Ginsenoside Rb3 reduces ox-LDL-induced injury in human aortic endothelial cells by regulating the miR-513a-5p/ZBTB20 axis

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Abstract

Background. Atherosclerosis (AS) is a common vascular disease, and its main influencing factor is endothelial damage caused by oxidized low-density lipoprotein (ox-LDL). As one of the main active ingredients of ginseng, ginsenoside Rb3 has anti-inflammatory and anti-oxidative effects. However, the role of ginsenoside Rb3 in endothelial injury induced by ox-LDL is not clear.

Objectives. This study aimed to evaluate the effect and potential mechanism of ginsenoside Rb3 action on ox-LDL-treated human aortic endothelial cells (HAECs).

Materials and methods. The HAECs treated with ox-LDL were used to establish an in vitro AS model. The viability of the HAECs was analyzed with Cell Counting Kit-8 (CCK-8). Flow cytometry was performed to assess the apoptosis. Oxidative stress, inflammation and endothelial dysfunction were evaluated using enzyme-linked immunosorbent assay (ELISA) and western blotting. The levels of miR-513a-5p were assessed using quantitative real-time polymerase chain reaction (qPCR). A dual-luciferase assay was performed to analyze the relationship between miR-513a-5p and a zinc finger and BTB domain-containing protein (ZBTB20).

Results. Exposure of HAECs to ox-LDL (50 μg/mL) reduced cell viability, superoxide dismutase (SOD) activity and endothelial nitric oxide synthase (eNOS) expression, while increasing the levels of malondialdehyde (MDA), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), and soluble intercellular adhesion molecule-1 (sICAM-1). The pretreatment with Rb3 markedly enhanced cell viability and decreased ox-LDL-induced oxidative stress, inflammation and endothelial dysfunction in HAECs. The ox-LDL decreased the level of miR-513a-5p, which was reversed by Rb3 pretreatment. The ZBTB20 was a target of miR-513a-5p in HAECs, and ox-LDL upregulated ZBTB20 expression, which was reversed by Rb3 pretreatment. The protective effect of Rb3 on ox-LDL-induced HAECs was diminished by miR-513a-5p inhibition, which was reversed by ZBTB20 knockdown.

Conclusions. Ginsenoside Rb3 reduces the effects of ox-LDL on HAECs by regulating the miR-513a-5p/ZBTB20 axis, which provides a theoretical basis for the treatment of AS.

Key words: atherosclerosis, oxidized low-density lipoprotein, miR-513a-5p, ZBTB20

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Background

Atherosclerosis (AS) is a condition characterized by a lipid metabolic imbalance and persistent inflammation.[1] It is a major cause of cardiovascular disease (CVD), which is the greatest cause of death worldwide.[2] Atherosclerosis lays down the groundwork for future strokes and heart attacks.[3] Aberrant human aortic endothelial cell (HAEC) injuries are regarded as one of the pathological characteristics in the progression of AS.[4] According to research, oxidized low-density lipoprotein (ox-LDL)-induced proliferation, migration and apoptosis of HAECs are linked to the progression of AS.[5,6]

Ginsenosides Rb1, Rd, F1, Rg1–Rg3, compound K, and total ginsenosides (TG) have all been shown to be anti-AS.[7] For example, Rb2 via the GPR120/AMPK/HO-1 pathway suppressed human umbilical vein endothelial cell (HUVEC) apoptosis induced by lipopolysaccharide (LPS) and inhibited THP-1 cell adhesion to HUVEC, resulting in a reduction in inflammation and endoplasmic reticulum (ER) stress.[8] Ginsenoside Rb3 (PubChem CID: 12912363) is one of ginseng's most common active components, and has shown anti-inflammatory and anti-oxidative properties.[9] The Rb3 strongly suppresses angiotensin II (Ang II)-induced vascular smooth muscle cell (VSMC) proliferation by arresting the cell cycle,[10] implying that it may play a positive function in CVD. However, the role of Rb3 in preventing AS is still unclear.

Non-coding RNA (ncRNA) has increasingly been recognized for its role in a range of illnesses, including CVD.[11] It has been established that ncRNA plays important regulatory roles in vascular inflammation and smooth muscle cells, thus constituting a novel treatment for AS.[12] MicroRNA (miRNA) is reported to inhibit gene expression resulting in mRNA degradation or translation inhibition.[13] Existing research indicates that miRNA is directly associated with inflammation, oxidative stress and apoptosis, which suggests its involvement in the underlying mechanism of AS development.[14] Interestingly, Rb1 and Rb2 have shown anti-aging and anti-inflammatory properties through the modulation of miRNA expression.[15] Based on these findings, ox-LDL-induced HAECs were used to construct an in vitro AS model in order to investigate the regulation of miRNA by Rb3 in AS.

Objectives

This study aimed to evaluate the effect and potential mechanism of ginsenoside Rb3 action on ox-LDL-treated HAECs.

Materials and methods

Cell treatment

The HAECs (Cat. No. 6100 from 3 individuals; ScienCell Research Laboratories, Carlsbad, USA) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS; Gibco, Waltham, USA) at 37°C in a 5% CO_2 humidified incubator with penicillin and streptomycin (Sigma-Aldrich, St. Louis, USA), at doses of 100 U/mL and 100 mg/mL, respectively. The HAECs were then cultured in a medium containing various concentrations (0–100 μg/mL) of ox-LDL (Biosynthesis Biotechnology Company, Beijing, China) for 24 h, or 50 μg/mL of ox-LDL for 0 h, 12 h, 24 h, or 48 h to establish the in vitro AS model. For Rb3 treatment, HAECs were treated with various concentrations (0–80 μM) of Rb3 (Cat. No. A0236; ≥98%; Must Biotechnology, Chengdu, China) for 24 h. For the combined treatment, HAECs were treated with Rb3 (20 μM or 40 μM) for 24 h, and then Rb3-treated HAECs were treated with ox-LDL (50 μg/mL) for 24 h.

Cell viability

Following the appropriate treatment, Cell Counting Kit-8 (CCK-8) reagent (10 μL) (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was added to the HAECs (5×10^4 cells/well) in a 96-well plate for 1 h, followed by the measurement of cell viability with the use of a LB960 microplate reader (450 nm; Titertek-Berthold, Pforzheim, Germany).

