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Visceral adipose tissue-derived serine protease inhibitor accelerates cholesterol efflux by up-regulating ABCA1 expression via the NF-κB/miR-33a pathway in THP-1 macropahge-derived foam cells

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ABSTRACT

Atherosclerosis is a dyslipidemia disease characterized by foam cell formation driven by the accumulation of lipids. Visceral adipose tissue-derived serine protease inhibitor (vaspin) is known to suppress the development of atherosclerosis via its anti-inflammatory properties, but it is not yet known whether vaspin affects cholesterol efflux in THP-1 macrophage-derived foam cells. Here, we investigated the effects of vaspin on ABCA1 expression and cholesterol efflux, and further explored the underlying mechanism. We found that vaspin decreased miR-33a levels, which in turn increased ABCA1 expression and cholesteorl efflux. We also found that inhibition of NF-κB reduced miR-33a expression and vaspin suppressed LPS-mediated NF-κB phosphorylation. Our findings suggest that vaspin is not only a regular of inflammasion but also a promoter of cholesterol efflux.

1. Introduction

Atherosclerosis is a critical etiology for cardiovascular diseases, and dyslipidemia is the major features in the pathogenesis of atherosclerosis [1]. It is well known that macrophage foam cell formation due to lipid accumulation is the hallmark of atherosclerosis [2]. ATP-binding cassette subfamily A member 1 (ABCA1) plays a central role in protection against atherosclerosis mainly by facilitating cholesterol efflux from macrophages to apoA-I. In addition, we also found a set of microRNAs have underlying effects on lipid metabolism [3], including microRNA (miR)-590, miR-19b, miR-486 and miR-27a/b [4–7]. MiR-33a is embedded within introns of the sterol regulatory element binding protein 2 (SREBP-2) gene and can act as a modulator of lipid metabolism, inflammatory response, and insulin sensitivity, affecting atherosclerosis [8]. It has been demonstrated that miR-33a suppresses cholesterol efflux by decreasing the ABCA1 level so as to affect lipid metabolism [9].

Inflammatory response is a critical part of atherosclerosis [10], Brown et al. provided evidence to support that nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)phosphorylation promotes atherosclerosis progression [11]. Further, inflammation regulates ABCA1 expression [12], for instance, we and others have reported that ABCA1 expression is suppressed by inflammatory cytokines such as IL-18, TNF-α and LPS [13]. Thus, inhibition of the NF-κB cascade may be an promising strategy to promote ABCA1-mediated cholesterol efflux.

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Adipose tissue-derived serine protease inhibitor (vaspin) is one member of the adipocytokine family that is secreted by the visceral adipose tissue. Several lines of evidence suggest that vaspin is found to retard cardiovascular diseases, and also has an anti-atherosclerosis properties in various cells including vascular smooth muscle cells, endotheliocytes [14–16]. Furthermore, vaspin was found to reduce inflammatory cytokine secretion by suppressing the NF-κB cascade [15,17]. Additionally, vaspin has closely relationship with the level of lipid in overweight children [18]. However, the mechansim of vaspin in lipid metabolism is remain elusive. Our results showed that vaspin inhibits NF-κB activation then down-regulates miR-33a levels and thereby promotes ABCA1-mediated cholesterol efflux from THP-1 macrophage-derived foam cells.

2. Material and methods

2.1. Cell culture and foam cell formation

Human THP-1 monocytes were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Primary human monocytes were isolated from 100 mL of heparin sodium-treated whole blood collected from healthy participants. Ficoll density centrifugation was used to obtain peripheral blood mononuclear cells. The collected cells were washed with Hanks' balanced salt solution for three times and allowed to aggregate in the presence of fetal bovine serum (FBS). After further purification, the human monocytes were resuspended and cultured in RPMI 1640 medium with recombinant human PMA (St. Louis, MO, USA) to differentiate into macrophages.

Human THP-1 monocytes were cultured in RPMI-1640 supplemented with 0.1% nonessential amino acids, penicillin (100 U/mL), streptomycin (100 mg/mL) and 10% FBS. Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2.

2.2. Oil red O staining

THP-1 cells were seeded on 6-well plates at a density of 2×10^6 cells/well and maintained in RPMI 1640 media (Solarbio, China) containing 10% FBS and 2% penicillin-streptomycin. All cultures were kept in an incubator with a humidified atmosphere of 5% CO_2 at 37 °C. Differentiation of THP-1 monocytes into macrophages was induced by treatment with 160 nmol/L of phorbol-12-myristate acetate (PMA) (Sigma, U.S.A) for 24 h. The medium was then changed to serum-free medium containing 50 mg/ml oxLDL for 48 h to promote foam cell formation. To confirm the formation of foam cells, the cells were fixed with 4% paraformaldehyde and then stained with 0.5% Oil red O. Hematoxylin was used for counterstaining. Cells were photographed at 40X.

