Disruption of adipocyte HIF-1α improves atherosclerosis through the inhibition of ceramide generation

Pengcheng Wang, Guangyi Zeng, Yu Yan, Song-yang Zhang, Yongqiang Dong, Yangming Zhang, Xingzhong Zhang, Huiying Liu, Zhipeng Zhang, Changtao Jiang, Yanli Pang

Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Peking University, Key Laboratory of Molecular Cardiovascular Science, Ministry of Education, Beijing 100191, China
Center of Basic Medical Research, Institute of Medical Innovation and Research, Peking University Third Hospital, Beijing 100191 China
Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing 100191 China
Center for Obesity and Metabolic Disease Research, School of Basic Medical Sciences, Peking University, Beijing 100191, China
General Surgery Department, Peking University Third Hospital, Beijing 100191, China

Received 12 July 2021; received in revised form 5 September 2021; accepted 14 September 2021

KEY WORDS
HIF-1α;

Abstract
Atherosclerosis is a chronic multifactorial cardiovascular disease. Western diets have been reported to affect atherosclerosis through regulating adipose function. In high cholesterol diet-fed

Abbreviations: HIF-1α/2α/3α, hypoxia-inducible factor 1/2/3 alpha; APOE, apolipoprotein E; PLS-DA, partial least squares discriminant analysis; VIP, variable importance for the projection; eWAT, epididymal white adipose tissue; SM, sphingomyelin; PC, phosphatidylcholine; SMase, sphingomyelinase; VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; MCP-1, monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor alpha; IL-6/1β, interleukin-6/1β; MAC-2, lectin, galactose binding, soluble 3; CXCL1, chemokine (C–X–C motif) ligand 1 protein; CCL5, chemokine (C–C motif) ligand 5; VEGF, vascular endothelial growth factor; SPTLC1/2/3, serine palmitoyltransferase long chain base subunit 1/2/3; DEGS1, delta(4)-desaturase, sphingolipid 1; SGMS1, sphingomyelin synthase 1; CERS2/4/5/6, ceramide synthase 2/4/5/6; SMPD1/2/3/4, sphingomyelin phosphodiesterase 1/2/3/4; ACER2/3, alkaline ceramidase 2/3; EIF5, eukaryotic translation initiation factor 5; ARNT, aryl hydrocarbon nuclear translocator; HREs, HIF-response elements; ChIP, chromatin immunoprecipitation; CoCl2, cobalt(II) chloride; GFP, green fluorescent protein.

*Corresponding authors.
E-mail addresses: jiangchangtao@bjmu.edu.cn (Changtao Jiang), yanlipang@bjmu.edu.cn (Yanli Pang).

These authors made equal contributions in this work.

Peer review under responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.
1. Introduction

Atherosclerosis is a universal problem in modern society that remains the leading cause of mortality worldwide. The pathogenesis of atherosclerosis is caused by a combination of risk factors, of which dyslipidemia is a major risk factor for atherosclerotic cardiovascular disease. Excessively expanded adipocytes and accumulated lipids cause obesity, which in turn leads to adipose tissue dysfunction. Importantly, as an energy storage organ, adipose tissue is also an endocrine organ, secreting adipocytokines that affect the function of cells and tissues throughout the body. Impaired lipid metabolism, such as elevated low-density lipoprotein (LDL) cholesterol, is the basis of atherosclerotic lesions. Changes in lipid components of adipose tissues have profound effects on inflammatory responses, which promotes atherosclerosis. However, repairing dysfunctional adipose tissue can improve systemic metabolic balance, thus decreasing atherosclerosis in mice. Therefore, targeting metabolic pathways in adipose tissue has become a potential treatment for atherosclerosis.

In the development of obesity, the underdeveloped vasculature cannot keep up with the expansion of adipose tissue, which leads to local hypoxia and activates hypoxia-inducible factors (HIFs). HIF is a heterodimer that consisting of one alpha subunit bound to the aryl hydrocarbon nuclear translocator (ARNT, also known as HIF-1β). HIF-α subunits contain three subtypes: HIF-1α, HIF-2α, and HIF-3α. During hypoxia, HIF-α avoids hydroxylation, ubiquitination and proteasome-mediated degradation, and dimerizes with HIF-1β to activate target genes such as vascular endothelial growth factor (VEGF), glucose transporters and glycolytic enzymes, through combining with the hypoxia response elements (HRE). Obesity-associated adipose hypoxia triggers increased expression of HIF-1α, which promotes the disease process. Overexpression of HIF-1α initiates adipose tissue fibrosis and induces adipocyte chemokine production with increased tissue inflammation, which results in glucose intolerance and insulin resistance. Genetic or pharmacologic inhibition of HIF-1α can prevent or reverse the associated pathophysiological processes. However, the role of adipose HIF-1α and the exact mechanisms in atherosclerosis are still unclear.

Ceramides, the main phospholipids for biofilm components, are important second messengers involved in cell growth inhibition and apoptosis, and interact with several pathways to promote insulin resistance, oxidative stress and inflammation, which are all linked to atherosclerosis. In preclinical studies of obesity, ceramides are considered as toxic lipids, and abnormal accumulation of ceramides is a putative intermediate link between excess adiposity and metabolic diseases. Notably, inhibiting the biosynthetic process or increasing the degradation of ceramides ameliorates metabolic disorders, including insulin resistance, atherosclerosis and steatohepatitis, in rodents.

Visceral adipose tissue is one of the major sources for ceramide synthesis. Ceramides are generally synthesized by three pathways in vivo, of which the sphingomyelinase (SMase) pathway has been reported to be involved in the pathogenesis of atherosclerosis. Type 2-neutral SMase (nSMase2, also called SMPD3), located in membrane structures by lipid bilayer insertion of palmitoylated residues and interaction with anionic phospholipid, catalyzes the hydrolysis of sphingomyelin to form ceramide and phosphocholine. Activation of SMPD3 by oxidized LDLs (oxLDLs), TNF-α, IL-1β, IFN-γ, and oxidative stress may contribute to endothelial activation and inflammation. Consequently, SMPD3 deficiency or the use of the specific inhibitor GW4869 inhibited atherosclerosis by reducing inflammatory responses partly through the NRF2 pathway in macrophages and endothelial cells, in which proved the role of ceramide metabolism controlled by SMPD3 gene in the whole body in atherosclerosis. However, deleting the SMPD3 in fibroblasts induced hyaluronan and cholesterol accumulation, which increased the pro-atherogenic risk. Therefore, the role of adipocyte-specific SMPD3 in atherosclerosis is still unclear.