Cell apoptosis

Flow cytometry was used to determine the apoptosis rate after HAECs (4×10^5 cells/well in 6-well plates) were stained with fluorescein-5-isothiocyanate (FITC)-conjugated annexin V and propidium iodide (PI; Keygen, Nanjing, China) for 10 min in the dark at room temperature.

Enzyme-linked immunosorbent assay

To measure the content of malondialdehyde (MDA), superoxide dismutase (SOD), soluble intercellular adhesion molecule-1 (sICAM-1), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α) in the culture media, enzyme-linked immunosorbent assay (ELISA) kits (Beyotime, Beijing, China) were used according to the manufacturer's protocol, followed by measuring the absorbance at 450 nm with the use of a LB960 microplate reader (Titertek-Berthold).

Western blot analysis

After lysing HAECs, the protein content was determined using a bicinchoninic acid (BCA) assay, and then the cells

were transferred to a polyvinylidene difluoride (PVDF) membrane and separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Prior to detection using Femto enhanced chemiluminescence substrates (Thermo Fisher Scientific, Waltham, USA) and a ChemiDoc MP Imaging System (Bio-Rad, Hercules, USA), the membranes were blocked with 5% non-fat milk at room temperature for 1 h, followed by the addition of anti-endothelial nitric oxide synthase (anti-eNOS) and anti-zinc finger and BTB domain-containing protein (anti-ZBTB20) antibodies, as well as an alkaline phosphatase-conjugated secondary antibody.

Microarray data and differential expression analysis

The microarray expression profiling analysis GSE137580 (platform GPL24741; https://www.ncbi.nlm. nih.gov/geo/query/acc.cgi?acc=GSE137580) was downloaded from the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/). The GEO annotation file for GPL21265 was obtained. Using the online analysis program GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r), the expression profiles of ox-LDL-treated HAECs (n = 3) and untreated HAECs (n = 3) were compared to identify differentially expressed miRNAs using the following criteria: $|\log_2 FC \text{ (fold change)}| > 1$ and $p < 0.05$.

Quantitative real-time polymerase chain reaction

Using the TRIzol reagent purchased from Invitrogen (Waltham, USA), RNA was extracted from the HAECs, and a RT First Strand Kit was subsequently used to synthesize cDNA, which was utilized as a template for quantitative real-time polymerase chain reaction (qPCR) amplification to examine miRNA expression using miRNA-specific primers (Ribobio, Guangzhou, China). Moreover, after using PrimeScriptTM RT Master Mix for mRNA expression of ZBTB20, the qPCR was carried out with a SYBR Green qPCR mix (Yeasen Biotechnology Co., Ltd., Shanghai, China) on an ABI 7500 system (Applied Biosystems, Foster City, USA). The miRNA expression was standardized to U6 while standardizing mRNAs to GAPDH. The $2^{-\Delta\Delta Ct}$ technique was used to examine the data.

Transfections

RiboBio developed and manufactured the miR-513a-5p mimics/inhibitors/negative control (NC), and Genepharma (Shanghai, China) provided small interfering RNAs (siRNAs) specific for ZBTB20 (si-ZBTB20, 5′- GAC TAG TTA AAT GGC GGA AGA TAA A-3′) and non-specific siRNA (si-NC) for the NC. Lipofectamine 2000 (Invitrogen) was used to transfect the HAECs.

Dual-luciferase assay

After using a QuikChange Kit (Qiagen, Hilden, Germany) to perform site-directed mutagenesis of the ZBTB20 3'-UTR within the probable miR-513-5p binding region, the ZBTB20-wt or ZBTB20-mut was amplified and cloned into a pMIR-REPORT luciferase vector (Ambion, Austin, USA). The HAECs were seeded in 24-well plates (2×105 cells per well) and co-transfected with ZBTB20-wt/ZBTB20-mut and miR-513-5p mimic/NC. The relative firefly luciferase activity was measured 48 h after transfection.

Statistical analyses

IBM SPSS v. 22.0 software (IBM Corp., Armonk, USA) was used to examine the data, which were expressed as mean and 95% confidence intervals (95% CIs). Each experiment was performed on 3 samples for each group, and each experiment was repeated in triplicate for every sample. To determine statistical significance, Student's t-test was used to compare the 2 groups. For the comparison of 3 or more groups, a bootstrap analysis of variance (ANOVA) with a bootstrap post hoc test was utilized. The distribution of data was analyzed using the Shapiro–Wilk test. Additionally, '0' in Fig. 1 represents the drug concentration of 0, i.e., untreated conventional HAECs. The heatmap was generated using the R package ComplexHeatmap (R Foundation for Statistical Computing, Vienna, Austria). The Venn diagram was generated using the R package Venn. A threshold p-value lower than 0.05 indicated a significant difference.

Results

Effects of ox-LDL and Rb3 treatment on HAECs

The ox-LDL is a major risk factor for AS, causing endothelial cell death and promoting the onset and progression of the disease.[16] The viability of HAECs was lowered in a dose- and time-dependent manner after exposure to ox-LDL (Fig. 1A,B), and the subsequent experiment used 50 μg/mL of ox-LDL to stimulate HAECs for 24 h in order to establish the in vitro AS model. Moreover, we found little effect of Rb3 on HAEC viability at different concentrations (0–80 μM) for 24 h (Fig. 1C,D). However, the effects of ox-LDL on HAEC activity and apoptosis were restored by Rb3 (20 μM or 40 μM, 2 h; Fig. 1E,F).

Rb3 attenuated the ox-LDL-induced injury of HAECs

Subsequently, ox-LDL-induced oxidative stress and inflammation in HAECs were found, with the increased production of MDA, the enhanced level of pro-inflammatory

