MicroRNA-30a-5p-Mediated Autophagy Regulates Formation of Foam Cells from THP-1-derived Macrophages

Chunhui Geng
First Affiliated Hospital of Harbin Medical University
Chao Wang
First Affiliated Hospital of Harbin Medical University
Guangming Su
First Affiliated Hospital of Harbin Medical University
Shengjiao Wang
First Affiliated Hospital of Harbin Medical University
Jiashan Li
First Affiliated Hospital of Harbin Medical University
Dan Liu
First Affiliated Hospital of Harbin Medical University
Xiuru Guan
First Affiliated Hospital of Harbin Medical University

Research Article

Keywords: miR-30a-5p, autophagy, foam cells, atherosclerosis, biomarker

DOI: https://doi.org/10.21203/rs.3.rs-267439/v1

Abstract

MicroRNAs are widely considered to be involved in the pathogenesis of atherosclerosis. Whereas the importance of miR-30a-5p as a tumor growth-promoting factor has been extensively studied, the relationship between this particular microRNA and atherosclerosis remains to be clarified. In this study, the role of miR-30a-5p in the formation of foam cells from THP-1-derived macrophages was investigated. It was found that miR-30a-5p could robustly regulate the pathological process of atherosclerosis by inhibiting autophagy and increasing the accumulation of lipids, the expression of inflammatory factors, and the apoptosis of macrophages. These results provide guidance for future assessments of the progression of atherosclerosis and for the development of intervention targets for the treatment of this disease.

1 Introduction

Atherosclerosis (AS) is a chronic inflammatory disease with a complex pathological mechanism. The mortality rate due to AS-caused cardiovascular disease has been increasing worldwide [1–3]. The main pathological features of AS are a large amount of lipid deposition under the intima, intimal fibrosis, and plaque formation. Enormous damage can be caused by the unstable plaques, which result in arterial thrombosis when broken [4]. Studies have shown that autophagic bodies are present in unstable plaques [5], and that microRNAs (miRNAs) affect the development of AS by targeting the proteins and affecting the pathways related to autophagy [6–10].

With the development of precision medicine, the early and accurate diagnosis of diseases would be of great significance for clinical tests. In recent years, miRNAs have been widely studied as biomarkers for the early diagnosis of diseases, for the following reasons: 1) miRNAs remain stable in peripheral blood. Cells secrete miRNAs through exosomes and extracellular vesicles. The miRNAs secreted remain stable in body fluids and can bind to protein complexes to avoid degradation [11]; 2) compared with some existing protein biomarkers (e.g., serum myoglobin, creatine kinase, and troponin), miRNAs exhibit high sensitivity and specificity during clinical tests. It has been reported that the miRNA miR-208A/B has higher sensitivity and specificity than troponin in the diagnosis of acute myocardial infarction [12]. One study suggested that the joint use of miR-150, miR-132, and miR-186 served as a better diagnostic biomarker of cardiovascular disease than four classical biomarkers (B-type natriuretic peptide, hypersensitive troponin I, C-reactive protein, and cysteine protease inhibitor C) in clinical tests [13]; and 3) miRNAs are considered to be a noninvasive means for the early diagnosis of tumors and cardiovascular diseases. The expression levels of miRNAs reflect the origin of specific types of tumors and even cell subpopulations, which means that miRNAs can be applied directly to clinical diagnosis on the basis of tumor classification [14].

It has been widely confirmed that miR-30a-5p plays a significant role in the proliferation and invasion of tumors [15, 16]. However, the relationship between miR-30a-5p and AS has not been clarified. Through bioinformatic analysis with miRbase (http://www/mirbase.org/) and some prophase experiments [17–

19], we had previously found that the expression of miR-30a-5p was significantly reduced in foam cells that had been formed through induction under increasing concentrations of oxidized low-density lipoprotein (ox-LDL). Furthermore, through a meta-analysis with miRTarBase (http://mirtarbase.mbc.nctu.edu.tw/), we showed that miR-30a-5p potentially regulates autophagy-related pathways in AS by regulating Beclin-1 [20, 21], which is a key autophagy regulator in mammals that forms an autophagy initiation complex with phosphatidylinositol 3-kinase class III (PtdIns3KC3 or VPS34) and ubiquitin-binding serine/threonine protein kinase (VPS15) [22]. Given these findings above, we surmised that miR-30a-5p-mediated autophagy regulates the formation of foam cells from THP-1-derived macrophages.

In this study, we carried out a series of cell and molecular experiments to investigate the regulatory effects of miR-30a-5p on AS. The results suggest that miR-30a-5p overexpression suppresses autophagy in foam cells by inhibiting Beclin-1 protein expression. We also found that miR-30a-5p affected the stability of AS plaques by increasing both the atherogenic inflammatory factor interleukin-18 (IL-18) and intracellular lipid droplet accumulation. In conclusion, miR-30a-5p plays an important role in the pathogenesis of AS and is suggested to be both a candidate diagnostic biomarker and a potential treatment target for this disease.

