Mechanism of lncRNA H19 in the Regulation of Lipid Accumulation and Inflammatory Response in Macrophages

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Abstract

Background and aims: Lipid accumulation of macrophages caused by oxidative stress is the key reason for the early pathological changes of atherosclerosis. LncRNA H19 repression downregulated NF- κ B activation, upregulated ABCA1 expression, intracellular lipid accumulation increased, but the role of lncRNA H19 in atherogenesis and the molecular mechanisms have not been defined. We aimed to explore if and how lncRNA H19 affects lipid accumulation of macrophages by regulating lipid metabolism and inflammatory response.

Methods and results: THP-1 macrophages were cultured with ox-LDL to form foam cells. THP-1-derived macrophages were incubated with H19 siRNA or not. Oil Red O staining was used for the determination lipid accumulation in macrophages. Enzymatic methods were performed to analyze cholesterol concentration. Both western blot and qRT-PCR were applied to detect target gene expression. ELISA was used to examine the levels of oxidative and inflammatory mediators. We found that lncRNA H19 repression reduced lipid accumulation by elevating efficiency of RCT and via upregulation of ABCA1 and PPAR α expression in THP-1 derived macrophages. Further, lncRNA H19 repression upregulated PGC-1 α and downregulated NF- κ B signaling pathway.

Conclusion: These results suggest that lncRNA H19 repression inhibits atherosclerosis by promoting RCT process and reducing inflammatory response via PGC-1 α and NF- κ B pathways, respectively.

1 Introduction

Atherosclerosis is the pathological basis of a variety of cardiovascular diseases. The main pathological characteristics of atherosclerosis are the accumulation of lipid in the blood vessels to form plaques, but the mechanism is not clear. It is an important cause of cardiovascular disease and death, seriously endangering human health [1]. Age, smoking, drinking, hypertension, hyperglycemia, hyperlipidemia, and increased blood viscosity are considered as key risk factors for atherosclerosis. At the same time, the prevalence and mortality of coronary heart disease caused by atherosclerosis showed a "blowout" growth [2]. The multi-provincial cohort showed that in the age of over 40 years old group, the detection rate of carotid ultrasound plaque was 36.2% [3, 4]. It is suggested that a new effective method for preventing and treating atherosclerosis, reducing incidence and mortality are urgent problems for medical practitioners.

Macrophages engulf large amounts of oxidized low density lipoprotein (ox-LDL) to form foam cells is the key step of atherosclerosis [5]. A large number of lipids in atherosclerotic plaques are mainly cholesterol, including free cholesterol (FC) and cholesterol ester (CE). In the formation of atherosclerosis, cholesterol metabolism is the most important segment [6, 7]. CD36 is the main receptor of cholesterol intake and ATP-binding cassette transporter A1 (ABCA1) is the main receptor of cholesterol outflow. At present, the main mechanism of anti-atherosclerosis is to promote reverse cholesterol transport (RCT), reduce lipid accumulation in macrophages, and reduce foam cells and plaque formation. The outflow of cholesterol from macrophage surface is the first step in the whole RCT process, and also the key limiting step [8, 9].

Peroxisome proliferators activated receptor α (PPAR α), which is highly expressed in liver, skeletal muscle, kidney, heart and vascular wall, is a critical gene regulating transcription factor in fatty acid oxidation process. Many target genes of PPAR α are related to lipid metabolism and participate in fatty acid uptake, binding, transportation, oxidation and lipoprotein assembly [10]. The RCT is a process of ATP participating in active energy consumption [11], and mitochondria is the main organelle producing ATP. Mitochondrial OXPHOS dysfunction leads to insufficient ATP supply and increased reactive oxygen species (ROS) content, which will increase macrophages phagocytosis of lipids and foaming [12]. Peroxisome proliferator activated receptor- γ coactivator α (PGC-1 α) is a nutrient deficient transcription factor that can interact with transcription factors or other co-activating factors to improve the transcription efficiency of target genes [13, 14]. Some studies have shown that PGC-1 α participates in regulating macrophage oxidative stress and mediates mitochondrial dysfunction [15].

Nuclear factor kappa B (NF- κ B) is a kind of transcription factor with multidirectional transcriptional regulation function, which widely exists in organism. Besides regulating various physiological and pathological processes such as inflammatory response, apoptosis, oxidative stress and immune response, the latest research found that this pathway also regulates energy metabolism [16]. Based on the previous studies on the NF- κ B signaling pathway in the process of atherosclerosis, as well as the role of macrophage inflammatory infiltration and lipid accumulation in atherosclerosis, inhibition of NF- κ B/ABCA1 pathway may be an effective treatment to block the vicious circle. However, the mechanism of NF- κ B regulating inflammation and RCT has not been fully elucidated [8].

