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MicroRNA-19b promotes macrophage cholesterol accumulation and aortic atherosclerosis by targeting ATP-binding cassette transporter A1

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ABSTRACT

Rationale: Macrophage accumulation of cholesterol leads to foam cell formation which is a major pathological event of atherosclerosis. Recent studies have shown that microRNA (miR)-19b might play an important role in cholesterol metabolism and atherosclerotic diseases. Here, we have identified miR-19b binding to the 3′UTR of ATP-binding cassette transporter A1 (ABCA1) transporters, and further determined the potential roles of this novel interaction in atherogenesis.
Objective: To investigate the molecular mechanisms involved in a miR-19b promotion of macrophage cholesterol accumulation and the development of aortic atherosclerosis.
Methods and results: We performed bioinformatics analysis using online websites, and found that miR-19b was highly conserved during evolution and directly bound to ABCA1 mRNA with very low binding free energy. Luciferase reporter assay confirmed that miR-19b bound to 3110-3116 sites within ABCA1 3′UTR. MiR-19b directly regulated the expression levels of endogenous ABCA1 in foam cells derived from human THP-1 macrophages and mouse peritoneal macrophages (MPMs) as determined by qRT-PCR and western blot. Cholesterol transport assays revealed that miR-19b dramatically suppressed apolipoprotein AI-mediated ABCA1-dependent cholesterol efflux, resulting in the increased levels of total cholesterol (TC), free cholesterol (FC) and cholesterol ester (CE) as revealed by HPLC. The excretion of ^{3}H-cholesterol originating from cholesterol-laden MPMs into feces was decreased in mice overexpressing miR-19b. Finally, we evaluated the proatherosclerotic role of miR-19b in apolipoprotein E deficient (apoE$^{-/-}$) mice. Treatment with miR-19b precursor reduced plasma high-density lipoprotein (HDL) levels, but increased plasma low-density lipoprotein (LDL) levels. Consistently, miR-19b precursor treatment increased aortic plaque size and lipid content, but reduced collagen content and ABCA1 expression. In

Abbreviations and Acronyms: ABCA1, ATP binding cassette transporter A1; apoAI, Apolipoprotein AI; RCT, reverse cholesterol transport.

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contrast, treatment with the inhibitory miR-19b antisense oligonucleotides (ASO) prevented or reversed these effects.
Conclusion: MiR-19b promotes macrophage cholesterol accumulation, foam cell formation and aortic atherosclerotic development by targeting ABCA1.

1. Introduction

Foam cell formation due to excessive accumulation of cholesterol in macrophages is a pathological hallmark of atherosclerosis. In cholesterolemia, macrophages can not restrain the uptake of cholesterol that infiltrates into arterial wall. Therefore, macrophages depend on the cholesterol efflux pathway to prevent their transformation into foam cells [1–3]. The facilitation of macrophage reverse cholesterol transport (RCT), the removal of cholesterol from peripheral macrophages and its transport to and eventual excretion from the liver, is an attractive target for antiatherogenic therapy [4–6]. Cholesterol efflux from macrophages is the first and potentially most important step in RCT. Accordingly, ATP-binding cassette transporter A1 (ABCA1) has been identified to be critical for this process in macrophage [7,8].

ABCA1 is an important membrane protein mediating the efflux of macrophage cholesterol to lipid-poor apolipoprotein (apoAI) as the preferred acceptor, defined as the first and rate-limiting step in RCT [9,10]. Previous studies from our laboratory and others have demonstrated that increased expression of ABCA1 plays critical roles in preventing cholesterol accumulation in macrophages, and deficiencies and mutations of this transporter lead to defects in cholesterol efflux and reduced plasma high-density lipoprotein (HDL) cholesterol (HDL-C) concentration [10–15]. As a primary transporter to eliminate intracellular cholesterol, ABCA1 can be tightly modulated at multiple levels, including transcriptional and posttranscriptional processes [16,17].