2 Materials And Methods

Cell Culture

The human monocytic cell line THP-1 was purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The THP-1 cells were maintained in RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum (both from Biological Industries, Shanghai, China) at 37°C under 5% CO_2. For the experiments, THP-1 cells were plated in 6-well culture plates at 1×10^6 cells/well and differentiated into macrophages through induction with 100 ng/mL phorbol-12- myristate-13-acetate (Merck KGaA; Sigma-Aldrich, St. Louis, MO, USA) for 24 h. Finally, ox-LDL (Guangzhou Yiyuan Biological Technology Co., Guangzhou, China) was added to a concentration of 75 μg/mL to induce foam cell formation. To explore the effect of ox-LDL on miR-30a-5p during foam cell formation, THP-1-derived macrophages were induced for 48 h with various concentrations of ox-LDL (0, 50, and 100 μg/mL, respectively). The expression of miR-30a-5p in the foam cells was then detected by qPCR and the obtained cycle threshold values were analyzed with the $2^{-\triangle\triangle Ct}$ method.

miR-30a-5p Transfection

Four groups of cells transfected with different miRNAs (i.e., miR-30a-5p mimics, miR-30a-5p inhibitor, and their respective negative controls (NCs)) were established, using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The sequences for the miRNAs were as follows: hsa-miR-30a-5p mimics: sense 5′-UGUAAACAUCCUCGACUGGAAG-3′ and antisense 5′-UCCAGUCGAGGAUGUUUACAUU-3′; mimics-NC: sense 5′-UUCUCCGAACGUGUCACGUTT-3′ and antisense 5′-ACGUGACACGUUCGGAGAATT-3′; has-miR-

30a-5p inhibitor: 5′-CUUCCAGUCGAGGAUGUUUACA-3′; and inhibitor-NC: 5′-CAGUACUUUUGUGUAGUACAA-3′ (GenePharma Co., Ltd, Shanghai, China). The prepared transfection complex was added to the cell-containing culture plates to a final concentration of 50 nM. After transfection for 8–12 h at 37°C in a 5% CO_2 incubator, the medium was removed.

Reverse Transcription and Real-Time Polymerase Chain Reaction

Total RNA was extracted from the cells using the miRcute miRNA Isolation Kit (Tiangen Biotech Co., Ltd, Beijing, China). Reverse transcription of the RNA was conducted by using the miRcute Plus miRNA First-Strand cDNA Kit (Tiangen Biotech Co., Ltd). The RNA expression levels were detected with the real-time polymerase chain reaction (qPCR), using the SYBR miRcute Plus miRNA qPCR Kit (Tiangen Biotech Co., Ltd). The PCR conditions were as follows: 95°C for 15 min; and 40 cycles of denaturation at 94°C for 20 s and annealing/elongation at 60°C for 34 s. The primer sequences for miR-30a-5p were as follows: forward 5′-GGGGTGTAAACATCCTCGACTG-3′ and reverse 5′-ATTGCGTGTCGT GGAGTCG-3′. The primer sequences for U6 were as follows: forward 5′-GCTTCGGCAGCACATATACTAAAAT-3′ and reverse 5′-CGCTTCACGAATTTGCGTGTCAT-3′ (GenePharma Co., Ltd). All expressed miRNA data are relative to the expression of a U6 small nuclear RNA from the same sample. Independent experiments were repeated three times. The relative mRNA expression levels were analyzed using the $2^{-\Delta\Delta Ct}$ method.

Protein Extraction and Western Blot Analysis

Total protein was extracted from the cells using radioimmunoprecipitation assay lysis buffer containing the protease inhibitor phenylmethylsulfonyl fluoride (Beyotime, Shanghai, China). A bicinchoninic acid assay kit (Solarbio, Beijing, China) was used to measure the protein concentration. The total protein samples (40 μg) were separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis and the protein bands were then transferred onto polyvinylidene difluoride membranes. Then, the membranes were blocked with 5% bovine serum albumin for 1 h, after which the target bands were incubated with the primary antibodies at 4°C overnight. This was followed by an incubation with the alkaline phosphatase- or horseradish peroxidase-conjugated secondary antibody for 1 h. Finally, Tanon GIS software was used to analyze the target protein content in the membrane strip. The dilution ratios for the various antibodies were as follows: anti-Beclin-1 antibody (1:3000) (Abcam, Cambridge, MA, USA), anti-sequestosome-1 antibody (SQSTM1, also p62; 1:1000) (Abcam, Cambridge, MA, USA), anti-microtubule-associated protein 1 light chain 3 beta antibody (LC3B; 1:3000) (Abcam, Cambridge, MA, USA), anti-β-actin antibody (1:5000) (Abcam, Cambridge, MA, USA), anti-caspase-3 antibody (1:5000) (Abcam, Cambridge, MA, USA), goat anti-mouse secondary antibody (1:10,000) (Abcam, Cambridge, MA, USA), and goat anti-rabbit secondary antibody (1:50,000) (Abcam, Cambridge, MA, USA).

Oil Red O Staining

The cells in each well of a 6-well plate were washed three times with phosphate-buffered saline (PBS) and then fixed with 4% paraformaldehyde for 5–10 min. The, 2 mL of Oil Red O solution was added to each well and the plate was incubated at 37°C for 15 min. The cells were washed three times with PBS,

differentiated through the addition of 60% isopropanol for 30 s, and then washed again. The PBS in the plate was poured out, and 100% isopropanol was added for dye extraction. After 10 min, the optical density of each well was measured at 490 nm

Enzyme-linked Immunosorbent Assay Analysis

To detect the IL-18 content in the cell culture medium, an enzyme-linked immunosorbent assay kit (Elabscience Biotechnology Co., Ltd, Wuhan, China) was used according to the manufacturer's instructions.