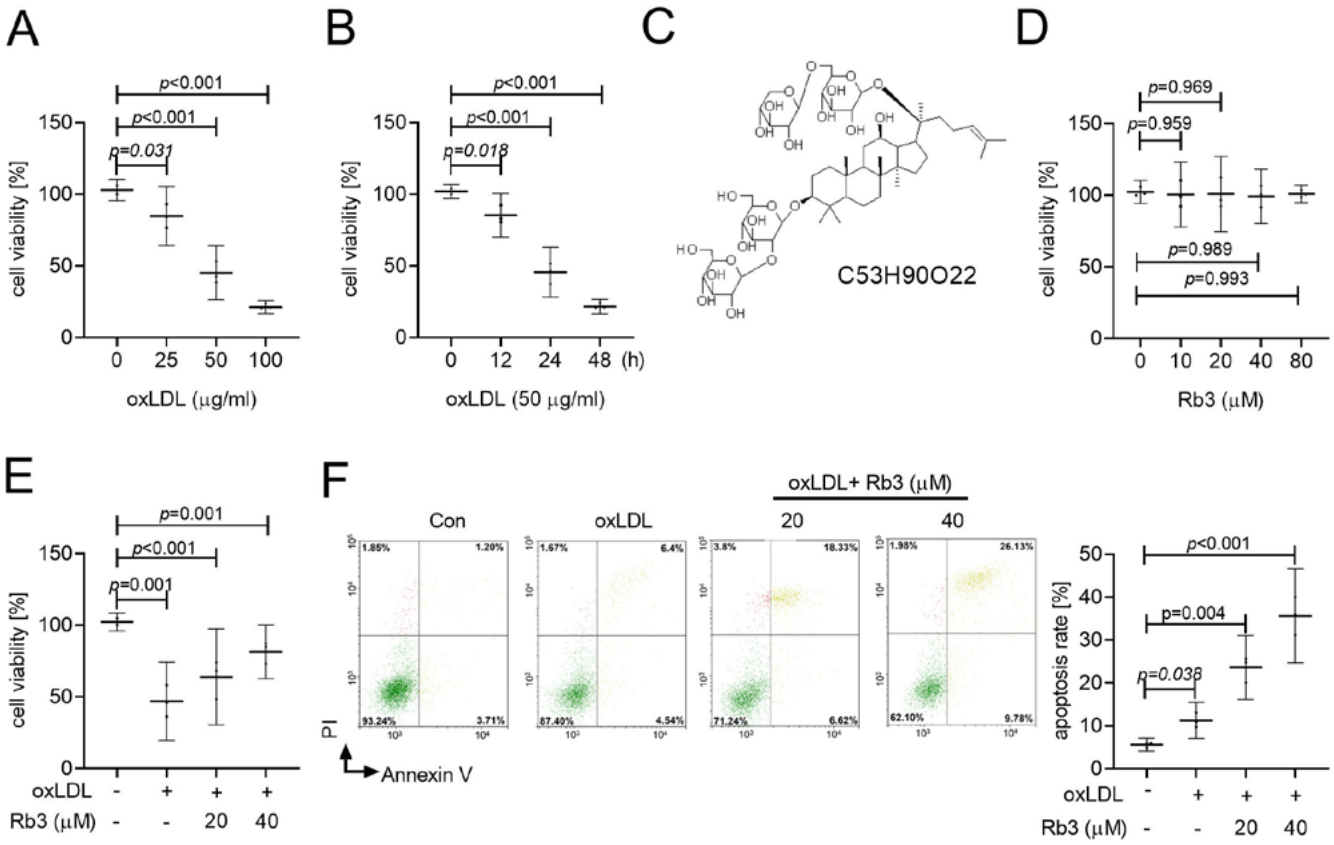

Fig. 1. The cell viability and apoptosis of human aortic endothelial cells (HAECs) after treatment with oxidized low-density lipoprotein (ox-LDL) or Rb3. A. HAECs were treated with 0–100 μg/mL of ox-LDL for 24 h, and their viability was assessed using the Cell Counting Kit-8 (CCK-8) assay. One-way analysis of variance (ANOVA) with Bonferroni post hoc multiple analysis was utilized; B. HAECs were treated with 50 μg/mL of ox-LDL for 0 h, 12 h, 24 h, or 28 h, and their viability was assessed using the CCK-8 assay. One-way ANOVA with Bonferroni post hoc multiple analysis was utilized; C. The chemical structure of Rb3; D. HAEC viability after treatment with 0–80 μM of Rb3 for 24 h; one-way ANOVA with Bonferroni post hoc multiple analysis was utilized; E. HAECs were pretreated with 20 μM or 40 μM of Rb3 for 24 h before being exposed to 50 μg/mL of ox-LDL for 24 h, and one-way ANOVA with Bonferroni post hoc multiple analysis was utilized; F. Apoptosis of HAECs was analyzed using flow cytometry, and one-way ANOVA with Bonferroni post hoc multiple analysis was utilized. Data were expressed as means and 95% confidence intervals (95% CIs)

cytokines and the decreased activity of SOD, while the activity of SOD was attenuated by Rb3 pretreatment (Fig. 2A–D). Additionally, ox-LDL increased the secretion of sICAM-1 and downregulated the expression of eNOS (Fig. 2E,F). The Rb3 pretreatment alleviated to a certain extent the endothelial dysfunction of HAECs caused by ox-LDL (Fig. 2E,F).

Rb3 upregulates miR-513a-5p in ox-LDL-treated HAECs

We used the online analysis program GEO2R to evaluate the differentially expressed miRNAs between ox-LDL-treated HAECs and untreated HAECs from the GEO database. In total, 7 downregulated miRNAs were discovered, including miR-7110-5p, miR-1202, miR-6749-5p, miR-154-3p, miR-513a-5p, miR-6068, and miR-4697-5p (Fig. 3A). Moreover, ox-LDL-treated HAECs showed a significantly reduced expression of miR-154-3p, miR-513a-5p and miR-7110-5p in our experiments (Fig. 3B). However, miR-513a-5p was markedly upregulated in ox-LDL-treated HAECs after Rb3 pretreatment (Fig. 3C). Moreover, miR-513a-5p inhibitor (inhib-miRNA) transfection prevented Rb3-induced elevation of miR-513a-5p in ox-LDL-treated HAECs (Fig. 3D–E).