The lncRNA H19 is located on human chromosome 11, which is a maternal imprinted gene in mammalian development [17]. Recent studies have shown that lncRNA H19 is closely related to coronary heart disease, obesity, diabetes and myocardial infarction. The mRNA level of lncRNA H19 was significantly higher in AMI patients than in healthy control, and elevated expression level of circulating H19 may be considered as novel biomarkers of AMI [18, 19]. However, the detailed molecular mechanism about lncRNA H19 mediate the inhibition of RCT in macrophages is still poorly understood. The purpose of this study was to investigate the mechanism of lncRNA H19 in the regulation of RCT and inflammation in macrophages.

2 Materials And Methods

Patient specimens

In the clinical experiment, we recruited the study cases of acute myocardial infarction (AMI) patients and healthy controls who were treated in the First Affiliated Hospital of Xinjiang Medical University from 2015 to 2017. The sample size is a group of 90 people. The study was reviewed by the hospital's ethics committee and the informed consent of patients was signed. The selected population recruited is strictly accordance with the diagnostic criteria of AMI, and serious complications and immune diseases were excluded. The peripheral blood of patients with AMI were the first blood samples after admission. Within 30 minutes of collection, the peripheral blood mononuclear cells (PBMCs) were mainly monocytes and

lymphocytes. PBMCs were prepared by adding lymphocyte separation solution. 3–5 ml peripheral blood anticoagulated with heparin tube was centrifuged at 3500 rpm for 10 minutes to extract monocytes, The total RNA was extracted from PBMCs [20].

Cell culture

Human THP-1 monocytes were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China) were maintained in 1640 medium supplemented with 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 mg/ml), at 37 °C and 5% CO₂. The differentiation of THP-1 monocytes into macrophages was induced by 150 nM phorbol-12-myristate acetate (PMA) for 24 h.

Preparation of stimuli

Cell models of ox-LDL, LPS and H₂O₂ were established respectively. For high fat model, ox-LDL (50 µg/ml) (Yiyuan biotech, China) were added to 1640 medium, and then incubated at 37°C for 48 h. For oxidative stress model, H₂O₂ (200 µmol/L) (Sigma, USA) were added to 1640 medium, and then incubated at 37°C for 12 h. And for inflammation model, LPS (100 ng/ml) (Sigma, USA) were added to 1640 medium, and then incubated at 37°C for 6 h. RPMI 1640 (with 10% FBS and 1% antibiotic) was employed as the blank control in this study. These changes in lncRNA H19 expression were further evaluated.

Cell transfection

THP-1 derived macrophages at 50–70% confluency were transfected with H19 siRNA or control siRNA using lipofectamine 3000 according to the manufacturer's instructions. The efficiency of gene transfection was evaluated by qRT-PCR. For preparation of lncRNA H19 siRNA and negative control (NC) siRNA cell transfection, they were added to 1640 medium before the stimuli, and then incubated at 37°C for 48 h. Concentrations of reagents were determined by pre-experiment.

Oil red O staining

THP-1 derived macrophages were seeded in 12 well plates. After treated with ox-LDL stimuli, macrophages were fixed with fresh 4% formalin for 30 min, and then stained by oil red O for intracellular lipids for 15 min. After washing with 60% isopropyl alcohol, nuclei of macrophages were stained by hematoxylin for 2 min, then observed and photographed under an optical microscope (Leica, Germany). Intracellular oil red O was extracted by 100% isopropyl alcohol, then optical absorbance of extracting solution at 520 nm was determined by enzyme-labeled instrument (Bio-Rad Benchmark Plus).

Cholesterol concentration in THP-1 derived macrophages

The enzymology method was used for the determination of cholesterol concentration in THP-1 derived macrophages. The measure is based on the classic GPO Trinder enzymology reaction [21, 22], the TC and FC were measured by kits (Applygen, China). The CE level was calculated by formula $CE = TC - FC$. Finally, all the results were corrected by cell protein content.

Determination of ATP level in cells

The ATP level of cells was measured with ATP detection kit (Biyuntian Biotechnology Co., Ltd. China). The operation steps were as follows: 1×10^7 cells was collected and put into centrifuge tube. 200 μ l ATP cracking liquid was added to ensure that cells were completely lysed. Centrifugation of 12000 g at 4 °C for 5 minutes, collect the supernatant. The protein concentration was determined by BCA method, and the samples were adjusted to an uniform concentration with ATP detection lysate, so as to eliminate the influence of different protein concentration. Add ATP detection working solution in 96 well plate according to 100 μ l/well, put it at room temperature for 5 minutes to consume background value. Then add 20 μ l standard product or tested sample to each hole respectively, and mixed. Luminometer function of full band enzyme scale was used to measure relative light unit (RLU). According to the RLU value of the standard product, the standard curve was established, and the ATP contents of the tested samples were calculated. The results of each group were statistically analyzed with the percentage of the control group.