Cell death assay

The lactate dehydrogenase (LDH)-Cytotoxicity Kit (Abcam, Cambridge, MA, USA) was used to detect cell death. LDH, a stable enzyme that is expressed in all cells, is released rapidly into cell culture fluids upon cell membrane damage. Therefore, the LDH kit is often used to assess the effect of toxic substances on the viability of cells, primarily by revealing the number of viable cells present. Approximately 10,000 THP-1 cells were plated into each well of 96-well plates. After treatment with ox-LDL and the miRNA transfection complex, 100 μL of LDH Reaction Mix reagent was added to each well and the cells were cultured for 1 h at 37°C. A LUmo microplate reader (PHOMO; Autobio Diagnostics Co., Ltd., Shanghai, China) was used to detect the optical density at 490 nm in each well.

Statistical analysis

All the data were analyzed using GraphPad Prism 8.0 software (GraphPad Software, Inc., La Jolla, CA, USA). The analysis of significant differences between two groups was performed using the paired *T* test. Three groups or more were compared by analysis of variance. The results of the statistical analyses are presented as the mean value ± standard deviation, and a *P* value of less than 0.05 was considered to be statistically significant.

3 Results And Discussion

The miR-30a-5p Levels Are Decreased in ox-LDL-treated Macrophages

The levels of miR-30a-5p expression in the 50 and 100 μg/mL ox-LDL groups were lower than that in the 0 μg/mL ox-LDL group (Fig. 1A). The miR-30a-5p expression level in the 100 μg/mL ox-LDL group was significantly lower than that in the 50 μg/mL ox-LDL group (Fig. 1A). These results indicated that the level of miR-30a-5p expression was negatively correlated with the formation of foam cells.

miR-30a-5p Inhibits Autophagy by Regulating the Expression of the Autophagy-related Protein Beclin-1

First, after transfecting the foam cells with the miR-30a-5p mimics or miR-30a-5p inhibitor or their respective NCs, the success of transfection was verified by qPCR (Fig. 1B). Second, the expression of autophagy-related proteins (including p62 and LC3B) was determined by western blot analysis. The

results showed that the expression of p62 was significantly increased in the miR-30a-5p mimics group compared with that in the mimic-NC group (Fig. 2B), whereas the LC3B expression level was significantly reduced (Fig. 2C). In the miR-30a-5p inhibitor group, p62 was significantly decreased compared with its level in the inhibitor-NC group (Fig. 2B), whereas the LC3II/LC3I levels were significantly increased (Fig. 2C). Next, we examined the level of Beclin-1 expression in the various groups. As shown in Fig. 3, compared with the level in the control group, the level was significantly lower in the miR-30a-5p mimics group (Fig. 2D), but significantly higher in the miR-30a-5p inhibitor group (Fig. 2D). These results suggested that miR-30a-5p reduced the expression of autophagy in macrophages by regulating the expression of Beclin-1.

miR-30a-5p Overexpression Increases Lipid Accumulation in Foam Cells and Induces the Expression of the Atherogenic Inflammatory Cytokine IL-18

To further explore the relationship between miR-30a-5p and foam cells, macrophages were treated with 75 μg/mL ox-LDL for 48 h after their transfection with the miR-30a-5p mimics or inhibitor for 12 h. Oil Red O staining was then used to observe the accumulation of lipids in the cells, and the enzyme-linked immunosorbent assay was applied to detect the IL-18 levels. The results indicated that miR-30a-5p could increase both lipid accumulation in the macrophage-derived foam cells (Fig. 3A, B) and IL-18 expression at the protein level (Fig. 3C).

miR-30a-5p Overexpression Aggravates Macrophage Apoptosis

To investigate the effect of miR-30a-5p on AS plaque stability, an LDH assay kit was used to detect macrophage apoptosis after the overexpression or inhibition of miR-30a-5p in the cells (Fig. 4A). The expression of the apoptotic-related protein caspase-3 was detected by western blot assay (Fig. 4B). Compared with the rate of apoptosis in the respective control groups, the rate was significantly higher in the miR-30a-5p mimics group (Fig. 4C) but significantly lower in the miR-30a-5p inhibitor group (Fig. 4C). These results indicated that miR-30a-5p had effectively induced macrophage apoptosis.

4 Conclusion

miRNAs have been shown to play an essential role in the regulation of autophagy in AS [23], which is consistent with our results. As a complex process, basal autophagy can suppress the development of AS in the early disease stage [24]. In 2017, Ma et al. found that rapamycin promoted the activation of autophagy by enhancing the expression of miR-155, which delayed the development of atherosclerotic plaques [25]. Another study suggested that miR-100 could suppress the expression of endothelial adhesion molecules by regulating rapamycin complex 1 signaling, which resulted in the stimulation of endothelial autophagy and attenuation of nuclear factor-kappa B signaling both in vitro and in vivo [26]. Our results indicate that miR-30a-5p acts as an inhibitor of autophagy in macrophages. We observed that under the high ox-LDL concentrations needed for foam cell induction, miR-30a-5p overexpression via the use of mimics could attenuate the autophagy of macrophages (Fig. 2). It has been shown that autophagy could be tightly regulated by more than 30 highly conserved *ATG* genes [27], and indeed, our results

supplemented the evidence for the influence of miR-30a-5p on the Beclin-1 (ATG6) and autophagy pathways [28–30].