In this study, we found that activation of adipocyte HIF-1α upregulated SMPD3-mediated ceramide production, and then aggravated atherosclerosis by suppressing cholesterol elimination and enhancing local and circulating inflammation levels. Moreover, Smpd3 was identified as a novel HIF-1α target gene. Pharmacological studies showed that inhibition of adipocyte HIF-1α by PX-478, a HIF-1α-specific inhibitor that functions at multiple levels through inhibition of both HIF-1α transcription and translation, had therapeutic effects on atherosclerosis in mice. Taken together, our study reveals a pivotal role of SMPD3 in the adipocyte--HIF-1α-induced atherosclerotic axis in a ceramide-dependent manner.

2. Materials and methods

2.1. Mice and experimental design

Adipocyte-specific HIF-1α knockout (Hif1a<sup>Adipo</sup>) mice and adipocyte-specific HIF-1α transgenic (AdHif1α<sup>Adipo</sup>) mice were generated using the Cre-loxP system, as previously described. ApoE<sup>−/−</sup> mice were obtained from the Animal Center of Peking University (Beijing, China). All the mice had a...
C57BL/6J background. We mated Hif1a<sup>fl/fl</sup> and Hif1a<sup>ΔAdipo</sup> mice with ApoE<sup>−/−</sup> mice to produce Hif1a<sup>fl/fl</sup> ApoE<sup>−/−</sup> and Hif1a<sup>ΔAdipo</sup> ApoE<sup>−/−</sup> mice, respectively. Eight-week-old male mice were used and acclimatized to new environment for 1 week before the experiments. Atherosclerosis lesion was induced by treating a Western diet (WD; 42% fat, 0.2% total cholesterol, TD.88137, Harlan Teklad) for 8 weeks. Mice were housed in an SPF environment with a 12-h light and 12-h dark cycle. All animal procedures were performed under protocols approved by the Animal Care and Use Committee of Peking University, and approval was obtained from the Animal Research Ethics Committees of Peking University. In lentivirus-SMPD3 injection experiment, LV-SMPD3 (Smpd3 cDNA was cloned into the lentivirus vector pLent-EF1a-FH-CMV-GFP-P2A-Puro) and LV-GFP (lentivirus vector pLent-EF1a-FH-CMV-GFP-P2A-Puro) were obtained from Hanheng (Shanghai, China). Eight-week-old Hif1a<sup>ΔAdipo</sup> ApoE<sup>−/−</sup> mice were administered recombinant lipovirus or the corresponding vector (1 × 10<sup>7</sup> infection units per mouse) in the epididymal fat pad. The detailed operation method was previously described. In the ceramide-treatment experiment, C16:0 ceramide (d18:1/16:0 ceramide; Avanti Polar Lipids, USA; #860516) was suspended in saline with 0.5% sodium carboxymethyl cellulose (NaCMC) and 5% Tween 80. Eight-week-old Hif1a<sup>ΔAdipo</sup> ApoE<sup>−/−</sup> mice were intraperitoneally injected with vehicle or C16:0 ceramide (10 mg/kg) every other day with a WD for 6 weeks. In the PX4-78 treatment experiment, PX-478 (5 mg/kg; MedChemExpress, China; #HY-10231) was dissolved in saline, and then, 8-week-old ApoE<sup>−/−</sup> mice were injected intraperitoneally with vehicle or PX-478 every other day for 8 weeks, respectively.

2.2. Cell culture

The 3T3-L1 cell line and HEK293T cell line were purchased from the Cell Resource Center of China (Beijing, China). The 3T3-L1 and HEK293T cells were cultured in Dulbecco’s modified Eagle’s medium—high glucose plus 10% fetal bovine serum in a 5% CO<sub>2</sub> atmosphere at 37 °C. For cells differentiation, 3T3-L1 cells were treated after reaching a confluence of 70%, and then covered cells were treated with insulin (5 μg/mL; Sigma—Aldrich; #R1077C), IMX (500 μmol/L; Abcam; #ab120840) and dexamethasone (0.1 μmol/L; Abcam; #ab120743) for 2 days. The medium was changed every other day.

2.3. Quantitative analysis of atherosclerotic lesion areas

To analyze atherosclerotic lesions, the entire en face aorta was visualized after staining with oil red O (Sigma—Aldrich). Pictures of aortas were captured by a digital camera that outfits a Canon EOS 650D lens (Canon) and then analyzed using ImageJ software. For histological analysis of atherosclerotic lesions in the aortic root, hearts were embedded in OCT compound. After solidification, the hearts were cut into serial 7-μm-thick sections using a Microm cryostat (for frozen blocks), stained with oil red O and counterstained with hematoxylin. Pictures were captured by an inverted microscope (Leica Microsystems Ltd., Milton Keynes, UK). The ratios of atherosclerotic lesions in the aortic roots were determined using ImageJ software.

2.4. Immunofluorescence analysis

Seven-μm-thick frozen cryosections of mouse aortic roots were used for detection of MAC-2 staining by immunofluorescence.

2.5. Immunological and biochemical assays

Plasma inflammatory cytokines were quantified using a cytommetric bead array inflammation kit (BD Biosciences, CA, USA) and analyzed by a BD FACSCalibur (BD Biosciences, CA, USA). Total cholesterol and triglyceride levels were examined using commercial kits (BioSino Bio-Technology and Science, Beijing, China) according to the manuscript. For FPLC analysis, pooled plasma samples were injected into the machine with a Superose S-6 10/300 GL column (GE Healthcare, Sweden), and all the sequential fractions were collected, then each fraction of cholesterol concentrations was measured as described above.

2.6. RNA isolation and quantitative real-time PCR

Tissues and cells were suspended in TRIzol Reagent (Invitrogen, CA, USA), and total RNA was extracted according to the manufacturer’s instructions. Reverse transcription was performed using a 5 × All-In-One RT MasterMix kit (Amb, China). Real-time PCR was performed using a TransStart SuperMix (with Dye) kit (TransGen Biotech, China). All the primer sequences are shown in Supporting Information Table S1. All PCR samples were quantitated by the comparative CT method to obtain relative quantitation that was normalized to 18S.