Quantitative RT-PCR

The qRT-PCR analysis included lncRNAs H19 and other RNAs. The amplification primers were in Table 1. Single-stranded cDNA was biosynthesized by transcribing 1 μ g tRNA using a cDNA transcription kit (thermos, USA) with random primer. Comparative test was carried out for the primer sequences by using primer comparison tool in NCBI, ensuring primers can specifically amplify target RNAs templates. The qRT-PCR by applying SYBR Green PCR Master (Applied Biosystems, USA), with 7500 HT Fast real-time PCR system (BIO-RAD, USA). Through the calculation of cycle threshold (Ct) value of each sample, the quantitative results are obtained according to $2^{-\Delta\Delta Ct}$ methods. GAPDH is the internal parameter.

Table 1
Sequences of primers for quantitative real-time PCR

Genes	Forward	Reverse
H19	5'- CCGGACACAAAACCCTCTAGCT - 3'	5'- TGTTCGATGGTGTCTTTGATG - 3'
ABCA1	5'- TATGAGGGCCAGATCACCTC - 3'	5'- GCTGGCTTGTTTTGCTTTTC - 3'
PPAR α	5'- CCGCAATGGACCATGTAAC - 3'	5'- CAGCTCTAGCATGGCCTTTT - 3'
GAPDH	5'- GCAAATTCATGGCACCGT-3'	5'- TCGCCCCACTTGATTTTGG - 3'

Table 2
Effects of H19 siRNA on cholesterol contents in THP-1 derived macrophage foam cells (mg/g protein)

group	TC	FC	CE	CE/TC(%)
CONTROL	236 $\pm$ 23	147 $\pm$ 16	90 $\pm$ 21	38 $\pm$ 7
ox-LDL	632 $\pm$ 26 [#]	253 $\pm$ 19 [#]	379 $\pm$ 22 [#]	60 $\pm$ 2 [#]
NC siRNA + ox-LDL	623 $\pm$ 42 [#]	252 $\pm$ 18 [#]	371 $\pm$ 53 [#]	59 $\pm$ 5 [#]
H19 siRNA + ox-LDL	517 $\pm$ 35 ^{**}	262 $\pm$ 15	256 $\pm$ 34 [*]	49 $\pm$ 4 [*]
Note: Values are the means $\pm$ SD from at least three separate experiments. [#] P < 0.001 vs control group, [*] P < 0.05 vs ox-LDL group, ^{**} P < 0.001 vs ox-LDL group, TC, total cholesterol; FC, free cholesterol; CE, cholesteryl ester.				

Cytokine assay

THP-1 derived macrophages were seeded in 96 well plates. After 48 hours incubation with stimuli, culture supernatant of macrophages were collected. Then activity of superoxide dismutase (SOD), concentration of malondialdehyde (MDA) and MCP-1 in the supernatant were measured by ELISA kits (NanJing JianCheng, China). Absorbance was detected by an enzyme-labeled instrument (Bio-Rad Benchmark Plus).

Measurement of protein expression by western blot analysis

Western blot were applied to detect target gene expression. The samples from THP-1 derived macrophages were harvested and lysed in RIPA buffer (Biyotime, China) supplemented with 0.5% protease inhibitors (Biyotime, China) and 1% phosphatase inhibitors (thermo, USA). Then the samples were placed on ice for 30 min. After centrifugation at 12,000 g for 10 min at 4°C, the supernatant was collected. Total proteins were denatured at 100°C for 10 min in SDS and 5 $\times$ loading buffer. Protein

concentrations were assessed with the BCA protein assay kit (thermo, USA). Equal amounts of protein (20 µg) were separated by 10% SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad, USA). Membranes were blocked in 5% nonfat milk in PBS with 0.1% Tween 20 at room temperature for 1 h. Size-separated proteins were transferred to PVDF membranes (Millipore, Germany), which then were incubated with primary antibodies. Expression of ABCA1, PPARα, PGC-1α, NF-κB p-p65, NF-κB p50 and GAPDH protein was detected by western blot analysis with the following primary antibodies at the given dilutions: ABCA1 (Abcam, 1:1000), PPAR-α (Abcam, 1:1000), PGC-1α (Abcam, 1:1000), NF-κB p-p65 (CST, 1:1000), NF-κB p50 (CST, 1:1000) and GAPDH (CST, 1:1000). They were shaken with primary antibody for 2 h. They were incubated at 4 °C overnight. After hatching with 1:1,000 diluted secondary antibody at 37 °C for 1 h, they were warm bathed with chemiluminescence reagent ECL and exposure, developing, and fixing were performed 1 min later. Quantity One software was used for analysis; the integral optical density value was calculated as the target protein divided by the internal reference GAPDH protein.

Statistical analysis

IBM SPSS Statistics 22.0 software was used for data analysis. Presentation format of continuous variances are as mean ± deviation (SD). The differences among groups were compared using One-way ANOVA. And significant difference was adjudged between the groups if the $p < 0.05$.