In addition to their mediation of the expression of autophagy, our study also emphasized the regulatory effect of miRNAs on the levels of inflammatory factors and their interference with lipid formation [31]. In 2013, Wei et al. found that macrophage-derived miR-342-5p could promote the development of AS and enhance the inflammatory stimulation of macrophages by suppressing the Akt1-mediated inhibition of miR-155 expression [32]. Further studies have shown that the blockage of autophagy could lead to lipid accumulation to promote the release of inflammatory factors. First, autophagy of the THP-1-derived macrophages is inhibited by ox-LDL in a concentration-dependent manner [33, 34]. Owing the inhibition of autophagy, the autophagosomes and lysosomes cannot be fused, and thereby autophagy is blocked. Second, lipids continue to be accumulated, leading to the development of AS [35]. Eventually, the release of inflammatory bodies known as inflammasomes, which are complexes of innate immune cells, will aggravate the rupture of atherosclerotic plaques [36]. The nucleotide-binding oligomerization domain (NOD)-like receptor protein 3 (NLRP3) inflammasomes are thought to be a bridge between lipid metabolism and inflammation [37]. They can be activated by cholesterol crystals and ox-LDL, whereupon IL-18 is secreted [38, 39]. In this study, miR-30a-5p was confirmed to have accelerated the development of AS through the foam cell accumulation of lipids and secretion of the inflammatory factor IL-18 and was probably involved in the generation of inflammasomes during AS pathogenesis (Fig. 3C).

In addition, macrophage apoptosis is an important feature of AS plaque development and can be induced by a variety of factors, such as oxidative stress, high concentrations of cytokines, and endoplasmic reticulum stress [40]. In our study, the regulatory mechanism of miR-30a-5p was confirmed to include that of macrophage apoptosis. Previous studies have shown that macrophage apoptosis in the early stages of AS was beneficial, as it limited the composition of diseased cells and inhibited the development of plaques. However, the effects of macrophage apoptosis on late lesions are extremely complicated [41]. Studies have reported that the failure to clear apoptotic cells could aggravate AS by inducing inflammation and expanding the necrotic core of the plaque [42]. Recent studies have observed the participation of miRNAs in AS progression through their regulation of macrophage apoptosis. In 2017, Canfran-Duque et al. found that miR-21 expression influenced the formation of foam cells, the sensitivity to endoplasmic reticulum stress-induced apoptosis, and the capacity for phagocytic clearance [43]. The absence of miR-21 resulted in accelerated AS, plaque necrosis, and vascular inflammation [43]. In the $ApoE^{-/-}$ mouse model, researchers observed that antagomiR-10b administration reduced the advanced plaque size and also enhanced plaque stability, which were considered to be associated with the increased plaque macrophage expression of ATP-binding cassette subfamilyA member 1 (ABCA1) and reduced plaque apoptosis and inflammation [44]. As the leading cause of cardiovascular diseases, AS urgently needs to be effectively treated. Interestingly, we found that miR-30a-5p significantly accelerated the apoptosis of macrophages, which also had an effect on the instability of the plaques (Fig. 5). Therefore, the antagonism of miR-30a-5p holds promise as an effective treatment intervention for AS.

Looking ahead, we will be carrying out the following studies to supplement our current findings. First. First, related in vivo experiments need to be carried out to further confirm our results in the vascular wall environment. Second, as noncoding RNAs, including long noncoding RNAs (lncRNAs), circular RNAs (circRNAs), and miRNAs, have been reported to participate in the pathological development of cardiovascular diseases through various mechanisms [45], it would be necessary for us to elucidate the miR-30a-5p-related lncRNA/circRNA–miRNA–mRNA axis in AS.

In conclusion, in this innovative investigation of the role of miR-30a-5p on foam cell formation from THP-1-derived macrophages, we found that the miRNA could robustly regulate the pathological processes of AS by inhibiting autophagy, and increasing the accumulation of lipids, the expression of inflammatory factors, and the apoptosis of macrophages (Fig. 5). These results should provide guidance for future assessments of AS and of intervention targets for its treatment.

Declarations

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Chunhui Geng, Chao Wang and Guangming Su conceived and designed the experiments. Chunhui Geng and Jiashan Li analyzed the data. Chunhui Geng wrote the manuscript. Xiuru Guan was responsible for the planning and guidance of the project. All authors read and approved the final manuscript.

Funding

The research was supported by the National Natural Science Foundation of China (81672084).

Acknowledgments

The authors thank editage(http://www.editage.cn/) for the improvement of this manuscript.