ZBTB20 as a target gene was regulated by miR-513a-5p in HAECs

Bioinformatics analysis conducted using miRDB (https://mirdb.org/), TargetScan (https://www.targetscan.org/vert_72/) and miRTarBase (https://mirtarbase.cuhk.edu.cn/~miRTarBase/miRTarBase_2022/php/index.php) (Fig. 4A), as well as the dual-luciferase reporter assay (Fig. 4B) confirmed that miR-513a-5p binds to the 3'UTR of ZBTB20 mRNA, and its mimics successfully upregulate the levels of miR-513a-5p in HAECs (Fig. 4C) while decreasing the expression of ZBTB20 mRNA and proteins. The transfection with an inhibitor had the opposite effects (Fig. 4D,E). Moreover, Rb3 treatment reduced ZBTB20 expression in ox-LDL-treated HAECs (Fig. 4F,G). Additionally, the upregulation of ZBTB20 expression in ox-LDL-treated

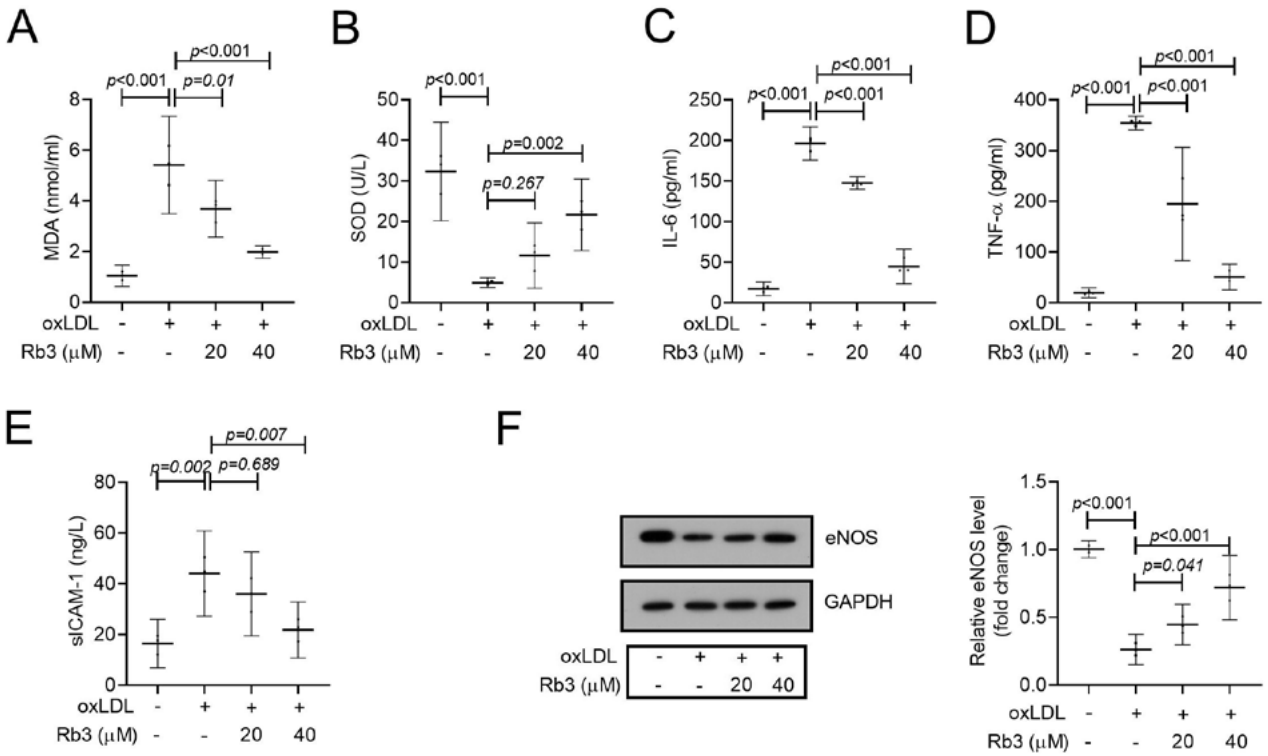

Fig. 2. Rb3 reduces human aortic endothelial cell (HAEC) injury caused by oxidized low-density lipoprotein (ox-LDL). A. The levels of malondialdehyde (MDA) were measured using enzyme-linked immunosorbent assay (ELISA); B. The levels of superoxide dismutase (SOD) were measured using ELISA; C. The levels of interleukin 6 (IL-6) were measured using ELISA; D. The levels of tumor necrosis factor alpha (TNF-α) were measured using ELISA; E. The levels of soluble intercellular adhesion molecule-1 (sICAM-1) were measured using ELISA; One-way analysis of variance (ANOVA) with Bonferroni post hoc multiple analysis was utilized; F. The expression of endothelial nitric oxide synthase (eNOS) in culture medium were determined using western blot and one-way ANOVA with Bonferroni post hoc multiple analysis was utilized. Data were expressed as means and 95% confdence intervals (95% CIs)