2.7. Protein extraction and Western blotting

The proteins of tissues and cells were obtained using RIPA buffer (Beyotime Biotechnology, China), which added with 1% protease and phosphatase inhibitors as well as PMSF according to the standard manufacturer’s instructions. Protein samples were quantified with BCA reagent (Pierce, USA), then separating total proteins by SDS-PAGE and electrophoretically transferred onto nitrocellulose membrane (NC). Chemiluminescence was used and then performed by using the LI-COR Odyssey Fc imager (LI-COR Biosciences, Lincoln, NE, USA). Analyses of proteins expression were performed using EIF5 as the internal control. Mouse monoclonal antibody against SMPD3 (Santa Cruz Biotechnology, Texas, USA; #sc-156637), mouse monoclonal antibody against HIF-1α (Novus, CO, USA; #NB100-105), rabbit polyclonal antibody against EIF5 (Santa Cruz Biotechnology, Texas, USA; #sc-282).

2.8. Luciferase reporter gene assays

The pGL3-basic luciferase reporter vector (Promega, WI, USA) was used, and the mouse Smpd3 promoter was amplified and then cloned into the Kpnl and XhoI sites of the vector. HEK293T cells were transfected with Smpd3 reporter vectors, phRL-TK Renilla luciferase control vector (Promega, WI, USA) and HIF-1αTM (constitutively active HIF-1α triple mutants) expression plasmid...
or the corresponding empty backbone vector (pcDNA3). Transfections were carried out using Lipofectamine 3000 (Invitrogen, CA, USA) according to the manufacturer’s instructions. A dual-luciferase assay system (Promega, WI, USA) was used for luciferase assays after 24 h. The Firefly/Renilla luciferase ratio was quantified as the fold change relative to the control group.

2.9. Chromatin immunoprecipitation

As previously reported, 200 mmol/L cobalt (II) chloride hexahydrate was used to incubate differentiated 3T3-L1 adipocytes for 12 h. Remove the growth medium from 10 cm dishes and add 5 mL of cross-linking solution (4% formaldehyde) to each of the dishes. Place the dishes on a rotary shaker for 10 min at room temperature, and then stop the cross-linking reaction by adding glycine to a final concentration of 125 mmol/L for 5 min. Gently mix by rotating the reaction mixture for 10 min at room temperature. After washing with buffers, scrape the cells and collect them with SDS ChIP lysis buffer. Using an M220 (Covaris, MA, USA) connected to a cooling system (4°C), sonicate the samples under high-power mode for ten cycles (30 s on/60 s off). Keep the samples on ice. Segregate the fragmented chromatin by centrifugation at 10,000 x g for 5 min at 4°C, and then dilute the supernatants in ChIP dilution buffer [1% Triton X-100, 2 mmol/L EDTA, 20 mmol/L Tris·HCl (pH 8.1), 150 mmol/L NaCl plus 10 mmol/L NAM and protease inhibitors]. Chromatin sections were rotated at 4°C with magnetic beads (Dynabeads Protein A or G, Invitrogen) prebound with 2 μg of antibody. Then, the ChIP reactions were washed in the following buffers: TSE I, TSE II, buffer III and Tris·EDTA buffer. Incubate all washing reactions in the rotating thermomixer for 10 min at 4°C. The sample was incubated under rotation overnight at 37°C and then for another 2 h at 70°C. DNA was purified by a PCR purification kit (Qiagen, Hilden, Germany) and quantified by quantitative PCR (qPCR). The primer sequences are shown in Table S1.

2.10. Metabolomics analysis

Lipidomic experiments were performed according to a procedure described previously. Briefly, for adipose tissue lipidomic analysis, approximately 20 mg of adipose tissue was homogenized in 200 μL of H2O and then extracted with 1000 μL of precooled chloroform:methanol (2:1) solution containing LM6002 (Avanti Polar Lipids, Alabaster, AL, USA) as an internal standard. After centrifuged at 18,000 x g for 5 min, the lower organic phase was collected to evaporate. For plasma lipidomic analysis, 200 μL of precooled chloroform:methanol (2:1) solution with LM6002 (Avanti Polar Lipids) was mixed with 50 μL of serum. Samples were centrifuged at 18,000 x g for 5 min, and the lower organic phase was collected to evaporate. Then, the organic residue was re-dissolved in isopropanol:acetoniitrile (1:1) solution for qualification. Eksigent LC100 coupled with an AB SCIESX Triple TOF 5600 system were used to analyze the samples. Peak extraction and integration were analyzed by Xcalibur 2.2 SP1.48 software (Thermo Fisher Scientific, Waltham, MA, USA).

2.11. Statistical analysis

Statistical analyses were performed with GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA). Experimental data are expressed as the mean ± standard error of mean (SEM). The Shapiro–Wilks normality test was used to determine the normal distribution of samples. Comparisons between two groups were performed by two-tailed unpaired Student’s t-test (normal distribution) or Mann–Whitney U test (non-normal distribution). Comparisons across multiple groups were assessed with one-way ANOVA (normal distribution) or Kruskal–Wallis test (non-normal distribution), followed by Tukey’s or Dunnett’s T3 post hoc correction, as indicated in each figure legend.

3. Results

3.1. Adipocyte-specific HIF-1α disruption alleviates atherosclerosis

To determine the role of adipocyte HIF-1α in WD-induced atherosclerosis, we generated adipocyte-specific HIF-1α and APOE double knockout (Hif1a+/−Adipo/ApoE−/−) mice and housed them with a WD for 8 weeks. In this model, Hif1a+/−Adipo/ApoE−/− mice show similar body weight compared to Hif1a+/−Adipo/ApoE−/− mice (Supporting Information Fig. S1A). Adipose HIF-1α deficiency significantly reduced atherosclerotic lesions area, as indicated by oil red O staining of heart outflow tract (Fig. 1A and S1B). In keeping with these data, Hif1a+/−Adipo/ApoE−/− mice showed decreased levels of plasma total cholesterol and triglyceride compared to those in control mice (Fig. 1B and C). Since high level of LDL-C is a clear risk factor, which positively correlated with the occurrence of atherosclerosis, we carried out fast protein liquid chromatography (FPLC) to examine cholesterol levels in different plasma lipoprotein fractions. As expected, the plasma levels of very low-density lipoprotein (VLDL) cholesterol and LDL cholesterol were both lower in the Hif1a+/−Adipo/ApoE−/− mice than those in the Hif1a+/−Adipo/ApoE−/− mice (Fig. 1D).