References

1. Gistera A, Hansson GK (2017) The immunology of atherosclerosis. Nat Rev Nephrol 13(6):368–380
2. Moriya J (2019) Critical roles of inflammation in atherosclerosis. J Cardiol 73(1):22–27
3. Zhang PY, Xu X, Li XC (2014) Cardiovascular diseases: oxidative damage and antioxidant protection. Eur Rev Med Pharmacol Sci 18(20):3091–3096
4. Tabas I, Garcia-Cardena G, Owens GK (2015) Recent insights into the cellular biology of atherosclerosis. J Cell Biol 209(1):13–22
5. Liu H et al (2015) Autophagy in atherosclerosis: a phenomenon found in human carotid atherosclerotic plaques. Chin Med J (Engl) 128(1):69–74
6. Wu W et al (2019) Overexpression of miR-223 inhibits foam cell formation by inducing autophagy in vascular smooth muscle cells. Am J Transl Res 11(7):4326–4336
7. Samidurai A, Kukreja RC, Das A (2018) Emerging Role of mTOR Signaling-Related miRNAs in Cardiovascular Diseases. Oxid Med Cell Longev 2018:6141902
8. Tang F, Yang TL (2018) MicroRNA-126 alleviates endothelial cells injury in atherosclerosis by restoring autophagic flux via inhibiting of PI3K/Akt/mTOR pathway. Biochem Biophys Res Commun 495(1):1482–1489
9. Feng S et al (2019) MiR-93 regulates vascular smooth muscle cell proliferation, and neointimal formation through targeting Mfn2. Int J Biol Sci 15(12):2615–2626
10. Zhang X et al., *MicroRNA-217-5p ameliorates endothelial cell apoptosis induced by ox-LDL by targeting CLIC4.* Nutr Metab Cardiovasc Dis, 2019
11. van de Vrie M et al (2017) Urinary MicroRNA as Biomarker in Renal Transplantation. Am J Transplant 17(5):1160–1166
12. Navickas R et al (2016) Identifying circulating microRNAs as biomarkers of cardiovascular disease: a systematic review. Cardiovasc Res 111(4):322–337
13. Paiva S, Agbulut O (2017) MiRroring the Multiple Potentials of MicroRNAs in Acute Myocardial Infarction. Front Cardiovasc Med 4:73
14. Lin J et al (2019) Tumour biomarkers-Tracing the molecular function and clinical implication. Cell Prolif 52(3):e12589
15. Zeng RC et al (2013) Down-regulation of miRNA-30a in human plasma is a novel marker for breast cancer. Med Oncol 30(1):477
16. Liang LB et al (2019) Plasma miR-30a-5p as an early novel noninvasive diagnostic and prognostic biomarker for lung cancer. Future Oncol 15(32):3711–3721
17. Fu XT et al (2018) MicroRNA-30a suppresses autophagy-mediated anoikis resistance and metastasis in hepatocellular carcinoma. Cancer Lett 412:108–117
18. Liu S et al., *Downregulation of miRNA-30a enhanced autophagy in osthole-alleviated myocardium ischemia/reperfusion injury*. J Cell Physiol, 2019

19. Bi R et al (2019) Endothelial cell autophagy in chronic intermittent hypoxia is impaired by miRNA-30a-mediated translational control of Beclin-1. J Cell Biochem 120(3):4214–4224
20. Zheng B et al (2015) MiRNA-30a-mediated autophagy inhibition sensitizes renal cell carcinoma cells to sorafenib. Biochem Biophys Res Commun 459(2):234–239
21. Xu K et al (2013) Reduced apoptosis correlates with enhanced autophagy in synovial tissues of rheumatoid arthritis. Inflamm Res 62(2):229–237
22. Zhao Y et al (2015) RACK1 Promotes Autophagy by Enhancing the Atg14L-Beclin 1-Vps34-Vps15 Complex Formation upon Phosphorylation by AMPK. Cell Rep 13(7):1407–1417
23. Zhao Y et al (2019) MicroRNAs play an essential role in autophagy regulation in various disease phenotypes. Biofactors 45(6):844–856
24. De Meyer GR et al (2015) Autophagy in vascular disease. Circ Res 116(3):468–479
25. Ma J et al (2017) Expression of miRNA-155 in carotid atherosclerotic plaques of apolipoprotein E knockout (ApoE(-/-)) mice and the interventional effect of rapamycin. Int Immunopharmacol 46:70–74
26. Pankratz F et al (2018) MicroRNA-100 Suppresses Chronic Vascular Inflammation by Stimulation of Endothelial Autophagy. Circ Res 122(3):417–432
27. Grootaert MOJ et al (2018) Vascular smooth muscle cell death, autophagy and senescence in atherosclerosis. Cardiovasc Res 114(4):622–634
28. Yang X et al (2017) Intensified Beclin-1 Mediated by Low Expression of Mir-30a-5p Promotes Chemoresistance in Human Small Cell Lung Cancer. Cell Physiol Biochem 43(3):1126–1139
29. Behura A et al (2019) ESAT-6 modulates Calcimycin-induced autophagy through microRNA-30a in mycobacteria infected macrophages. J Infect 79(2):139–152
30. Kumar S et al., *Merkel cell polyomavirus oncoproteins induce microRNAs that suppress multiple autophagy genes*. Int J Cancer, 2019
31. Martens CR, Bansal SS, Accornero F (2019) Cardiovascular inflammation: RNA takes the lead. J Mol Cell Cardiol 129:247–256
32. Wei Y et al (2013) The microRNA-342-5p fosters inflammatory macrophage activation through an Akt1- and microRNA-155-dependent pathway during atherosclerosis. Circulation 127(15):1609–1619
33. Liang X et al., *p62/mTOR/LXRalpha pathway inhibits cholesterol efflux mediated by ABCA1 and ABCG1 during autophagy blockage.* Biochem Biophys Res Commun, 2019. **514**(4): p. 1093–1100
34. Ma Y et al (2018) A novel antioxidant Mito-Tempol inhibits ox-LDL-induced foam cell formation through restoration of autophagy flux. Free Radic Biol Med 129:463–472
35. Torisu K et al (2016) Intact endothelial autophagy is required to maintain vascular lipid homeostasis. Aging Cell 15(1):187–191
36. Raggi P et al (2018) Role of inflammation in the pathogenesis of atherosclerosis and therapeutic interventions. Atherosclerosis 276:98–108