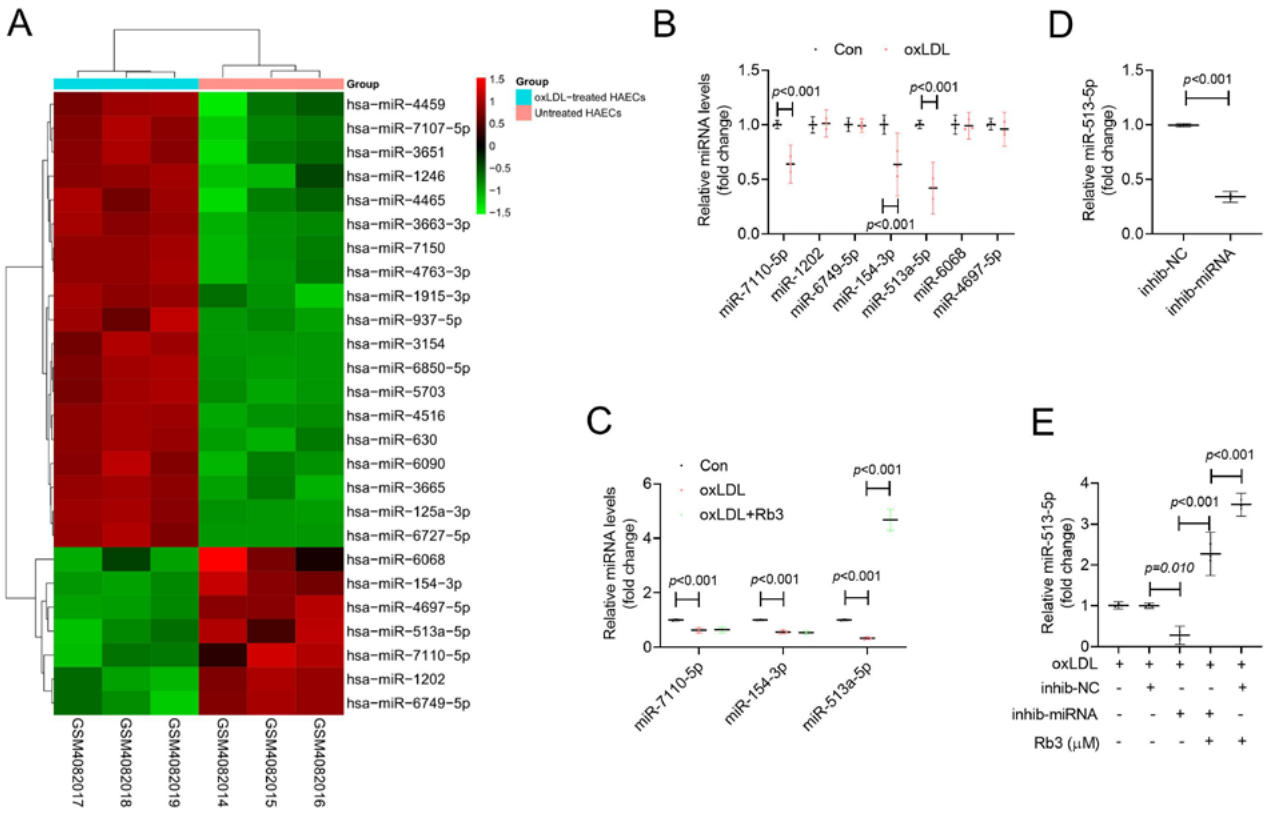

Fig. 3. Rb3 upregulated miR-513a-5p in human aortic endothelial cells (HAECs) stimulated by oxidized low-density lipoprotein (ox-LDL). A. A heatmap of 27 differentially expressed miRNAs in HAECs with or without stimulation by ox-LDL; B. The expression of downregulated miRNAs in HAECs was caused by ox-LDL, and Student's t-test was utilized; C. miRNA expression in HAECs treated with Rb3 and ox-LDL, and one-way analysis of variance (ANOVA) with Bonferroni post hoc multiple analysis was utilized; D. miR-513a-5p expression in HAECs transfected with miR-513a-5p inhibitor (inhib-miRNA) and negative control (inhib-NC), and Student's t-test was utilized; E. HAECs were transfected with an inhibitor of miR-513a-5p for 24 h; then, they were pretreated with Rb3 for 24 h before being exposed to ox-LDL for 24 h. The expression of miR-513a-5p was measured using quantitative real-time polymerase chain reaction (qPCR), and one-way ANOVA with Bonferroni post hoc multiple analysis was utilized. Data were expressed as means and 95% confidence intervals (95% CIs)