It has been reported that ABCA1 is comprehensively regulated by intracellular microRNAs [16,18–20]. MicroRNAs comprise a class of small noncoding RNAs of approximately 22 nucleotides in length and represent an elegant mechanism underlying posttranscriptional control of gene expression [21–24]. Recently, several lines of evidence suggest that miR-19b contributes to atherosclerosis progression. MiR-19b is upregulated in human atherosclerotic plaques and rat abdominal aortic aneurysms [25,26]. However, the underlying mechanism is currently unclear. We performed bioinformatics analysis and found that miR-19b directly binds to 3' untranslated region (3′UTR) of ABCA1 mRNA. Subsequently, we showed that miR-19b promotes cholesterol accumulation *in vitro* in human THP-1 macrophages. Thus, we hypothesized that miR-19b promotes macrophage cholesterol accumulation and development of aortic atherosclerosis by targeting ABCA1.

To test this hypothesis, we investigated the effects and underlying mechanisms of miR-19b on ABCA1 expression and efflux of intracellular lipids in the foam cells derived from human THP-1 macrophages and mouse peritoneal macrophages (MPM), and also determined the potential effects of miR-19b on plasma lipid levels, aortic lipid deposition and development of atherosclerotic lesions in apoE$^{-/-}$ mice.

2. Materials and methods

2.1. Cell culture

THP-1 and HEK 293T cell lines were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). THP-1 cell line was cultured in RPMI 1640 (Solarbio, China) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco BRL, America) at 37 °C and 5% CO_2. Differentiation of THP-1 cells into macrophages was induced using 160 nM phorbol-12-myristate acetate (PMA, Sigma, America) for 24 h. HEK 293T cell line was cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS. MPMs from adult C57BL/6J mice (Jackson Laboratories) were obtained by peritoneal lavage 4 d after the intraperitoneal injection of 2.0 ml thioglycollate broth (4%, w/v). The thioglycollate-elicited MPMs were maintained in RPMI 1640 medium containing 10% FBS. Finally, macrophages were incubated in RPMI 1640 containing 0.2% (w/v) bovine serum albumin (BSA, Sigma, America) and 50 μg/ml acetylated-low density lipoprotein (acLDL) for 48 h, for full differentiation of THP-1 macrophages/MPMs to foam cells in all experiments analyzing ABCA1 expression and cholesterol metabolism (unless otherwise indicated). Foam cell formation was verified by staining with 0.5% (w/v) Oil red O and hematoxylin and eosin after fixation with 4% (v/v) paraformaldehyde.

2.2. Bioinformatics analyses

The miRNA sequences were obtained from the miRBase (http://www.mirbase.org/search.shtml). Target predictions were performed with miRDB (http://mirdb.org/miRDB/) and miRanda (http://www.microrna.org/microrna/home.do). MiR-19b binding sites within ABCA1 3′UTR were analyzed with miRnaViewer (http://cbio.mskcc.org/cgi-bin/mirnaviewer/mirnaviewer.pl) and TargetScan (http://www.targetscan.org/). The minimum free energy of the hybridization of ABCA1 3′UTR and miR-19b was predicted with RNAhybrid (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/submission.html).

2.3. DNA constructs, 3′UTR luciferase reporter assays and MiR-19b/anti-miR-19b transfection

A cDNA fragment corresponding to the entire 3′UTR of ABCA1 was amplified by reverse transcription-polymerase chain reaction (RT-PCR) from total RNA extracted from THP-1 cells with XhoI and NotI linkers. The PCR products were directionally cloned downstream of renilla luciferase open reading frame of the psiCHECK™-2 vector (Promega, America) that also contains a constitutively expressed firefly luciferase gene, for transfection normalization. Site-directed mutagenesis introduced in the seed region of predicted miR-19b binding sites within the 3′UTR of ABCA1 was generated using Multisite-QuickChange (Stratagene, America) according to the manufacturer's protocol. All constructs were confirmed by commercial sequencing (Ribobio, China). HEK 293T cells were seeded at 1×10^6 cells/well into 12-well plates (Costar, America) and co-transfected with 1 μg of the indicated 3′UTR luciferase reporter vectors and miR-19b mimic utilizing RiboFECT™CP reagent (Ribobio, China) for 24 h. Luciferase activity was measured using the Dual-Glo Luciferase Assay System (Promega, America). Relative luciferase activity of each group was expressed as a percentage of renilla luciferase activity normalized to the corresponding firefly luciferase activity.