37. Zhang X et al (2020) Development of small molecule inhibitors targeting NLRP3 inflammasome pathway for inflammatory diseases. Eur J Med Chem 185:111822
38. Hoseini Z et al (2018) NLRP3 inflammasome: Its regulation and involvement in atherosclerosis. J Cell Physiol 233(3):2116–2132
39. Stinson EJ, Krakoff J, Gluck ME (2018) Depressive symptoms and poorer performance on the Stroop Task are associated with weight gain. Physiol Behav 186:25–30
40. Tabas I, Bornfeldt KE (2016) Macrophage Phenotype and Function in Different Stages of Atherosclerosis. Circ Res 118(4):653–667
41. Martinet W et al (2019) Macrophage Death as a Pharmacological Target in Atherosclerosis. Front Pharmacol 10:306
42. Kolodgie FD et al (2000) Localization of apoptotic macrophages at the site of plaque rupture in sudden coronary death. Am J Pathol 157(4):1259–1268
43. Canfran-Duque A et al (2017) Macrophage deficiency of miR-21 promotes apoptosis, plaque necrosis, and vascular inflammation during atherogenesis. EMBO Mol Med 9(9):1244–1262
44. Wang D et al (2018) Apoptotic cell induction of miR-10b in macrophages contributes to advanced atherosclerosis progression in ApoE-/- mice. Cardiovasc Res 114(13):1794–1805
45. Li M et al (2019) Long noncoding RNA/circular noncoding RNA-miRNA-mRNA axes in cardiovascular diseases. Life Sci 233:116440

Figures

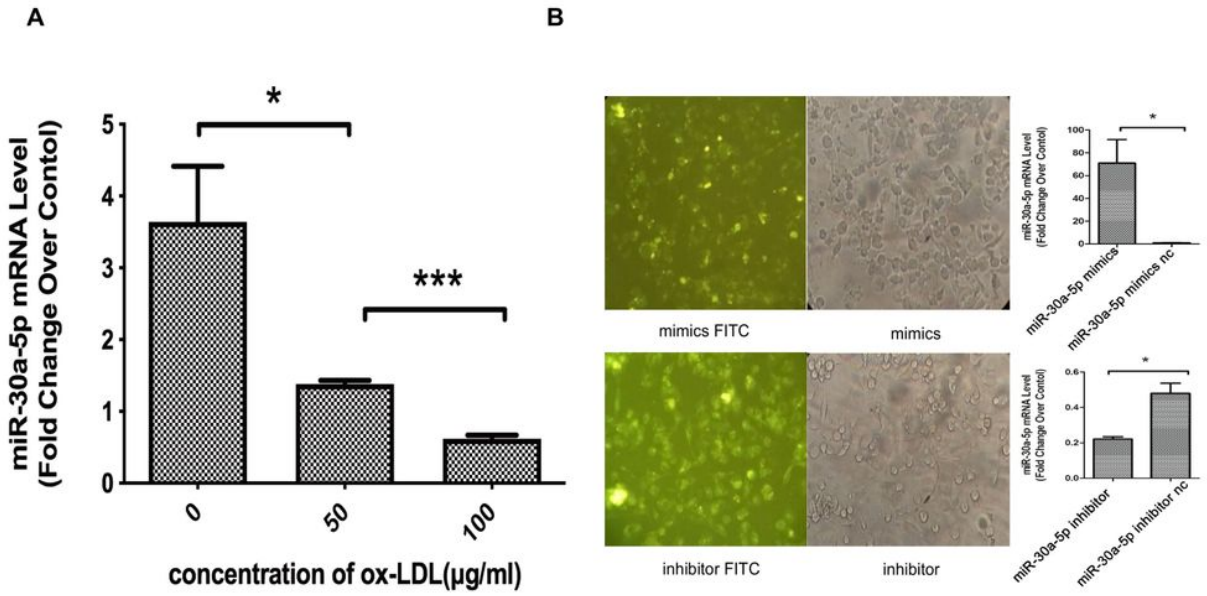

Figure 1

Ox-LDL induces a decrease in miR-30a-5p expression. A: THP-1 derived macrophages were treated with different concentrations of ox-LDL for 48h, and the expression of miR-30a-5p was detected by real-time PCR. *P<0.05, **P<0.01, ***P<0.001. B: MiR-30a-5p mimics, miR-30a-5p inhibitor and their blank carriers were transfected into cells respectively. The transfection was confirmed by fluorescence microscopy, ordinary light microscopy and real-time PCR.