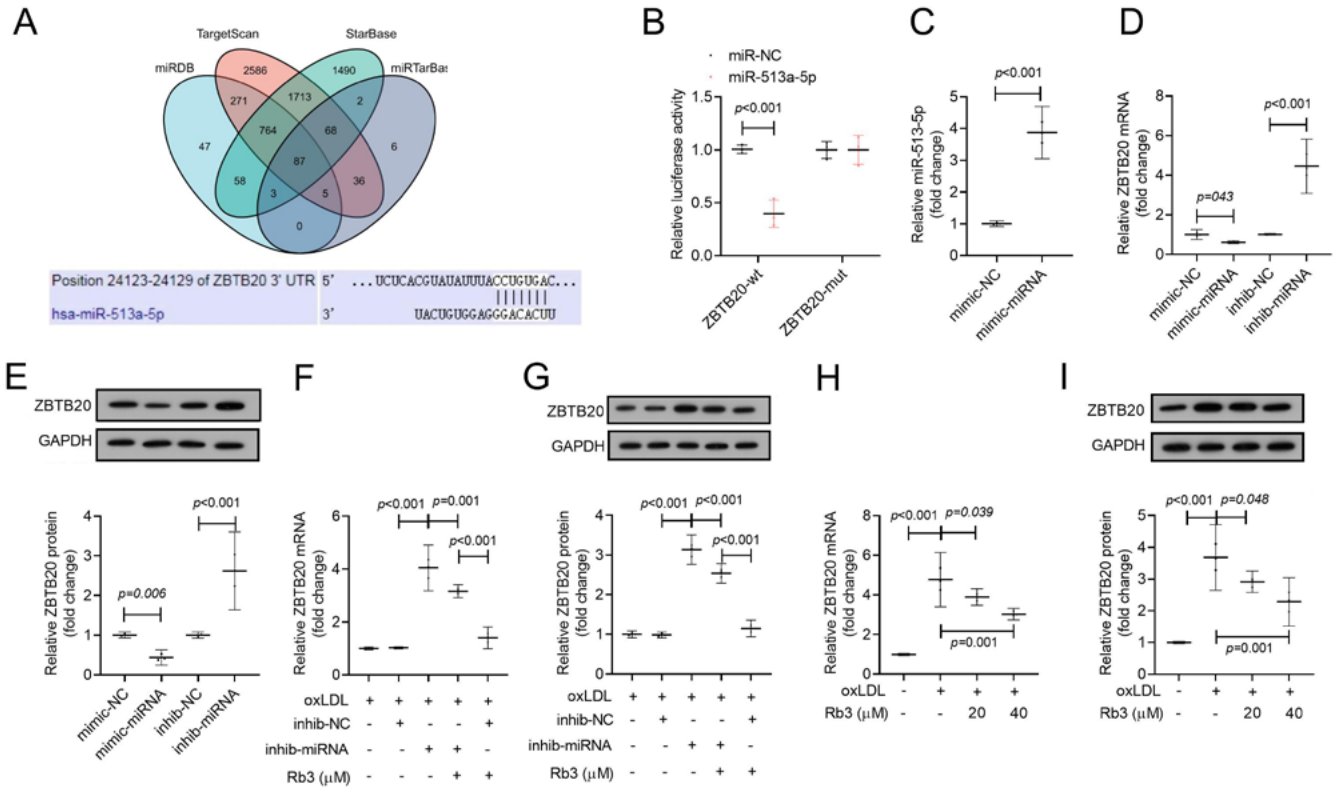

Fig. 4. *ZBTB20* as a target gene was regulated by miR-513a-5p in human aortic endothelial cells (HAECs). A. Binding sites for ZBTB20 and miR-513a-5p predicted using miRDB (https://mirdb.org/), TargetScan (https://www.targetscan.org/vert_72/) and miRTarBase (https://mirtarbase.cuhk.edu.cn/~miRTarBase/miRTarBase_2022/php/index.php); B. The assessment of luciferase activity in HAECs transfected with ZBTB20-wt/ZBTB20-mut and miR-513-5p mimic/negative control (NC); Student's t-test was utilized; C. miR-513a-5p expression in HAECs transfected with miR-513a-5p mimics; Student's t-test was utilized; D,E. ZBTB20 mRNA and protein expression in HAECs treated with miR-513a-5p mimics and inhibitors; Student's t-test was utilized; F,G. HAECs were transfected with miR-513a-5p inhibitor for 24 h, and then pretreated with Rb3 for 24 h, followed by exposure to ox-LDL for 24 h; the expression of ZBTB20 mRNA and protein were analyzed using quantitative real-time polymerase chain reaction (qPCR) and western blot assay. One-way analysis of variance (ANOVA) with Bonferroni post hoc multiple analysis were utilized; H,I. HAECs were pretreated with 20 μM or 40 μM of Rb3 for 24 h, and then exposed to 50 μg/mL of oxidized low-density lipoprotein (ox-LDL) for 24 h; the expression of ZBTB20 mRNA and protein were analyzed using qPCR and western blot assay, and one-way ANOVA with Bonferroni post hoc multiple analysis were utilized. Data were expressed as means and 95% confidence intervals (95% CIs)

HAECs enhanced by the miR-513a-5p inhibitor was abolished after Rb3 treatment (Fig. 4F,G). Furthermore, ox-LDL upregulated the expression of ZBTB20 in HAECs, which was reversed by Rb3 treatment (Fig. 4H,I).

The effect of Rb3 on endothelial dysfunction of HAECs induced by ox-LDL is mediated by miR-513a-5p/ZBTB20 axis

As shown in Fig. 5A, ZBTB20 siRNA transfection markedly downregulated the expression of ZBTB20 in HAECs. The inhibition of miR-513a-5p eliminated the anti-oxidative, anti-inflammatory and anti-endothelial dysfunction effects of Rb3 pretreatment in ox-LDL-treated HAECs, while the effects of miR-513a-5p overexpression were reversed by ZBTB20 siRNA (Fig. 5B–G). Collectively, Rb3 reduced oxidative stress, inflammation and endothelial damage caused by ox-LDL through the miR-513a-5p/ZBTB20 axis.

Discussion

Endothelial dysfunction and apoptosis are thought to be the initial elements in the development of AS, contributing to the pathophysiology of the disease.[3] Inhibiting ox-LDL-induced endothelial dysfunction and endothelial cell death is thought to be a possible therapeutic treatment for AS.[17] The management of the inflammatory response is critical for the treatment of AS, and inhibiting pro-inflammatory cytokines is a promising technique for preventing AS.[18] Except for the inflammatory response, which has an impact on lipid metabolism in AS and vice versa,[19] endothelial dysfunction also appears to be a frequent hallmark of AS, and the endothelial dysfunction could be induced by ox-LDL via the acceleration of oxidative stress.[20] A previous study reported that tofacitinib has shown vascular protective properties in HAECs by inhibiting ox-LDL-induced inflammation and oxidative stress.[21] In addition, saxagliptin-mediated endothelial protection is associated with the inhibition