Results

H19 overexpression was associated with high fat stress

LncRNA H19 is upregulated in PBMCs from AMI patients, and is highly expressed in THP-1 derived high fat model and inflammation model. Firstly, we tested the H19 expression status in all the 90 AMI PBMCs samples and 90 non-AMI samples. The qRT-PCR data indicated that LncRNA H19 expression in PBMCs is 3.21-fold higher than in the non-AMI samples ($P < 0.001$, Fig. 1A). Secondly, these changes in H19 expression were further evaluated within the THP-1 derived macrophages model of ox-LDL, LPS and H₂O₂ respectively. Compared with the control group, the expression of LncRNA H19 was upregulated after LPS ($P < 0.05$) or ox-LDL ($P < 0.01$) induction (Fig. 1B).

H19 repression reduced the lipid accumulation and the cholesterol ester level of macrophages

To explore the role of H19 in the pathogenesis of atherosclerosis, we investigated the potential effect of H19 on the ratio of CE/TC in THP-1 derived macrophages. We found that H19 siRNA significantly inhibited intracellular lipid accumulation as determined by Oil Red O staining ($P < 0.05$). Furthermore, the increased intracellular lipid content was also evidenced by a commonly used enzymatic method (Fig. 2). H19 siRNA treatment significantly decreased TC, FC and CE content, and the ratio of CE/TC was

significantly decreased (Table 1). These results indicate that H19 repression inhibits the ox-LDL induced lipid accumulation of macrophage.

H19 repression relieved mitochondrial dysfunction and oxidative stress

The ATP level of H19 siRNA group was significantly higher ($P < 0.05$) compared with the NC siRNA group, and the ATP level of ox-LDL group was significantly lower ($P < 0.001$) compared with the control group. The results are reported in Fig. 3A. To assess the inflammation and oxidative stress effects of H19, concentration of MCP-1, activity of SOD and concentration of MDA were tested. Compared with the control group, the activity of SOD in the ox-LDL group decreased significantly ($P < 0.001$), MDA increased significantly ($P < 0.01$), MCP-1 increased significantly ($P < 0.001$). Compared with the NC siRNA group, the activity of SOD in the H19 siRNA group increased significantly ($P < 0.05$), MDA decreased significantly ($P < 0.05$), the level of MCP-1 decreased significantly ($P < 0.001$), and see Fig. 3B, C, D for details.

The stimulatory effects of ox-LDL on lipid accumulation were regulated by H19 repression

ABCA1 is a key transporter that regulates cholesterol efflux from macrophages. PPAR α is an important transcription factor in fatty acid oxidation process. We therefore evaluated the mechanism by which H19 enhances lipid accumulation by examining the expression of ABCA1 and PPAR α in macrophages. The data indicated that H19 siRNA significantly increased the ABCA1 and PPAR α expression in macrophages compared with that of the NC siRNA group (Fig. 4A, B, C). Similar results were observed by qRT-PCR (Fig. 4D). These results confirmed that lncRNA H19 promoted lipid accumulation by reducing ABCA1 and PPAR α expression.

LncRNA H19 promotes lipid accumulation by regulating NF- κ B and PGC-1 α signaling pathway

We had found that lncRNA H19 could promote lipid accumulation via regulating mitochondrial function and RCT in THP-1 derived macrophage. Further studies have found that NF- κ B and PGC-1 α signaling pathway were involved in regulating the function of macrophage. We found that overexpressed H19 could lead to decreased expression of PGC-1 α , the increased expression of NF- κ B p-p65, decreased expression of NF- κ B p50 which was the key factors of NF- κ B signaling pathway (Fig. 5A, C, D), suggesting that lncRNA H19 could activate NF- κ B signaling pathway and downregulated the PGC-1 α pathway in THP-1 derived macrophage.

Discussions

The present study investigated the effect of lncRNA H19 on lipid accumulation of macrophages caused by metabolic stress. We analyzed mRNA expression of lncRNA H19 in PBMCs from whole blood specimens of AMI participants and non-AMI volunteers. Then we tested the expression of lncRNA H19 in the model of ox-LDL, LPS and H₂O₂ of THP-1 derived macrophages. Compared with the control group, the expression of lncRNA H19 was upregulated after LPS or ox-LDL induction. It suggests that inflammation and oxidative stress are the main mechanisms for H19 to influence the macrophages transfer to foam cells. In the ox-LDL induced foam cell model, we have made several important findings. H19 repression downregulated oxidative stress by increasing the activity of SOD, reducing levels of MDA. And H19 repression reduces lipid accumulation by reducing cellular TC, FC and EC. Meanwhile, H19 repression increases RCT conferred cardiovascular protection in THP-1 derived macrophages. H19 repression upregulated mitochondrial function inferred ATP production and ABCA1-mediated cholesterol efflux, attenuating oxidative stress and inflammatory infiltration. And this was achieved at least in part by depressions of NF-κB signaling pathway and enhanced PGC-1α signaling pathway.

