells ($\bar{x}\pm s$, n=9).

Group	Apoptosis rate (%)	Bcl-2 protein	Bax protein
oxLDL+miR-NC	33.14±9.22	0.27±0.03	0.59±0.05
oxLDL+miR-455-5p	15.56±1.33[a]	0.61±0.05[a]	0.29±0.03[a]
t	5.662	17.493	15.435
p	0.000	0.000	0.000

Note: Compared with the oxLDL+miR-NC group, [a]$p<0.05$.

Table 6. Interference with miR-455-5p expression reverses the effect of total flavones of *Clematis filamentosa Dunn* (300μg/mL) on oxLDL-induced vascular smooth muscle cell injury (x±s, n=9).

Group	miR-455-5p	IL-1β(pg/mL)	TNF-α(pg/mL)	Apoptosis rate (%)	Bcl-2 protein	Bax protein
oxLDL+TFCD +anti-miR-NC	1.00±0.05	26.94±2.78	43.57±3.97	10.62±1.04	0.67±0.05	0.21±0.02
oxLDL+TFCD +anti-miR-455-5p	0.55±0.05[a]	54.89±5.75[a]	81.91±7.56[a]	25.93±2.41[a]	0.38±0.03[a]	0.47±0.04[a]
t	19.092	13.129	13.470	14.498	14.920	17.441
p	0.000	0.000	0.000	0.000	0.000	0.000

Note: Compared with oxLDL+TFCD+anti-miR-NC group, [a]$P<0.05$.

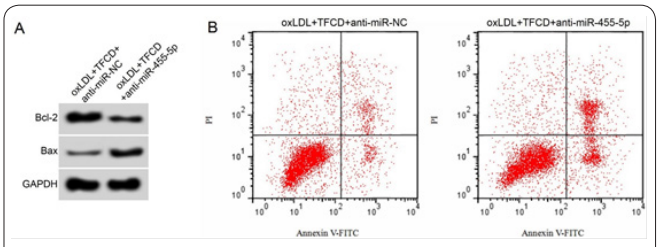

Figure 3. Interference with miR-455-5p expression reverses the effect of total flavones of *Clematis filamentosa Dunn* on oxLDL-induced apoptosis of vascular smooth muscle cells. A: Apoptosis-related protein expression; B: Flow cytometry of apoptosis.

smooth muscle cells are measured as shown in Table 6 and Figure 3. Compared with the oxLDL+TFCD+anti-miR-NC group, the oxLDL+TFCD+anti-miR-455-5p group has significantly reduced miR-455-5p expression level, significantly increased IL-1β, TNF-α expression levels and apoptosis rate, significantly decreased Bcl-2 protein expression level and significantly increased Bax protein expression level ($p<0.05$).

Discussion

Vascular smooth muscle cells play a key role in atherosclerosis (10) as the main cellular determinant of arterial wall disease. In atherosclerosis, vascular smooth muscle cells first carry lipid retention and lipid overload through the formation and death of foam cells. As vascular smooth muscle cells help the synthesis of matrix components to form fibrous caps of atherosclerotic plaques, induction of apoptosis in vascular smooth muscle cells increases the vulnerability and medial degeneration of plaques, leading to plaque thrombosis (11). OxLDL plays an important role in the early stages of atherosclerosis because activation of its receptor (LOX-1) increases oxidative stress and apoptosis in the blood vessels, inducing endothelial dysfunction that leads to inflammation. Therefore, oxLDL-stimulated vascular smooth muscle cells are taken as the model of atherosclerotic cells in this study to investigate the regulatory mechanisms involved in atherosclerosis.

Atherosclerosis is a chronic inflammatory response to arterial wall damage. Endothelial inflammation will stimulate the production of pro-inflammatory cytokines and adhesion molecules like IL (interleukin)-6. These effectors cause adherence of monocytes and T cells as well as infiltration of neointimal lesions so that pro-inflammatory cytokines are secreted therefrom, such as TNF-α and interferon γ (12). Monocyte-derived macrophages produce foam cells via phagocytosis of oxLDL, which shows characteristics of fatty streaks. Blocking inflammatory factor IL-1β is a promising therapy that can prevent the occurrence and progression of atherosclerotic plaques and thereby prevent cardiovascular disease. TNF-α is an early inflammatory biomarker of atherosclerosis, which can predict cardiovascular events in the short term (13). In this study, it was observed that oxLDL induced vascular smooth muscle cells, resulting in significantly increased expression of inflammatory cytokines IL-1β, TNF-α. At the same time, there was increased apoptosis, increased expression of pro-apoptotic protein Bax, and decreased expression of anti-apoptotic protein Bcl-2, suggesting that vascular smooth muscle cells were injured by oxLDL. In clinical care, keeping a healthy lifestyle is the most important measure to prevent atherosclerosis, so several unhealthy behaviors should be avoided, such as smoking, sedentary lifestyle, overweight and bad dietary habits (14). Considering the key role of inflammation in the pathogenesis of atherosclerosis, anti-inflammatory therapy is considered as a promising option for the treatment of atherosclerosis.