As abundant evidence proved, the interaction of LDL with macrophages in atherosclerotic plaques leads to increased inflammation. We found the mRNA levels of the proinflammatory genes Tnf, Mcp1, Il1b, and Ccl5 were lower in the aortas of the Hif1a+/−Adipo/ApoE−/− mice than the Hif1a+/−Adipo/ApoE−/− mice (Fig. 1E). Consistently, the circulating inflammatory cytokines MCP-1, TNF-α, and IL-6 were much lower in the Hif1a+/−Adipo/ApoE−/− mice than in control mice (Fig. 1F–H). Infiltration of macrophages in the atherosclerotic plaques reflects severe inflammation and atherosclerotic plaque damage. MAC-2 expression of the aortic root sections detected by immunofluorescence demonstrated reduced macrophage contents in the Hif1a+/−Adipo/ApoE−/− mice compared to those of control mice (Fig. 1I).

3.2. Loss of adipocyte HIF-1α reduces proinflammatory ceramide levels

The harmful effects of adipocyte HIF-1α on atherosclerosis led us to investigate which key factor may alter the development of atherosclerosis. We then determined the pro-inflammatory lipid profiles. Partial least squares discriminant analysis (PLS-DA) modeling of lipid metabolites in epididymal white adipose tissue (eWAT) was different between the Hif1a+/−Adipo/ApoE−/− and Hif1a+/−Adipo/ApoE−/− mice after fed a WD for 8 weeks (Fig. 2A). The variable importance for the projection (VIP) score analysis showed that the primary metabolites leading to clustering were ceramides and sphingolipids (Fig. 2B). We further quantified the concentrations of sphingolipids in the adipose tissue and plasma of
the \( \text{Hif}1\alpha^{\Delta\text{Adipo}} \) \( \text{Apoe}^{-/-} \) and \( \text{Hif}1\alpha^{\text{fl/fl}} \) \( \text{Apoe}^{-/-} \) mice by targeted lipidomics. The total and different species of ceramides and dihydroceramides were lower in the eWAT (Fig. 2C and D) and plasma (Fig. 2E and F) of the \( \text{Hif}1\alpha^{\Delta\text{Adipo}} \) \( \text{Apoe}^{-/-} \) mice than those of the \( \text{Hif}1\alpha^{\text{fl/fl}} \) \( \text{Apoe}^{-/-} \) mice. While levels of several sphingolipid metabolites such as SM16:0 and SM24:0 were higher in the eWAT (Supporting Information Fig. S2A) of the \( \text{Hif}1\alpha^{\Delta\text{Adipo}} \) \( \text{Apoe}^{-/-} \) mice than the \( \text{Hif}1\alpha^{\text{fl/fl}} \) \( \text{Apoe}^{-/-} \) mice, there was no difference in plasma (Fig. S2B). These findings indicate that defective lipid metabolism in atherosclerosis and adipocyte HIF-1\( \alpha \) deficiency contributes to the protective response through decreasing proinflammatory ceramides level.

3.3. Adipocyte HIF-1\( \alpha \) modulates ceramide generation by regulating the expression of SMPD3

To further elucidate the function of adipocyte HIF-1\( \alpha \) in ceramide disorder, we measured the relative expression of genes involved in ceramide metabolism. In adipocyte specific HIF-1\( \alpha \) overexpression (\( \text{AdHif}1\alpha^{\text{LSL/LSL}} \)) mice, we observed significantly elevated mRNA levels of \( \text{Smpd}3 \), which regulates ceramide synthesis, in the eWAT (Supporting Information Fig. S2A) of the \( \text{AdHif}1\alpha^{\text{LSL/LSL}} \) mice compared to vehicle (\( \text{Hif}1\alpha^{+/+} \)) mice, while unaltered expression of ceramide catabolism genes, such as \( \text{Acers} \) (Fig. 3A). In addition, we also found lower \( \text{Cers}6 \) and \( \text{Smpd}3 \) expression in the \( \text{Hif}1\alpha^{\Delta\text{Adipo}} \) \( \text{Apoe}^{-/-} \) mice than in the littermate.
control mice (Fig. 3C). Similarly, the protein level of SMPD3 in eWAT was consistently changed between the compared groups (Fig. 3B and D). Based on these results, Smpd3 may be a potential target gene of adipocyte HIF-1α. To confirm this hypothesis, we analyzed the Smpd3 promoter and identified putative HIF-response elements (HREs) in the promoter region of Smpd3 (Fig. 3E). Thus, we performed chromatin immunoprecipitation (ChIP) assays to validate whether HIF-1α bound to this site, and under CoCl2 stimulated condition, mimicking a hypoxia environment, enhanced the binding of HIF-1α to the HRE in 3T3-L1 cells (Fig. 3F). Similarly, luciferase reporter assays further revealed increased transcriptional activity of the Smpd3 promoter after overexpression of a constitutively active HIF-1α triple mutant (HIF-1αTM) expression plasmid in HEK293T cells, while the increased reporter
gene activity was eliminated when the HRE was mutated (Fig. 3G). These data indicate that HIF-1α directly regulated Smpd3 transcription expression via binding to the HRE of the Smpd3 promoter.

3.4. Overexpression of SMPD3 in adipose tissue reverses the alleviation of atherosclerosis induced by adipocyte HIF-1α deficiency

Next, we explored the role of SMPD3 on HIF-1α-induced ceramide production and secretion in vivo. Specific lentivirus carrying the mouse Smpd3 cDNA sequence was designed and injected directly into the eWAT of the Hif1afl/flAdipoApoEe/e and Hif1aΔAdipoApoEe/e mice fed a WD for 8 weeks (n = 6 per group). Two-tailed Student’s t-test: **P < 0.01 compared to the Hif1afl/flApoEe/e mice. (D) The protein levels of HIF-1α and SMPD3 in the eWAT of the Hif1afl/flAdipoApoEe/e mice fed a WD for 8 weeks (n = 3 per group). (E) Schematic diagram of the mouse Smpd3 promoter illustrating the predicted HRE in the regulatory region. The upstream regions are numbered in relation to the transcription initiation site, which is designated +1. (F) ChIP assays of 3T3-L1 adipocytes treated with CoCl2 (200 μmol/L) for 12 h (n = 4 per group). One-way ANOVA with Tukey’s post hoc test: **P < 0.01 compared to the IgG-Ab group. (G) Luciferase reporter gene assay of Smpd3 promoter activity (n = 4 per group). One-way ANOVA with Dunnett’s T3 post hoc test: **P < 0.01 compared to the Smpd3 promoter plasmid with vector group; ##P < 0.01 compared to the Smpd3 promoter plasmid with HIF-1α TM group.