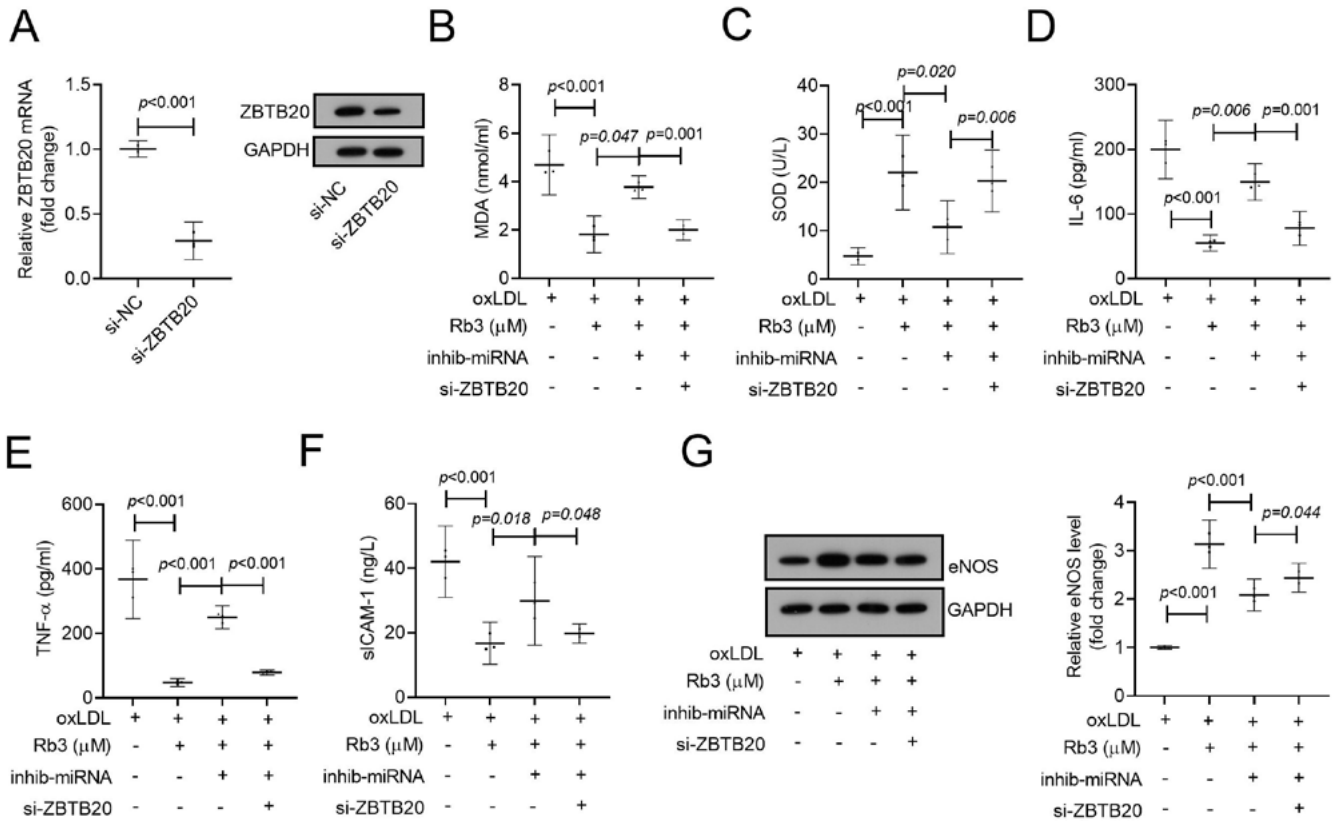

Fig. 5. The effect of Rb3 on oxidized low-density lipoprotein (ox-LDL)-caused human aortic endothelial cell (HAEC) injury is mediated by the miR-513a-5p/ZBTB20 axis. A. ZBTB20 expression in HAECs treated with ZBTB20 siRNA; Student's t-test was utilized; B. The levels of malondialdehyde (MDA) were measured using enzyme-linked immunosorbent assay (ELISA); C. The levels of superoxide dismutase (SOD) were measured using ELISA; D. The levels of interleukin 6 (IL-6) were measured using ELISA; E. The levels of tumor necrosis factor alpha (TNF-α) were measured using ELISA; F. The levels of soluble intercellular adhesion molecule-1 (sICAM-1) were measured using ELISA; one-way analysis of variance (ANOVA) with Bonferroni post hoc multiple analysis was utilized; G. The expression of endothelial nitric oxide synthase (eNOS) in culture medium were determined using western blot w, and one-way ANOVA with Bonferroni post hoc multiple analysis were utilized

Data were expressed as means and 95% confidence intervals (95% CIs).

of the inflammatory response induced by ox-LDL.[22] In the current investigation, we discovered that Rb3 pretreatment reduced MDA formation and SOD caused by ox-LDL, as well as inhibited the release of IL-6 and TNF-α, indicating that Rb3 had anti-inflammatory and anti-oxidative effects in cellular models of AS. Furthermore, we discovered that Rb3 boosted the viability and eNOS expression of ox-LDL-treated HAECs while inhibiting the generation of sICAM-1, indicating that Rb3 prevented endothelial dysfunction in cellular models of AS. Our results revealed an anti-AS effect of Rb3 in vitro.

The significance of miRNA in vascular disorders has recently gained a lot of interest.[23] The miRNA is associated with various pathophysiological processes, including growth, proliferation, apoptosis, and autophagy. Recent research has found that aberrant miRNA expression occurs at various phases of AS.[24] Increased plasma levels of miR-216a were found in elderly CVD patients, and were responsible for triggering endothelial cell inflammation via the Smad3 pathway.[25] In addition, miR-513a-5p was downregulated in human aortic VSMCs stimulated by IκBα,[26] as well as HAECs treated with ox-LDL, while the expression of miR-513a-5p could be increased with Rb3 treatment. Furthermore, it has been revealed that miR-513a-5p plays a role in TNF-α/endotoxin-induced apoptosis by suppressing the production of endothelial cell apoptosis protein X-linked inhibitors,[27] thus being a vital miRNA in regulating cell damage caused by an inflammatory response. In this study, we not only found that Rb3 treatment increased the expression of miR-513a-5p, which was inhibited by ox-LDL stimulation, but also determined that the beneficial effects of Rb3 were eliminated by miR-513a-5p inhibitors. This indicates that miR-513a-5p had a novel role in regulating Rb3-mediated anti-oxidative, anti-inflammatory and anti-endothelial damage in AS.

The ZBTB20, as a member of the large complex tramtrack bric-a-brac (BTB)/poxvirus and zinc finger (POZ) family,[28] has the ability to suppress *IκBα* gene transcription while also promoting nuclear factor-κB (NF-κB) activation.[29] In addition, ZBTB20 levels are elevated in human AS lesions and ox-LDL-stimulated macrophages. The ZBTB20 knockdown has been shown to decrease the oxidative stress level and inflammatory response of ox-LDL-stimulated macrophages.[30] Therefore, we hypothesize that miR-513a-5p could play a role by inhibiting ZBTB20. Our results showed that miR-513a-5p targeted

ZBTB20 in ox-LDL-treated HAECs. Additionally, the enhanced ZBTB20 expression caused by ox-LDL was blocked by Rb3 treatment. Moreover, the inhibition of miR-513a-5p eliminated the anti-oxidative, anti-inflammatory and anti-endothelial dysfunction effects of Rb3 in ox-LDL-treated HAECs, while ZBTB20 knockdown could have enhanced the anti-oxidative, anti-inflammatory and anti-endothelial dysfunction effects. Therefore, Rb3 might exert its anti-AS effect through the miR-513a-5p/ZBTB20 axis.

Limitations

The intracellular signal transduction pathway is very complicated. In the present study, miR-513a-5p was selected as a candidate miRNA based only on the relevant literature. In future studies, we should select more miRNAs or miRNA expression profiles to explore the protective role of Rb3 in AS. Furthermore, the role of Rb3 in animal models and clinical research needs to be further investigated.

Conclusions

In conclusion, we proved that Rb3 reduced oxidative stress, inflammation and endothelial damage caused by ox-LDL through the miR-513a-5p/ZBTB20 axis. These results supported the beneficial role of Rb3 as an anti-AS agent.