2.3. Lipid content assay by high-performance liquid chromatography (HPLC)

THP-1-derived macrophages were washed three times with PBS. 1 ml of 0.9% NaCl was added to the cells before sonication using the ultrasonic processor (Sonics, U.S.A) for 6 bursts of 4 s with 25-s intervals under 600 W on the ice. Protein concentrations of cell lysate were measured using BCA Protein Assay kit (Beyotime, China). Cholesterols were dissolved in isopropanol as 1 mg cholesterol/ml and stored at 20 C as the stock solution. The stock solution was then diluted to produce cholesterol standard calibration solution ranging from 0 to 40 mg of cholesterol/ml. The 0.1 ml of each sample (cholesterol standard calibration solution or cell solution) was supplemented with 10 μl reaction mixture containing 500 mM MgCl2, 500 mM Tris-HCl (pH7.4), 10 mM dithiothreitol, and 5% NaCl. Meanwhile, 0.4 U cholesterol oxidase in 10 μl 0.5% NaCl was added to each one for free cholesterol determination, or 0.4 U cholesterol oxidase plus 0.4 U of cholesterol esterase for total cholesterol measurement. The total reaction solution in each tube was incubated at 37 C for 30 min, and then 100 ml of methanol: ethanol (1:1) was added to stop the reaction. Each solution was placed on ice for 30 min and then centrifuged at 1500 rpm for 10 min at 4 C. Thereafter, 10 μl of supernatant was applied to a 2790 Chromatographer (Waters, U.S.A). The column was eluted using isopropanol:n-heptane:acetonitrile (35:13:52) at a flow rate of 1 ml/min for 12 min. Absorbance at 216 nm was monitored. Data were analyzed with Total Chrom software from Perkin Elmer.

2.4. Bioinformatics prediction and dual luciferase reporter assay

The online databases including miRDB (http://mirdb.org/miRDB/) and TargetScan (http://www.targetscan.org/) were used to predict the binding sites between miR-33a and ABCA1 3′ untranslated region (UTR). In addition, the full ABCA1 3′UTR was amplified by RT-PCR from THP-1 cells. These amplified products were then cloned into a pMIR-REPORT luciferase reporter vector using restriction sites XhoI and NotI (Promega, Madison, WI, USA). Meanwhile, QuikChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA, USA) was used to mutate the miR-33A seed sequence located in ABCA1 3′UTR. Subsequently, HEK 293T cells were cotransfected with the above plasmids (1 μg) and miR-33a mimic for 24 h. The luciferase activities were measured using Dual-Luciferase reporter system (Promega).

2.5. Real-time quantitative PCR

To evaluate gene expression in the liver or peritoneal macrophages, total RNA was extracted by TRIzol reagent in accordance with manufacturer's instructions and converted into complementary DNA by reverse transcriptase (TOYOBO, Japan). Real-time quantitative PCR (qRT-PCR) was undertaken on an ABI Prism 7900 Fast Realtime PCR system with SYBR Green detection and TaqMan microRNA detection. The relative mRNAs levels of the ABCA1 and SREBP RNAs were calculated with β-actin mRNA as the invariant control. The relative levels of the miR-33a were calculated with U6 RNA's level as the invariant control. Relative transcript expression was determined using control sample as a calibrator and the ΔΔCt method. Human ABCA1 primers were: 5′-GTCCTCTTTCCCGATTATCTGG-3′ and downstream 5′-CACTCACTCTCGCTCGCA AT-3'. Human SREBP-2 primers were: 5′-AGGAGAACATGGTGCTGA-3'and downstream 5′-TAAAGGAGAGGCACAGGA-3'.

2.6. Western blot analysis

Cells were rinsed with PBS and lysed in RIPA buffer (Beyotime, China) on ice for 25 min. Lysates were centrifuged at 12,000 g for 10 min at 4 C, and resolved by SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore Corporation, U.S.A). Membranes were blocked with 5% nonfat dry milk (NFDM) in TBST (Tris 0.05% Tween-20), incubated overnight at 4 C with primary antibodies in TBST containing 5% NFDM, washed with TBST, and incubated with HRP-conjugated secondary antibodies diluted in TBST containing 5% NFDM for 1 h at room temperature. Immunoreactive bands were visualized with Tanon 5500 (China) and BeyoECL Plus (Beyotime, China).

2.7. Transfection of miR33a mimic/inhibitor

Human miR33a mimic/inhibitor were obtained from the Dharmacon company. THP-1 macrophage-derived foam cells (2×10^6 cells/well) were transfected with the inhibitor of miR33a

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