Figure 3 The ceramide-generation-related gene Smpd3 is a novel target gene of adipocyte HIF-1α. (A) The mRNA levels of genes related to ceramide metabolism, including synthesis, transportation and elimination in the eWAT of the AdHif1aLSL/LSL and Hif1a+/+ mice (n = 6 per group). Two-tailed Student’s t-test: **P < 0.01 compared to the Hif1a+/+ mice. (B) The protein levels of HIF-1α and SMPD3 in the eWAT of the AdHif1aLSL/LSL and Hif1a+/+ mice (n = 3 per group). (C) The mRNA levels of genes related to ceramide metabolism, including synthesis, transportation and elimination in the eWAT of the Hif1a+/+ApoE−/− and Hif1aΔAdipoApoE−/− mice fed a WD for 8 weeks (n = 6 per group). Two-tailed Student’s t-test: **P < 0.01 compared to the Hif1a+/+ApoE−/− mice. (D) The protein levels of HIF-1α and SMPD3 in the eWAT of the Hif1a+/+ApoE−/− and Hif1aΔAdipoApoE−/− mice fed a WD for 8 weeks (n = 3 per group). (E) Schematic diagram of the mouse Smpd3 promoter illustrating the predicted HRE in the regulatory region. The upstream regions are numbered in relation to the transcription initiation site, which is designated +1. (F) ChIP assays of 3T3-L1 adipocytes treated with CoCl2 (200 μmol/L) for 12 h (n = 4 per group). One-way ANOVA with Tukey’s post hoc test: **P < 0.01 compared to the IgG-Ab group. (G) Luciferase reporter gene assay of Smpd3 promoter activity (n = 4 per group). One-way ANOVA with Dunnett’s T3 post hoc test: **P < 0.01 compared to the Smpd3 promoter plasmid with vector group; ##P < 0.01 compared to the Smpd3 promoter plasmid with HIF-1α TM group.
whole aortic and cross-sections of aortic roots (Fig. 4D and Fig. S3C), and the lower total cholesterol and triglyceride levels, especially VLDL and LDL cholesterol, in the plasma of the Hif1aΔAdipo ApoE−/− mice than the Hif1aΔfl/fl ApoE−/− mice were also aggravated due to rescued SMPD3 overexpression (Fig. 4E–G). Together, these results demonstrate that adipocyte HIF-1α promotes atherosclerosis progression via Smpd3 expression regulation.

### 3.5 Overexpression of SMPD3 in adipose tissue exacerbates the inflammation in atherosclerosis improved by adipocyte HIF-1α deficiency

The latest studies and clinical evidence indicated that atherosclerosis is a chronic inflammatory disease. We then determined the effect of overexpression of Smpd3 in adipose tissue on inflammation. As assessed by biochemical analysis, detection of MCP-1, TNF-α, and IL-6 levels in plasma showed that the Hif1aΔAdipo ApoE−/− + SMPD3 mice reversed the anti-inflammatory effect of the Hif1aΔAdipo ApoE−/− + GFP mice (Fig. 5A–C). Consistently, upregulation of the macrophage marker MAC-2 was observed in the atherosclerotic lesions of Hif1aΔAdipo ApoE−/− + SMPD3 mice compared to the Hif1aΔAdipo ApoE−/− + GFP mice (Fig. 5D), suggesting adipocyte SMPD3 overexpression exacerbated inflammation level in atherosclerosis through ceramide generation.

### 3.6. Ceramide aggravates WD-induced dyslipidemia

In order to determine the mechanisms underlying of ceramide in diet-induced atherosclerosis, we quantified the effect of additional administration of C16:0 ceramide on the progression of atherosclerosis (Fig. 6A). We found several species of ceramide in the plasma and eWAT of the Hif1aΔAdipo ApoE−/− mice with C16:0 ceramide increased after injection (Fig. 6B and C), which reversed the decreased ceramide profiles in the Hif1aΔAdipo ApoE−/− mice. The levels of plasma total cholesterol and triglyceride, especially VLDL and LDL cholesterol, were higher in the Hif1aΔAdipo ApoE−/− mice treated with C16:0 ceramide than in the Hif1aΔfl/fl ApoE−/− mice (Fig. 6D–F). As described previously, ceramide was found to reduce hepatic cholesterol elimination and aggravate dyslipidemia. Then we further observed higher hepatic cholesterol and triglyceride levels in the Hif1aΔAdipo ApoE−/− mice treated with C16:0 ceramide than in the Hif1aΔAdipo ApoE−/− mice (Supporting Information Fig. S4A and S4B). Moreover, the mRNA expression of the Cyp7a1 and Abcg5 genes involved in cholesterol elimination was lower in the Hif1aΔAdipo ApoE−/− mice treated with C16:0 ceramide than in

---

**Figure 4** Overexpression of SMPD3 in adipose tissue aggravates the progression of atherosclerosis. Eight-week-old Hif1aΔfl/fl ApoE−/− and Hif1aΔAdipo ApoE−/− mice were injected with lentivirus carrying mouse Smpd3 cDNA sequence or green fluorescent protein (GFP) and were fed a WD for 8 weeks. (A) Schematic diagram of the mouse model illustrating the concept of mouse injection with lentivirus-SMPD3/GFP. (B, C) Quantitation of ceramide concentrations in plasma (B) and eWAT (C), n = 6 per group. (D) Representative oil red O staining of cross-sections of aortic roots (n = 6–7 per group). Left, representative examples of cross-sections from the aortic root stained with oil red O. Scale bar, 500 μm. Right, quantification of aortic root lesion areas. (E, F) The levels of plasma total cholesterol (E) and triglyceride (F) in mice (n = 6–7 per group). (G) FPLC analysis of plasma cholesterol levels in mice. All data are presented as the mean ± SEM. One-way ANOVA with Tukey’s post hoc test (B–F). *P < 0.05, **P < 0.01 compared to the Hif1aΔfl/fl ApoE−/− + GFP group; *P < 0.05, **P < 0.01 compared to the Hif1aΔAdipo ApoE−/− + GFP group. See also Fig. S3.