Atherosclerosis is the common pathological basis of many cardiovascular and cerebrovascular disease, it is an important cause of cardiovascular disease and death, seriously endangering human health [23]. Macrophages play a key role in lipid accumulation to form plaque, produce necrotic core and maintain plaque stability [24]. High fat stress induces oxidative stress and lipid accumulation in macrophages. In our study, we found that H19 repression reduced the lipid accumulation and the CE level of the THP-1 derived macrophages. H19 siRNA treatment significantly decreased TC, FC and CE content and the ratio of CE/TC. These results indicate that H19 repression inhibits the ox-LDL induced lipid accumulation of macrophage. Meanwhile, H19 repression relieved the inflammation and oxidative stress. There are several reasonable explanations for this result. Firstly, under the exogenous stimulation of ox-LDL and inflammatory factors, a large amount of ROS were produced in vivo. The imbalance between ROS production and anti-oxidation results was oxidative stress. A large number of ROS can lead to lipid peroxidation [25]. Secondly, the increase of ROS also promote the secretion of matrix metalloproteinase, and with the degradation of matrix, mediate the migration of vascular smooth muscle cells, accelerate the progress of atherosclerosis [26]. Therefore, inhibiting excessive oxidative stress and maintaining macrophage homeostasis are very important for the prevention and treatment of atherosclerosis.

Our study found ATP content decreased in the THP-1 derived macrophages, RCT was blocked, and intracellular lipid accumulation increased, the H19 repression relieved it. The reasons are as follows, RCT in macrophages and the polar state are tightly coupled with the energy metabolism of mitochondria, which is the core link of foam accumulation in macrophages [27, 28]. Mitochondrion is the place of oxidative phosphorylation and the production of ATP. The electron transport chain and ATP synthetase of OXPHOS produce ATP. Once the dysfunction of mitochondria occurs, it will cause the metabolic disorder [29]. It had been shown that inhibition of oxidative stress of mitochondria in macrophage would slow down atherosclerosis and inhibit inflammatory monocyte infiltration [14]. Karunakaran D et al [30] reported that the energy state of mitochondria regulate cholesterol outflow, miR-33 is involved in the regulation of mitochondrial functional genes, inhibition of mitochondrial ATPase activity significantly,

reduced the cholesterol outflow capacity of macrophages. And anti-miR33 expression can inhibit the target gene PGC-1 α , PDK4 and slc25a25, promote mitochondrial respiration and ATP production, reduce atherosclerosis pathological development. Previous research showed that the damage of mitochondrial DNA of monocytes and the decrease of the activity of mitochondrial respiratory chain complexes I and IV in mouse atherosclerosis model [31]. However, the effective intervention for ROS accumulation, DNA damage and mitochondrial dysfunction of macrophages is still limited.

In the study, we also found that H19 repression relieved the gene and protein expression of ABCA1 and PPAR α , promoted the RCT. LncRNA H19 promotes lipid accumulation by regulating NF- κ B and PGC-1 α signaling pathway. As we known, the process of cholesterol efflux is a strictly regulated energy consumption process mediated by ABCA1 or ABCG1. ABCA1 is the most important membrane transporter, and its cholesterol outflow accounts for more than 70% of the total outflow [32]. In addition, many target genes of PPAR α are related to lipid metabolism and participate in fatty acid uptake, binding, transportation, oxidation and lipoprotein assembly [33]. Carnitine palmitoyltransferase-1 (CPT-1) is a key enzyme for the activation and oxidation of fatty acids, which transports fatty acids to mitochondria. CPT-1 is also a direct downstream target gene of PPAR α , and its expression is up-regulated by PPAR α transcription [34]. Up-regulation of CPT-1 expression can promote fatty acid transport to mitochondria and promote oxidative phosphorylation and decomposition of lipids [35]. The NF- κ B p50 (NFKB1) is a key subunit of NF- κ B signaling pathway. In recent years, studies have shown that in different ethnic groups, the ATTG deletion mutation at the promoter region-94 of NFKB1 gene is significantly related to the occurrence of vascular endothelial function, cardiomyopathy, left ventricular dysfunction and heart failure [36]. Tang CK team's study [9] found that NF- κ B can inhibit the expression of ABCA1 and cholesterol outflow by regulating the expression of SREBPs, and promote the lipid accumulation of macrophages. Therefore, NF- κ B signaling pathway plays an important role in macrophage inflammatory response and lipid metabolism. Our previous study found that adiponectin can slow down the development of atherosclerosis in ApoE^{-/-} mice by inhibiting NF- κ B signaling pathway [37]. In the study, we also found that H19 repression promoted the RCT by upregulating PGC-1 α signaling pathway. PGC-1 plays an important role in the mitochondrial biosynthesis and functional regulation. Some studies have shown that PGC-1 α has a strong inducing effect on the gene expression of mitochondrial nuclear respiratory factor (NRFs) [38]. PGC-1 α can regulate the co-expression of OXPHOS components through NRFs and mitochondrial transcription factor A (mtTFA), which is an important transcription activator in mitochondrial biosynthesis. Therefore, PGC-1 α promotes the mitochondrial respiratory chain function in different energy supply and demand states [39, 40]. Consistent with these reports, the current study indicates LncRNA H19 repression abates ox-LDL induced macrophages dysfunction by suppressing oxidative stress, adhesion molecules, and inflammation. Targeting the NF- κ B and PGC-1 α pathway provides new ideas for early diagnosis and targeted therapy of atherosclerosis.