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References

1. Evans MA, Sano S, Walsh K. Cardiovascular disease, aging, and clonal hematopoiesis. *Annu Rev Pathol Mech Dis*. 2020;15(1):419–438. doi:10.1146/annurev-pathmechdis-012419-032544
2. Falk E. Pathogenesis of atherosclerosis. *J Am Coll Cardiol*. 2006;47 (8 Suppl):C7–C12. doi:10.1016/j.jacc.2005.09.068
3. Tedgui A, Mallat Z. Cytokines in atherosclerosis: Pathogenic and regulatory pathways. *Physiol Rev*. 2006;86(2):515–581. doi:10.1152/physrev.00024.2005
4. Wu X, Zhang H, Qi W, et al. Nicotine promotes atherosclerosis via ROS-NLRP3-mediated endothelial cell pyroptosis. *Cell Death Dis*. 2018;9(2):171. doi:10.1038/s41419-017-0257-3
5. Zhang Y, Liu X, Bai X, et al. Melatonin prevents endothelial cell pyroptosis via regulation of long noncoding RNA MEG3/miR-223/NLRP3 axis. *J Pineal Res*. 2018;64(2):e12449. doi:10.1111/jpi.12449
6. Libby P, Bornfeldt KE, Tall AR. Atherosclerosis: Successes, surprises, and future challenges. *Circ Res*. 2016;118(4):531–534. doi:10.1161/CIRCRESAHA.116.308334
7. Xue Q, He N, Wang Z, et al. Functional roles and mechanisms of ginsenosides from Panax ginseng in atherosclerosis. *J Ginseng Res*. 2021;45(1):22–31. doi:10.1016/j.jgr.2020.07.002
8. Sun JL, Abd El-Aty AM, Jeong JH, Jung TW. Ginsenoside Rb2 ameliorates LPS-induced inflammation and ER stress in HUVECs and THP-1 cells via the AMPK-mediated pathway. *Am J Chin Med*. 2020;48(4):967–985. doi:10.1142/S0192415X20500469
9. Chen X, Wang Q, Shao M, et al. Ginsenoside Rb3 regulates energy metabolism and apoptosis in cardiomyocytes via activating PPARα pathway. *Biomed Pharmacother*. 2019;120:109487. doi:10.1016/j.biopha.2019.109487
10. Yang L, Liu Q, Yu Y, Xu H, Chen S, Shi S. Ginsenoside-Rb3 inhibits endothelial–mesenchymal transition of cardiac microvascular endothelial cells. *Herz*. 2019;44(1):60–68. doi:10.1007/s00059-017-4628-4
11. Sun M, Ji Y, Li Z, et al. Ginsenoside Rb3 inhibits pro-inflammatory cytokines via MAPK/AKT/NF-κB pathways and attenuates rat alveolar bone resorption in response to *Porphyromonas gingivalis* LPS. *Molecules*. 2020;25(20):4815. doi:10.3390/molecules25204815
12. Wang T, Yu XF, Qu SC, Xu HL, Sui DY. Ginsenoside Rb3 inhibits angiotensin II-induced vascular smooth muscle cells proliferation. *Basic Clin Pharmacol Toxicol*. 2010;107(2):685–689. doi:10.1111/j.1742-7843.2010.00560.x
13. Uchida S, Dimmeler S. Long noncoding RNAs in cardiovascular diseases. *Circ Res*. 2015;116(4):737–750. doi:10.1161/CIRCRESAHA.116.302521
14. Pierce JB, Feinberg MW. Long noncoding RNAs in atherosclerosis and vascular injury: Pathobiology, biomarkers, and targets for therapy. *Arterioscler Thromb Vasc Biol*. 2020;40(9):2002–2017. doi:10.1161/ATVBAHA.120.314222
15. Zhang Z, Salisbury D, Sallam T. Long noncoding RNAs in atherosclerosis. *J Am Coll Cardiol*. 2018;72(19):2380–2390. doi:10.1016/j.jacc.2018.08.2161
16. Zhou T, Ding JW, Wang XA, Zheng XX. Long noncoding RNAs and atherosclerosis. *Atherosclerosis*. 2016;248:51–61. doi:10.1016/j.atherosclerosis.2016.02.025
17. Li B, Dasgupta C, Huang L, Meng X, Zhang L. MiRNA-210 induces microglial activation and regulates microglia-mediated neuroinflammation in neonatal hypoxic-ischemic encephalopathy. *Cell Mol Immunol*. 2020;17(9):976–991. doi:10.1038/s41423-019-0257-6
18. Feinberg MW, Moore KJ. MicroRNA regulation of atherosclerosis. *Circ Res*. 2016;118(4):703–720. doi:10.1161/CIRCRESAHA.115.306300
19. Karunakaran D, Rayner KJ. Macrophage miRNAs in atherosclerosis. *Biochim Biophys Acta*. 2016;1861(12):2087–2093. doi:10.1016/j.bbalip.2016.02.006
20. Churov A, Summerhill V, Grechko A, Orekhova V, Orekhov A. MicroRNAs as potential biomarkers in atherosclerosis. *Int J Mol Sci*. 2019;20(22):5547. doi:10.3390/ijms20225547
21. Yang X, Wan M, Cheng Z, Wang Z, Wu Q. Tofacitinib inhibits ox-LDL-induced adhesion of THP-1 monocytes to endothelial cells. *Artif Cells Nanomed Biotechnol*. 2019;47(1):2775–2782. doi:10.1080/21691401.2019.1573740
22. Ma S, Bai Z, Wu H, Wang W. The DPP-4 inhibitor saxagliptin ameliorates ox-LDL-induced endothelial dysfunction by regulating AP-1 and NF-κB. *Eur J Pharmacol*. 2019;851:186–193. doi:10.1016/j.ejphar.2019.01.008
23. Chen Y, Wang S, Yang S, et al. Inhibitory role of ginsenoside Rb2 in endothelial senescence and inflammation mediated by microRNA-216a. *Mol Med Rep*. 2021;23(6):415. doi:10.3892/mmr.2021.12054
24. Lu H, Zhou X, Kwok HH, et al. Ginsenoside-Rb1-mediated anti-angiogenesis via regulating PEDF and miR-33a through the activation of PPAR-γ pathway. *Front Pharmacol*. 2017;8:783. doi:10.3389/fphar.2017.00783
25. Kattoor AJ, Kanuri SH, Mehta JL. Role of Ox-LDL and LOX-1 in atherogenesis. *Curr Med Chem*. 2019;26(9):1693–1700. doi:10.2174/0929867325666180508100950
26. Zhang Y, Cao X, Zhu W, et al. Resveratrol enhances autophagic flux and promotes Ox-LDL degradation in HUVECs via upregulation of SIRT1. *Oxid Med Cell Longev*. 2016;2016:7589813. doi:10.1155/2016/7589813
27. Patrikoski M, Juntunen M, Boucher S, et al. Development of fully defined xeno-free culture system for the preparation and propagation of cell therapy-compliant human adipose stem cells. *Stem Cell Res Ther*. 2013;4(2):27. doi:10.1186/scrt175
28. Liu H, Deng Y, Wu L, et al. Interleukin-1β regulates lipid homeostasis in human glomerular mesangial cells. *J Nutr Health Aging*. 2020;24(3):246–250. doi:10.1007/s12603-019-1302-y
29. Tumurkhuu G, Dagvadorj J, Porritt RA, et al. *Chlamydia pneumoniae* hijacks a host autoregulatory IL-1β loop to drive foam cell formation and accelerate atherosclerosis. *Cell Metab*. 2018;28(3):432–448.e4. doi:10.1016/j.cmet.2018.05.027
30. Kattoor AJ, Pothineni NVK, Palagiri D, Mehta JL. Oxidative stress in atherosclerosis. *Curr Atheroscler Rep*. 2017;19(11):42. doi:10.1007/s11883-017-0678-6