---

**Figure 5** Overexpression of SMPD3 in adipose tissue aggravates the progression of atherosclerosis. Eight-week-old Hif1aΔfl/fl ApoE−/− and Hif1aΔAdipo ApoE−/− mice were injected with lentivirus carrying mouse Smpd3 cDNA sequence or green fluorescent protein (GFP) and were fed a WD for 8 weeks. (A) Schematic diagram of the mouse model illustrating the concept of mouse injection with lentivirus-SMPD3/GFP. (B, C) Quantitation of ceramide concentrations in plasma (B) and eWAT (C), n = 6 per group. (D) Representative oil red O staining of cross-sections of aortic roots (n = 6–7 per group). Left, representative examples of cross-sections from the aortic root stained with oil red O. Scale bar, 500 μm. Right, quantification of aortic root lesion areas. (E, F) The levels of plasma total cholesterol (E) and triglyceride (F) in mice (n = 6–7 per group). (G) FPLC analysis of plasma cholesterol levels in mice. All data are presented as the mean ± SEM. One-way ANOVA with Tukey’s post hoc test (B–F). *P < 0.05, **P < 0.01 compared to the Hif1aΔfl/fl ApoE−/− + GFP group; *P < 0.05, **P < 0.01 compared to the Hif1aΔAdipo ApoE−/− + GFP group. See also Fig. S3.

---

**Figure 6** Overexpression of SMPD3 in adipose tissue aggravates the progression of atherosclerosis. Eight-week-old Hif1aΔfl/fl ApoE−/− and Hif1aΔAdipo ApoE−/− mice were injected with lentivirus carrying mouse Smpd3 cDNA sequence or green fluorescent protein (GFP) and were fed a WD for 8 weeks. (A) Schematic diagram of the mouse model illustrating the concept of mouse injection with lentivirus-SMPD3/GFP. (B, C) Quantitation of ceramide concentrations in plasma (B) and eWAT (C), n = 6 per group. (D) Representative oil red O staining of cross-sections of aortic roots (n = 6–7 per group). Left, representative examples of cross-sections from the aortic root stained with oil red O. Scale bar, 500 μm. Right, quantification of aortic root lesion areas. (E, F) The levels of plasma total cholesterol (E) and triglyceride (F) in mice (n = 6–7 per group). (G) FPLC analysis of plasma cholesterol levels in mice. All data are presented as the mean ± SEM. One-way ANOVA with Tukey’s post hoc test (B–F). *P < 0.05, **P < 0.01 compared to the Hif1aΔfl/fl ApoE−/− + GFP group; *P < 0.05, **P < 0.01 compared to the Hif1aΔAdipo ApoE−/− + GFP group. See also Fig. S3.
the Hif1aDAdipoApoEe/e mice (Fig. S4C). Consistently, oil red O staining exhibited increased atherosclerosis lesions, and proinflammatory cytokine levels and macrophage infiltration rate were also higher in the Hif1aDAdipoApoEe/e mice treated with C16:0 ceramide than those treated with vehicle (Fig. 6 G, H, and Fig. S4D–S4F).

3.7. Administration of PX-478 reduces atherosclerosis in a HIF-1α-dependent manner

Because of the harmful role of adipocyte HIF-1α in atherosclerotic development, we explored the therapeutic effect of selective HIF-1α inhibitor PX-478, which inhibits HIF-1α protein levels in cancer models and improved diet-induced adipose tissue dysfunction51, on atherosclerosis. PX-478 was intraperitoneally administered to ApoE−/− mice (Fig. 7A). The mRNA level of Smpd3 and the protein levels of HIF-1α and SMPD3 were inhibited in the eWAT of the ApoE−/− mice after PX-478 administration compared to those of the vehicle group (Supporting Information Fig. S5A and S5B). Analysis of whole aortas and sections demonstrated that the PX-478-treated ApoE−/− mice had smaller lesions than the vehicle-treated mice (Fig. 7B and Fig. S5C). Furthermore, PX-478 treatment attenuated the ceramide levels in the plasma and eWAT of the ApoE−/− mice (Fig. 7C and D). The levels of plasma total cholesterol and triglyceride were lower after PX-478 treatment in the ApoE−/− mice than in the vehicle-treated mice (Fig. 7E–G). After staining for macrophage infiltration of the aortic root, we also found a lower level of the macrophage marker MAC-2 in the ApoE−/− mice after PX-478 administration than in the control mice (Fig. 7H). The plasma MCP-1, TNF-α, and IL-6 levels were also reduced in the ApoE−/− mice after PX-478 administration than the control mice (Fig. S5D–S5F). Hence, these results demonstrate the potential therapeutic function of PX-478 in atherosclerosis through inhibiting HIF-1α.
4. Discussion

The main finding of this study is that adipocyte HIF-1α accelerated WD-induced atherosclerosis by increasing ceramide levels. Loss-of-function and gain-of-function studies showed that adipocyte HIF-1α directly regulates Smpd3, which encodes neutral SMase, an enzyme that generates the bioactive lipid ceramide. These findings indicate a central role for the adipocyte HIF-1α-ceramide signaling axis in the pathogenesis of atherosclerosis. Furthermore, we observed that PX-478 played a protective role in the progression of atherosclerosis through inhibition of adipocyte ceramide production.

Figure 6 Administration of ceramide reverses the protective effects of adipose HIF-1α deficiency on atherosclerosis through increased inflammation and cholesterol. Eight-week-old Hif1a<sup>+/+</sup> ApoE<sup>−/−</sup> and Hif1a<sup>ΔAdipo</sup> ApoE<sup>−/−</sup> mice were injected with ceramide C16:0 or vehicle and were fed a WD for 6 weeks. (A) Schematic diagram of the mouse model illustrating the concept of mouse injection with ceramide C16:0 or vehicle. (B, C) Quantitation of ceramide concentrations in plasma (B) and eWAT (C), n = 6 per group. (D, E) The levels of plasma total cholesterol (D) and triglyceride (E), n = 6–7 per group. (F) FPLC analysis of plasma cholesterol levels in mice. (G) Left, representative oil red O staining of cross-sections of aortic roots. Scale bar, 500 μm. Right, quantification of aortic root lesion areas. n = 6–7 per group. (H) Immunofluorescence staining of atherosclerotic lesions with MAC-2 antibody and calculated MAC-2 positive areas in the plaques (n = 3 per group). Scale bar, 500 μm. All data are presented as the mean ± SEM. One-way ANOVA with Tukey’s post hoc test (B, D, E, H) and Kruskal–Wallis test (C and G): *P < 0.05, **P < 0.01 compared to the Hif1a<sup>+/+</sup> ApoE<sup>−/−</sup> vehicle group; #P < 0.05, ##P < 0.01 compared to the Hif1a<sup>ΔAdipo</sup> ApoE<sup>−/−</sup> + vehicle group. See also Fig. S4.
An increased visceral white adipose tissue (WAT) mass enhances the risk of developing cardiovascular diseases. Due to limited O₂ diffusion into hypertrophic adipocytes, cellular hypoxia is observed in adipose tissue. Recent reports have demonstrated that adipose tissue HIF-2α reduces atherosclerosis via promoting ceramide catabolism and thus increasing hepatic cholesterol elimination and thermogenesis. Unlike adipocyte HIF-1α, HIF-2α is significantly inhibited with treated western diet.