Conclusions

In conclusion, the present study demonstrates that under high fat stress conditions H19/NF- κ B and H19/PGC-1 α regulate cholesterol reverse transport and inflammatory response in macrophages, resulting in lipid accumulation and foaming of macrophages, promoting vascular plaque formation and atherosclerosis damage. Intervention of its key molecules could reduce or reverse the occurrence of atherosclerosis. On the one hand, lncRNA H19 binds to NF- κ B in the nucleus, intensifies the abnormal activation of NF- κ B signaling pathway, promotes inflammatory response, downregulates gene and protein expression of ABCA1, inhibits cholesterol outflow. On the other hand, lncRNA H19 repression upregulate the gene and protein expression of PGC-1 α . Targeted intervention in the axial pathway of H19/PGC-1 α could reduce or reverse the lipid accumulation and plaque formation. Collectively, these findings provide insights to potential therapeutic targets that ultimately could be directed to reducing cardiovascular morbidity and mortality.

Limitations of our study

In this experiment study, we measured the high expression of H19 in high fat stress. Inhibiting the expression of H19 can reduce the lipid accumulation of macrophages, which may be achieved by increasing mitochondrial ATP production, inhibiting NF- κ B signaling pathway and increasing the expression of reverse cholesterol transporter ABCA1 by upregulating PGC-1 α signal pathway. But there is no direct evidence of RNA protein interaction. Further exploration is needed. Meanwhile, we need to increase the evidence of animal experiments, and seek the accurate target and dose-response regulation.

Declarations

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Conflicts of interest

There are no contradiction and conflict among all authors.

Author Contributions

Fen Liu, Ying Cao and Jie-Ying Wang for technical contributions related to the clinical experiment and cell models assays; Dr. Xue-Mei Wang and Xiao-Ming Gao for conceived and designed the experiments; and Yuan Xing for and statistical analysis.

References

1. HU Shengshou GR, LIU Lisheng, ZHU Manlu, WANG Wen, WANG Yongjun, WU Zhaosu, LI Huijun, GU Dongfeng, YANG Yuejin, ZHENG Zhe, CHEN Weiwei: **Summary of the 2018 Report on Cardiovascular Diseases in China** *Chinese Circulation Journal* 2019, **34**:209-219.
2. L P, Z Y, Y W, RX Y, Y L, J W, B G, L Z: **The prevalence, awareness, treatment and control of dyslipidemia among adults in China.** *Atherosclerosis* 2016, **248**:2-9.
3. CL S, D M, H C, HA S, A ZAH, DM H, TM H: **Association of Heart Rate Variability With Cognitive Performance: The Multi-Ethnic Study of Atherosclerosis.** *Journal of the American Heart Association* 2020, **9**:e013827.
4. DP L, PG J, M M, SS A, KK T, JD S, S Y: **Reducing the Global Burden of Cardiovascular Disease, Part 2: Prevention and Treatment of Cardiovascular Disease.** *Circulation research* 2017, **121**:695-710.
5. H L, Y X, L T, F Z, G H, JM X, AM X, RP D, ZG Z: **Adipocyte Fatty Acid-Binding Protein Promotes Palmitate-Induced Mitochondrial Dysfunction and Apoptosis in Macrophages.** *Frontiers in immunology* 2018, **9**:81.
6. W K, K W, P P, JC S, AC C, D S, AW G: **Impairment of Macrophage Cholesterol Efflux by Cholesterol Hydroperoxide Trafficking: Implications for Atherogenesis Under Oxidative Stress.** *Arteriosclerosis, thrombosis, and vascular biology* 2015, **35**:2104-2113.
7. G W, JH G, LH H, XH Y, ZW Z, J Z, FJ W, L Z, XJ W, CK T: **Fargesin alleviates atherosclerosis by promoting reverse cholesterol transport and reducing inflammatory response.** *Biochimica et biophysica acta Molecular and cell biology of lipids* 2020, **1865**:158633.
8. XW H, D Y, WL L, Z Z, CL L, C L, P L, CQ X, XF H, XP J: **Anti-atherosclerotic potential of baicalin mediated by promoting cholesterol efflux from macrophages via the PPAR γ -LXR α -ABCA1/ABCG1 pathway.** *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 2016, **83**:257-264.
9. C T, BA H, C S, RC L: **Both STAT3 activation and cholesterol efflux contribute to the anti-inflammatory effect of apoA-I/ABCA1 interaction in macrophages.** *Journal of lipid research* 2016, **57**:848-857.
10. N H, I D, C P, J V, E W, V T, S C, E V, S L, P L, B S: **The novel selective PPAR α modulator (SPPARM α) pemafibrate improves dyslipidemia, enhances reverse cholesterol transport and decreases inflammation and atherosclerosis.** *Atherosclerosis* 2016, **249**:200-208.
11. Y Z, C W, Y J, Q Y, Q M, Q L, Y D, L C, Z L, K L, H S: **Activating the PGC-1/TERT Pathway by Catalpol Ameliorates Atherosclerosis via Modulating ROS Production, DNA Damage, and Telomere Function: Implications on Mitochondria and Telomere Link.** *Oxidative medicine and cellular longevity* 2018, **2018**:2876350.
12. G U, DA H: **The importance of PGC-1 α in contractile activity-induced mitochondrial adaptations.** *American journal of physiology Endocrinology and metabolism* 2011, **300**:E361-371.
13. AO K, DS C, K A-A, DD G: **Role of PGC-1 α in Vascular Regulation: Implications for Atherosclerosis.** *Arteriosclerosis, thrombosis, and vascular biology* 2016, **36**:1467-1474.
14. J L, C T, X C, Z R, H Q, R C, Z T: **Impacts of Aerobic Exercise on Depression-Like Behaviors in Chronic Unpredictable Mild Stress Mice and Related Factors in the AMPK/PGC-1 α Pathway.** *International journal of environmental research and public health* 2020, **17**.