Clematis filamentosa Dunn is traditional Chinese medicine in China with rich flavonoids, which is often used in the treatment of hypertension and coronary heart disease (15). Data show that total flavones of *Clematis filamentosa Dunn* have been proved to have a protective effect on the nerve damage caused by hydrogen peroxide (16), which can interfere with the mitochondrial pathway of apoptosis via inhibition of lipid peroxidation, thereby reducing the myocardial toxicity of epirubicin (17). Total flavones of *Clematis filamentosa Dunn* can alleviate myocardial ischemia-reperfusion injury in rats by oxidation resistance, promoting the production of nitric oxide and activating Phosphoinositide 3-kinase (PI3K)/Protein kinase B (Akt) pathway (18). In this experiment, the detection results of vascular smooth muscle cell injury show that treatment with different concentrations of total flavones of *Clematis filamentosa Dunn* significantly inhibits oxLDL-induced IL-1β, TNF-α expression, apoptosis rate, Bax protein expression in vascular smooth muscle cells, and significantly promotes oxLDL-induced Bcl - 2 protein expression in vascular smooth muscle cells. It is worth noting that total flavones of *Clematis filamentosa Dunn* show concentration dependence in these effects, indicating that total flavones of *Clematis filamentosa Dunn* can alleviate oxLDL-induced inflammatory reaction of vascular smooth muscle cells and inhibit cell apoptosis, showing certain activity in the prevention or treatment of atherosclerosis.

Total flavones of *Clematis filamentosa Dunn* at different concentrations significantly increased oxLDL-induced miR-455-5p expression in vascular smooth

muscle cells, suggesting that the protection of total flavones of *Clematis filamentosa Dunn* against oxLDL injury in vascular smooth muscle cells may be related to the up-regulation of miR-455-5p. miRNA can bind to mRNA in 3' -untranslated region (UTR), thereby regulating gene expression at the post-transcriptional level (19). Previous reports have shown that miR-455 expression is down-regulated in primary neurons undergoing oxygen and glucose deprivation (OGD), and the overexpression of miR-455 protects neurons from death by down-regulating tumor necrosis factor receptor-related factor 3 (20). miR-455-5p has low expression in high glucose-induced retinal pigment epithelium injury, and up-regulating it can increase the activity of high glucose-induced retinal pigment epithelium, inhibit apoptosis, and inhibit the secretion of inflammatory cytokines IL-1β, IL-6 and TNF-α, thereby effectively alleviating high glucose-stimulated inflammatory response (21). These studies demonstrate the protective effect of miR-455-5p in different cell injury. However, so far, the functional significance of miR-455-5p in oxLDL-treated vascular smooth muscle cells remains unknown. In this study, it was detected that overexpression of miR-455-5p significantly reduced IL-1β, TNF-α expressions, apoptosis rate, Bax protein expression, and significantly increased Bcl-2 protein expression in oxLDL-induced damaged vascular smooth muscle cells, which is consistent with the previous studies. In oxLDL-induced injury of vascular smooth muscle cells, miR-455-5p can also protect vascular smooth muscle cells from oxLDL injury. In addition, this study investigates the mechanism of action of total flavones of *Clematis filamentosa Dunn*, finding that after interfering with miR-455-5p expression, the inhibitory effect of total flavones of *Clematis filamentosa Dunn* on oxLDL-induced IL-1β, TNF-α expression, apoptosis, and Bax protein expression in vascular smooth muscle cells was reversed, and its promoting effect on Bcl-2 protein expression was also reversed. These results suggest that the protection of total flavones of *Clematis filamentosa Dunn* against oxLDL-induced vascular smooth muscle cell injury is achieved by up-regulating miR-455-5p expression. The subject of gene expression and its regulation has always been an interesting topic for molecular genetic researchers, and research in this area has yielded interesting results. Gene editing has been one of the most important achievements of human research that can greatly control gene expression (22-33). In this experiment, the miR-455-5p expression was targeted.

In conclusion, this study demonstrates the protection of total flavones of *Clematis filamentosa Dunn* against oxLDL-induced vascular smooth muscle cell injury. Total flavones of *Clematis filamentosa Dunn* can inhibit the expression of inflammatory factors and reduce cell apoptosis. In addition, its mechanism of action has a relation to the up-regulation of miR-455-5p expression.

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