![Graphs and images showing the effect of PX-478 on atherosclerosis](image)

**Figure 7**  Inhibition of HIF-1α by PX-478 improves atherosclerosis through the SMPD3—ceramide axis. Eight-week-old ApoE⁻/⁻ mice were administered with PX-478 (5 mg/kg) or vehicle every other day and fed a WD for 8 weeks. (A) Schematic diagram of the mouse model illustrating the concept of mouse injection with PX-478. (B) Left, representative oil red O staining of cross-sections of aortic roots. Scale bar, 500 μm. Right, quantification of aortic root lesion areas. n = 7 per group. (C, D) Quantitation of ceramide concentrations in plasma (C) and eWAT (D), n = 6 per group. (E, F) The levels of plasma total cholesterol (E) and triglyceride (F), n = 7 per group. (G) FPLC analysis of plasma cholesterol levels in mice. (H) Immunofluorescence staining of atherosclerotic lesions with MAC-2 antibody and calculated MAC-2 positive areas in the plaques (n = 3 per group). Scale bar, 500 μm. All data are presented as the mean ± SEM. Mann–Whitney U test (B, D), Two-tailed Student’s t-test (C, E, F, H): *P < 0.05, **P < 0.01 compared to the vehicle group. See also Fig. S5.
tissue is significantly activated, which indicates that adipose HIF-1α activation is associated with metabolic disorders. Furthermore, HIFs are themselves regulated in a non-equivalent manner, where HIF-2α is typically stabilized at higher relative O2 concentrations compared with HIF-1α. They also exert different regulation on divergent target genes in interaction with alternative transcription factors and coregulators.

HIF-1α deletion in macrophages specifically reduced atherosclerosis and necrotic core formation through limiting macrophage necroptosis and necrotic core formation in ApoE−/− mice. In occurrence of atherosclerosis, except for the vascular lesion in situ, the crosstalk between multiple organs is of profound impact as well. Although it has been reported that adipose HIF-1α activation is associated with metabolic disorders such as insulin resistance and inflammation in obesity and independently related to coronary artery disease. Genetic oncerase metabolism is directly activated by HIF-1α, the crosstalk between multiple organs is of profound impact 

Lipidomic analysis of different lipid fractions demonstrated that epididymal adipose tissue and plasma-derived ceramides and dihydroceramides were key mediators in the progression of atherosclerosis in our studies. Sphingolipid metabolites, particularly ceramide, sphingosine-1-phosphate (S1P) and sphingomyelin, are important signal molecules to regulate several cellular processes in immunity and inflammatory disorders. Unlike ceramide, S1P has potent but opposing regulatory roles in numerous cell types, whereas S1P promotes vasodilatation and is atheroprotective. Meanwhile, human plasma SM levels are positively and independently related to coronary artery disease. Genetic ablation of the Sgms2 gene in mice with or without Apoe knockout reduced plasma-membrane levels of sphingomyelin, increased insulin sensitivity, and ameliorated features of atherosclerosis. Ceramide has also been regarded as a primary lipid mediator of obesity and inflammation. While the change of ceramides was the most significant among sphingolipid metabolites in our animal models. Then, we examined the pathways of ceramide metabolism regulated by de novo synthesis, sphingomyelin hydrolysis and salvage pathways in different models. In the present study, we found that the impact of HIF-1α on ceramide metabolism is associated with neutral SMase 2 (nSMase2), a strongly altered enzyme that generates the bioactive lipid ceramide through the hydrolysis of the membrane lipid sphingomyelin in different models. The previous literature has proved SMPD3 knocked out systemically could reduce atherosclerosis. Meanwhile, Angel and his colleague found that hypoxia increased ceramide content in isolated rat pulmonary artery smooth muscle cells which was abrogated by inhibition of nSMase but not aSMase. Ceramide and reactive oxygen species production were increased by hypoxia in pulmonary arteries. Based on previous studies and our results, adipose-derived ceramide is most likely mediated by HIF-1α-SMPD3 induction.

Ceramide has been shown to stimulate TNF-α and IL-6 production in cultured macrophages through NF-κB activation. Furthermore, inflammation can reversely exacerbate the ceramide synthesis. Interestingly, IL-1β was first reported to induce nSMase activity in rat hepatocytes, and nSMase2-derived ceramide also shows bioactivity itself in response to TNF-α and IFN-γ. In agreement with Tom group who reported that nSMase2 deficiency or inhibition could reduce atherosclerosis with a decrease in macrophage infiltration and lipid deposition. We also found that administration of C16:0 ceramide increased plasma cytokines of MCP-1, TNF-α and IL-6, as well as MAC-2 expression in atherosclerotic lesions in Hif1α+/−Adipo cardioe−/− mice, which reversed the improvement of atherosclerosis (Fig. 6H and Fig. S4D–S4F). We also observed that induced changes in ceramide species of eWAT and plasma in our animal models (Fig. 6B and C). In general, ceramides exacerbated atherosclerosis at least partially through raising inflammatory cytokine production in the circulation.

Previously, hepatic cholesterol was shown to be excreted directly and indirectly through bile; thus, activation of hepatic cholesterol elimination through biliary excretion can reduce plasma cholesterol levels. Based on our study, a similar inhibitory effect of ceramide on hepatic cholesterol elimination via suppression of hepatic Cyp7a1 and Abcg5 was observed (Fig. S4C). As described previously, the ceramide catabolism enzyme ACER2 is inhibited in eWAT, which in turn leads to the accumulation of ceramide. In our model, HIF-1α in adipose tissue is significantly accumulated, which activates the synthesis pathway of sphingomyelin to ceramide and increases the content of ceramide through activated SMPD3. We also confirmed the role of ceramide in causing atherosclerosis to promote inflammation and increase the content of cholesterol. All of these findings can be explained in part by ceramide-induced atherosclerosis via increased inflammation and impaired cholesterol metabolism. Based on these results, we postulate that the regulation of lipid metabolism and inflammation by ceramides could be one of the possible mechanisms of diet-induced atherosclerosis.