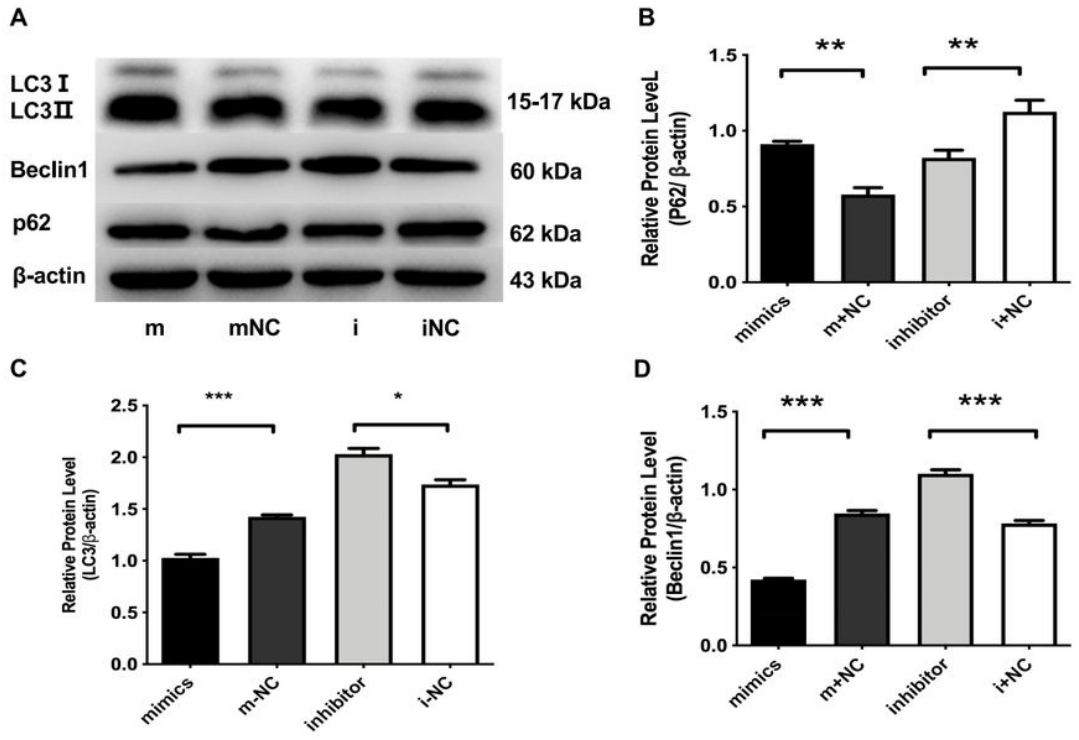

Figure 2

MiR-30a-5p promotes macrophage autophagy. The transfected cells were incubated with 75 μg/ml ox-LDL for 48 hours, the protein expression level was detected by WB. A: The expression levels of p62, LC3B and Beclin1. B, C, D: Quantitative analysis of protein expression levels, *P<0.05, **P<0.01, ***P<0.001.

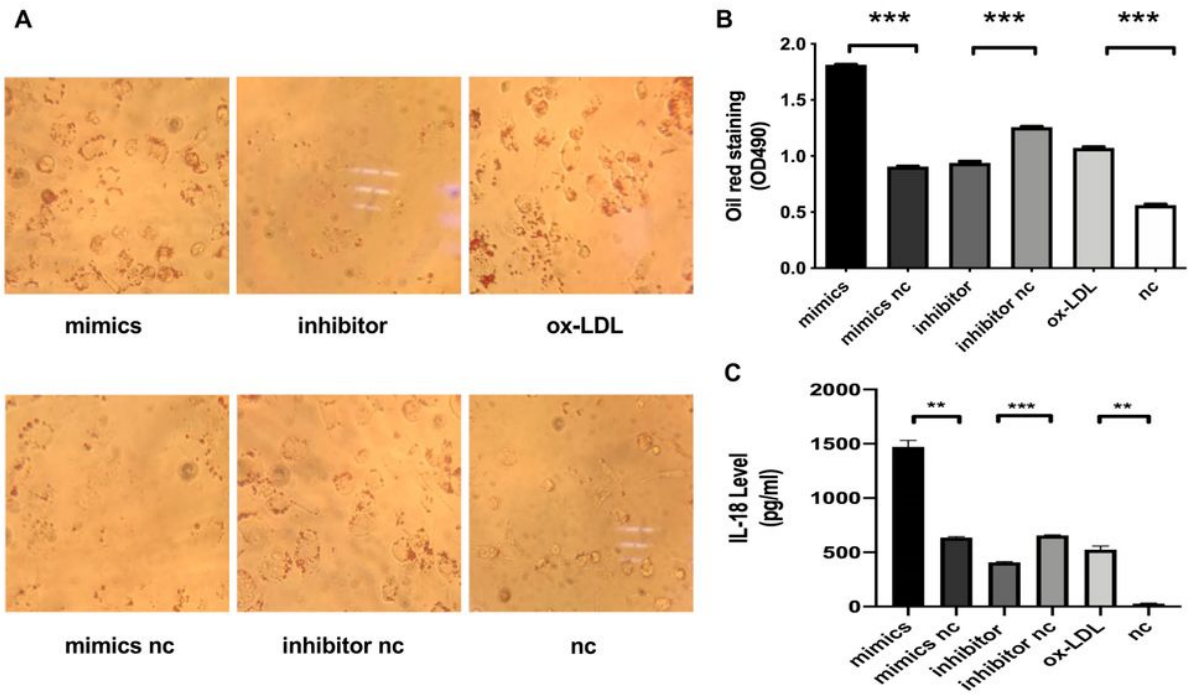

Figure 3

Overexpression of miR-30a-5p increased lipid accumulation in foam cells. A: The transfected cells were treated with 75 μg/ml ox-LDL for 48 hours and then conducted oil red staining. The ox-LDL group was treated with only 75 μg/ml ox-LDL, and the nc group was treated with medium. The Oil Red O-stained macrophages (40X). B: Quantitative analysis of oil red staining at OD490, *P<0.05, **P<0.01, ***P<0.001. C: IL-18 was detected after the transfection (at a final concentration of 50nM) for 24 hours and the stimulation under 75μg/ml ox-LDL for 48 hours (*P<0.05, **P<0.01, ***P<0.001).