15. Y W, GZ W, PS R, I T: **Macrophage mitochondrial oxidative stress promotes atherosclerosis and nuclear factor- κ B-mediated inflammation in macrophages.** *Circulation research* 2014, **114**:421-433.
16. L T, AK T, J B, M M, G F: **The nuclear factor kappa B signaling pathway: integrating metabolism with inflammation.** *Trends in cell biology* 2012, **22**:557-566.
17. M O, H L, C I, M P, D M, M G, V S, K S, X Z, M C, et al: **H19 Noncoding RNA, an Independent Prognostic Factor, Regulates Essential Rb-E2F and CDK8- β -Catenin Signaling in Colorectal Cancer.** *EBioMedicine* 2016, **13**:113-124.
18. XM W, XM L, N S, H Z, XM G, YN Y: **Long non-coding RNAs H19, MALAT1 and MIAT as potential novel biomarkers for diagnosis of acute myocardial infarction.** *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 2019, **118**:109208.
19. JX P: **LncRNA H19 promotes atherosclerosis by regulating MAPK and NF- κ B signaling pathway.** *European review for medical and pharmacological sciences* 2017, **21**:322-328.
20. H Z, XM L, F L, BD C, H Z, XM W, W L, QJ C, YT M, YN Y: **Expression pattern of genome-scale long noncoding RNA following acute myocardial infarction in Chinese Uyghur patients.** *Oncotarget* 2017, **8**:31449-31464.
21. D B, P T: **An improved colour reagent for the determination of blood glucose by the oxidase system.** *The Analyst* 1972, **97**:142-145.
22. P T: **Oxidase determination of plasma cholesterol as cholest-4-en-3-one using iso-octane extraction.** *Annals of clinical biochemistry* 1981:64-70.
23. T Z, F AR, V J, C K: **Atherosclerosis and the Capillary Network; Pathophysiology and Potential Therapeutic Strategies.** *Cells* 2019, **9**.
24. M J, T Z, DR T, J L, Y S, X L, T P, Y Y, MB B, L J, et al: **Dietary fenofibrate attenuated high-fat-diet-induced lipid accumulation and inflammation response partly through regulation of ppara and sirt1 in juvenile black seabream (*Acanthopagrus schlegelii*).** *Developmental and comparative immunology* 2020:103691.
25. R G, S P, S K: **Metformin treatment prevents SREBP2-mediated cholesterol uptake and improves lipid homeostasis during oxidative stress-induced atherosclerosis.** *Free radical biology & medicine* 2018, **118**:85-97.
26. A P, I C, L B, S R, C V, V P, M G, M C, J B, I V, et al: **Insulin resistance is associated with preclinical carotid atherosclerosis in patients with type 1 diabetes.** *Diabetes/metabolism research and reviews* 2020:e3323.
27. AN O, AV P, IA S, NN N, EA I: **Mitochondrion as a selective target for treatment of atherosclerosis: Role of mitochondrial DNA mutations and defective mitophagy in the pathogenesis of atherosclerosis and chronic inflammation.** *Current neuropharmacology* 2019.
28. TE R, CA S, TD G, EE S, PD N, JM M: **Targeted Expression of Catalase to Mitochondria Protects Against Ischemic Myopathy in High-Fat Diet-Fed Mice.** *Diabetes* 2016, **65**:2553-2568.
29. Z P, LF Z, YJ C, YM C, CW J, ZZ M, P X, HY L, DY W, XB C: **The Protective Effects of Salidroside from Exhaustive Exercise-Induced Heart Injury by Enhancing the PGC-1 α -NRF1/NRF2 Pathway and**