“Ceramide reduction therapies”, such as inhibiting ceramide biosynthesis or catalyzing ceramide degradation in rodents, is a good strategy to ameliorate many metabolic disorders. We demonstrated the ceramide-lowering effects of PX-478, a selective HIF-1α inhibitor. PX-478 inhibits HIF-1α at multiple levels through inhibition of both HIF-1α transcription and translation and is approved for advanced solid tumors/lymphoma in Phase I. Although these effects have not been confirmed to be driven by inhibition of adipocyte-derived ceramide production, an alternative source of ceramide is SM hydrolysis by SMase, which is directly activated by HIF-1α, and the present data suggested that this pathway is likely affected by PX-478 inhibition of HIF-1α signaling. Previous studies showed PX-478 significantly reduced plasma and total cholesterol levels in mice administered a high-fat diet. Furthermore, PX-478-treated mice displayed decreased macrophage infiltration, and the local mRNA levels of IL-6 were significantly suppressed by PX-478 treatment, suggesting that this treatment reduces inflammation. These results indicate that PX-478 could be an anti-atherosclerotic agent. We further demonstrated that plasma cholesterol and circulating pro-inflammatory cytokines reduced after PX-478 treatment, accompanied by reduced macrophage infiltration in the plaque. PX-478 suppressed atherosclerosis, which could mainly depend on HIF-1α-ceramide metabolic regulation.
to atherosclerotic plaque formation. Pharmacological HIF-1α inhibition could attenuate ceramide-induced atherosclerosis in ApoE−/− mice. It’s worth noting that the inhibitory effect of PX-478 on adipose HIF-1α is remarkable (Fig. S5A and S5B), but the inhibition effect is universal by intraperitoneal injection. Therefore, it cannot be ruled out that reduced expression of HIF-1α in other tissues participated in the anti-atherosclerotic effects of PX-478. In addition, due to the vital role of hypoxia signaling in growth and development, regulation of ceramide metabolism by adipose HIF-1α may responsible for aggravating atherosclerosis partially, it is possible that other mechanisms are involved in the pro-atherosclerotic effects of adipose HIF-1α. Thus, the selective inhibitory effect of adipose HIF-1α can be used as a good strategy for atherosclerosis and exploring an adipose targeted drug delivery system will raise the security and practicability in clinical treatment. Taken together, our study identifies an important role for adipocyte HIF-1α in regulating lipid metabolism in atherosclerosis and provides a rationale for using HIF-1α specific inhibitor PX-478, as an adjunctive medical therapy, combined with current therapies to reduce the risk of atherosclerosis.

5. Conclusions

Reducing ceramide levels is a good strategy to ameliorate many metabolic disorders. This study partially clarifies the role of adipose-derived ceramide in the onset of atherosclerosis and provides a new strategy and drug candidate for the treatment of atherosclerosis. Adipocyte HIF-1α could be an effective target for treatment of metabolic dysregulation, and HIF-1α specific inhibitor PX-478 could exert metabolic benefits on atherosclerosis through inhibiting HIF-1α.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (2018YFA0800700 and 2018YFC1003900) and the National Natural Science Foundation of China (Nos. 91857115, 31925021, 82022028 and 81921001).

Author contributions

Pengcheng Wang, Guangyi Zeng, Yu Yan, Song-yang Zhang, Yongqiang Dong, Yangming Zhang, Xingzhong Zhang, Huiying Liu and Zhipeng Zhang performed the experiments and analyzed the data. Yanli Pang and Changaotao Jiang designed the study. Pengcheng Wang, Guangyi Zeng, Changaotao Jiang and Yanli Pang wrote the manuscript. Pengcheng Wang and Guangyi Zeng contributed equally to this work. All authors edited the manuscript and approved the final manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2021.10.001.
Chaurasia B, Summers SA. Ceramides
Sasset L, Zhang Y, Dunn TM, Di Lorenzo A. Sphingolipid
Pagadala M, Kasumov T, McCullough AJ, Zein NN, Kirwan JP. Role
Pavoine C, Pecker F. Sphingomyelinases: their regulation and roles in
Neeland IJ, Singh S, McGuire DK, Vega GL, Roddy T, Reilly DF, et al. Metabolic messengers: ceramides. Nat Metab 2019;1:1051–8.
Gertow J, Kjellqvist S, Stahlman M, Cheung L, Gottfries J, Itohara S, et al. Role for matrix metalloproteinase-2 in oxidized low-density lipoprotein-induced activation of the saphenous vein/Ceramide pathway and smooth muscle cell proliferation. Circulation 2004;110:571–8.
Lallemand T, Rouahi A, Swiader A, Grazide MH, Geoffre N, Alayrac P, et al. NSmase2 (type 2-neutral sphingomyelinase) deficiency or inhibition by GW4869 reduces inflammation and atherosclerosis in Apoe^-/- mice. Arterioscler Thromb Vasc Biol 2018;38:1479–92.
Canals D, Perry DM, Jenkins RW, Hannun YA. Drug targeting of sphingolipid metabolism: sphingomyelins and ceramidases. Br J Pharmacol 2011;163:694–712.
Qin J, Berdychev E, Poizner C, Schwartz NB, Dawson G. Neutral sphingomyelinase 2 deficiency increases hyaluronan synthesis by up-regulation of hyaluronan synthase 2 through decreased ceramide production and activation of Akt. J Biol Chem 2012;287:13620–32.
Kim WY, Safran M, Buckley MR, Ebert BL, Glickman J, Bosenberg M, et al. Failure to prolyl hydroxylate hypoxia-inducible factor alpha phenotypes VHL inactivation in vivo. EMBO J 2006;25:4650–62.
Xue X, Ramakrishnan S, Anderson E, Taylor M, Zimmermann EM, Spence JR, et al. Endothelial PAS domain protein 1 activates the inflammatory response in the intestinal epithelium to promote colitis in mice. Gastroenterology 2013;145:831–41.
Zhang X, Zhang Y, Wang F, Zhang SY, Dong Y, Zeng G, et al. Adipocyte hypoxia-inducible factor Zalpah suppresses atherosclerosis by promoting adipose ceramide catabolism. Cell Metab 2019;30:937–951 e935.
Li L, Shi L, Yang S, Yan R, Zhang D, Yang J, et al. SIRT7 is a histone desuccinyllase that functionally links to chromatin compaction and genome stability. Nat Commun 2016;20:12235.