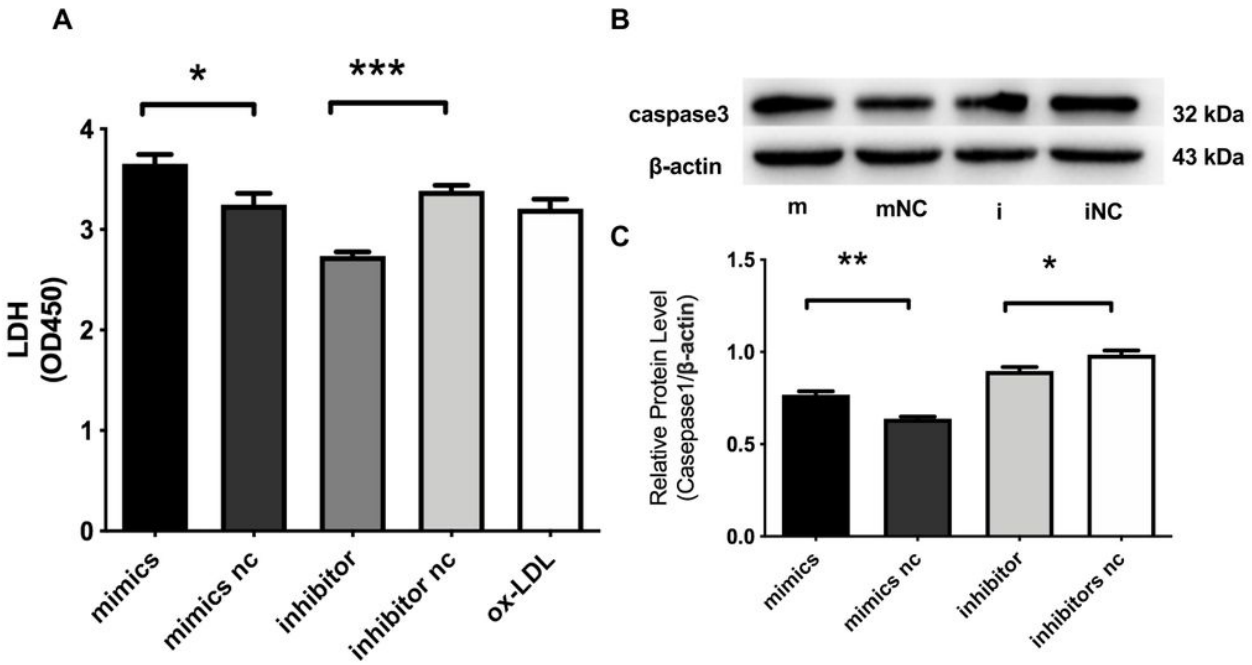

Figure 4

Macrophage viability under miR-30a-5p treatment. A: Analysis of LDH. B, C: WB imaging and expression analysis of caspase3, *P<0.05, **P<0.01, ***P<0.001.

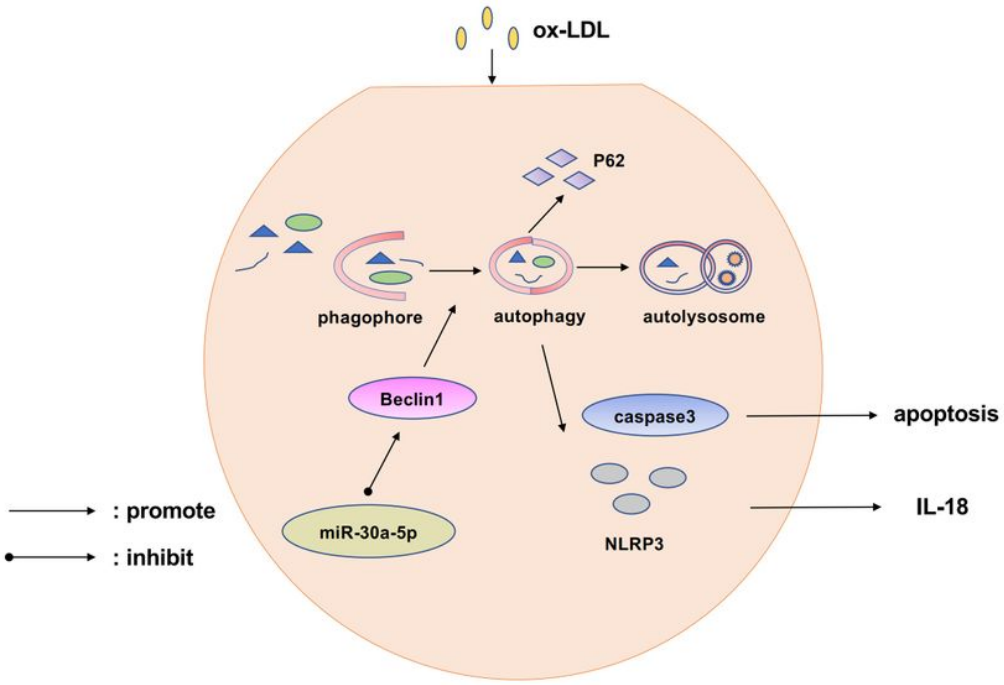

Figure 5

After an excess of ox-LDL was added to THP-1 derived macrophages, overexpressed miR-30a-5p inhibits Beclin1 expression then suppresses autophagy. MiR-30a-5p increases the release of IL-18 and induce the apoptosis of macrophage by activating Caspase-3 mediated apoptosis pathway.