- Mitochondrial Respiratory Function in Rats.** *Oxidative medicine and cellular longevity* 2015, **2015**:876825.
30. D K, AB T, MA N, L R, M G, R S, E R, P S, M O, JP P, et al: **Macrophage Mitochondrial Energy Status Regulates Cholesterol Efflux and Is Enhanced by Anti-miR33 in Atherosclerosis.** *Circulation research* 2015, **117**:266-278.
 31. Y W, W W, N W, AR T, I T: **Mitochondrial Oxidative Stress Promotes Atherosclerosis and Neutrophil Extracellular Traps in Aged Mice.** *Arteriosclerosis, thrombosis, and vascular biology* 2017, **37**:e99-e107.
 32. C S, G B, M F, N DM, K P, M S, C N: **Epigenetic-sensitive pathways in personalized therapy of major cardiovascular diseases.** *Pharmacology & therapeutics* 2020:107514.
 33. D K, A B: **Targeting Peroxisome Proliferator Activated Receptor α (PPAR α) for the Prevention of Mitochondrial Impairment and Hypertrophy in Cardiomyocytes.** *Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology* 2018, **49**:245-259.
 34. JL P, JW W, PY H, JS J, C Y: **HOTAIR alleviates ox-LDL-induced inflammatory response in Raw264.7 cells via inhibiting NF- κ B pathway.** *European review for medical and pharmacological sciences* 2018, **22**:6991-6998.
 35. D N, S L, TJ L, RC M, K E, N F, G G, B B: **Inhibition of macrophage fatty acid β -oxidation exacerbates palmitate-induced inflammatory and endoplasmic reticulum stress responses.** *Diabetologia* 2014, **57**:1067-1077.
 36. X G, Y S, L F, HY Z, H Y, P H, XH G, YW S, M Y: **Polymorphic variation of inflammation-related genes and risk of non-Hodgkin lymphoma for Uygur and Han Chinese in Xinjiang.** *Asian Pacific journal of cancer prevention : APJCP* 2014, **15**:9177-9183.
 37. X W, Q C, H P, Q W, M D, C Z, T J, X S, J Z, Y Y: **Adiponectin improves NF- κ B-mediated inflammation and abates atherosclerosis progression in apolipoprotein E-deficient mice.** *Lipids in health and disease* 2016, **15**:33.
 38. KN M, JP C, SA M, PR H, MS B, SA H, NB J, TW R, DM P, KW E, et al: **PGC-1 α integrates a metabolism and growth network linked to caloric restriction.** *Aging cell* 2019, **18**:e12999.
 39. A C-B, R L-B, RJ V-B, A G-G, MJ C, S L-M, S P-Z, MR M-V, AM R-V, C L, et al: **Cisplatin resistance involves a metabolic reprogramming through ROS and PGC-1 α in NSCLC which can be overcome by OXPHOS inhibition.** *Free radical biology & medicine* 2019, **135**:167-181.
 40. M A, GA K: **Diabetic animal fed with high-fat diet prevents the protective effect of myocardial ischemic preconditioning effect in isolated rat heart perfusion model.** *Journal of biochemical and molecular toxicology* 2020, **34**:e22457.

Figures

Figure 1

Expression of lncRNA H19 (A) circulating levels of lncRNA H19 in patients with AMI and non-AMI participants (n=90). (B) lncRNA H19 expression in the THP-1 derived macrophages model of H₂O₂, LPS and ox-LDL respectively. Data are expressed as mean $\pm$ SD at least three separate experiments. *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 2

H19 repression attenuates ox-LDL-induced lipid accumulation in macrophage foam cells (A) THP-1 derived macrophages were pretreated with H19 siRNA or NC siRNA for 48 h, and then stimulated with 50 $\mu\text{g/mL}$ ox-LDL for 48 h. After incubation, the extent of lipid loading was assessed by Oil Red O staining. The cells were observed with bright-field microscopy. The magnification of each panel is $\times 400$. (B) The lipid content in THP-1 derived macrophages after treating with or without H19 siRNA. Values are the means $\pm$ SD from at least three separate experiments. * $P < 0.05$ vs the control was treated with vehicle (zero ox-LDL and H19 siRNA).

Figure 3

H19 repression increase ATP contents mitochondrial function and reduced oxidative stress in vitro. A. comparison of the ATP level of cells in different groups. B: activity of superoxide dismutase. C: serum concentration of malondialdehyde. D. MCP-1 concentration of culture supernatant of macrophages. Data are expressed as mean $\pm$ SD at least three separate experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 4

Stimulatory effects of ox-LDL on ABCA1 expression and lipid accumulation were mediated by H19 repression. A. the ABCA1 and PPARα protein expression of the THP-1 derived macrophages in different groups. B: protein expression of ABCA1. C: protein expression of PPARα. D. mRNA expression of ABCA1 and PPARα. Data are expressed as mean $\pm$ SD at least three separate experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 5

H19 repression inhibits lipid accumulation by downregulating NF-κB and upregulating PGC-1α signaling pathway. A. the PGC-1α, NF-κB p-p65 and NF-κB p50 protein expression of the THP-1 derived macrophages in different groups. B: protein expression of PGC-1α. C: protein expression of NF-κB p-p65. D. protein expression of and NF-κB p50. Data are expressed as mean ± SD at least three separate experiments. *P < 0.05, **P < 0.01, ***P < 0.001.