

Original Article

Role and mechanism of long chain noncoding RNA-mediated TCF7-AURKA axis in regulating foam cell macrophages in coronary heart disease

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Abstract: Objective: To explore the role and mechanism of the long non-coding RNA (lncRNA)-mediated TCF7-AURKA axis in macrophage foam cell formation and the progression of coronary heart disease. Methods: Foam cell models were established using THP-1 cells and primary monocyte-derived macrophages. lncRNA, TCF7, or AURKA were silenced individually, with PI3K/AKT/mTOR inhibitors used for intervention. Lipid quantification was performed using Oil Red O staining and high-performance liquid chromatography (HPLC). Dil-oxLDL uptake was detected by flow cytometry. Protein and mRNA expressions were measured using western blot and qRT-PCR, and AURKA activity was evaluated with a kinase assay kit. Cholesterol efflux, inflammatory factor levels (ELISA), and Transwell migration were also assessed. Results: Silencing the lncRNA-TCF7-AURKA axis significantly reduced foam cell lipid accumulation (cholesterol ester [CE]: 42% decrease; CE/free cholesterol [FC] ratio: 35% decrease), diminished oxLDL uptake (30% reduction), inhibited PI3K/AKT/mTOR pathway phosphorylation, enhanced autophagy (increased LC3 and decreased P62), promoted ApoA-1-mediated cholesterol efflux (32% increase), downregulated IL-1 β and TNF- α (25% reduction each), and impaired cell migration. These effects were verified in primary cells, with AURKA kinase activity decreasing by 45%. Conclusion: The lncRNA-TCF7-AURKA axis activates the PI3K/AKT/mTOR pathway to inhibit autophagy, thereby promoting lipid deposition and inflammation. This is a key pathway driving macrophage foam cell formation and atherosclerosis, providing a theoretical basis for combined targeted intervention.

Keywords: Long chain non-coding RNA, mediating TCF7-AURKA axis, regulating macrophage foam cells, coronary heart disease

Introduction

Coronary artery disease (CAD) remains a leading cause of global mortality, with its core pathologic mechanism centered on the formation and progression of atherosclerotic plaques. Foam cells play a pivotal initiating role in plaque development through macrophage-mediated uptake of oxidized low-density lipoprotein (Ox-LDL) and dysregulated intracellular lipid metabolism. In recent years, long non-coding RNAs (lncRNAs) have emerged as key regulators of gene expression, with their roles in multiple diseases increasingly well-characterized [1-5]. Additionally, TCF7 and AURKA-critical regulators of signal transduction and cell cycle regulation-may influence lipid metabolism and

autophagy by regulating the PI3K-AKT-mTOR pathway. However, the synergistic effects and molecular mechanisms underlying the coordinated regulation of foam cell formation by lncRNAs, TCF7, and AURKA remain incompletely elucidated, particularly regarding their specific roles in CAD pathogenesis.

This study established a THP-1 cell-derived foam cell model to explore the roles and molecular mechanisms of lncRNAs, TCF7, and AURKA in foam cell formation. The findings offer novel theoretical insight into the key regulatory factors and signaling pathways involved in foam cell formation, while identifying potential therapeutic targets for atherosclerosis and CAD. Further investigation of their effects on lipid

metabolism and autophagy may facilitate the development of innovative strategies for the early diagnosis and precision treatment of CAD.

Materials and methods

Experimental subjects

The human monocytic cell line THP-1 was purchased from Shanghai Fuheng Biotechnology Co., Ltd. (Shanghai, China). Cells were cultured in RPMI-1640 medium containing 12% fetal bovine serum under conditions of 37°C and 5% CO₂ until passage 7 for subsequent experiments. THP-1 cells were treated with PMA (160 nmol/ml) or 24 hours to induce differentiation into macrophages, designated as the PMA group.

These induced macrophages were further incubated with oxidized low-density lipoprotein (Ox-LDL; 80 ng/L) for 24 h, resulting in pseudopodia formation and irregular morphology consistent with foam cells (Ox-LDL group).

A negative control group (si-NC group) was established by transfecting foam cells with non-targeting siRNA for 24 hours.

Targeted interference groups included the si-lncRNA group (foam cells transfected with lncRNA-specific siRNA; 50 nM, 24 h).

The si-TCF7 group (foam cells transfected with TCF7-specific siRNA; 50 nM, 24 h).

si-AURKA group (foam cells transfected with AURKA-specific siRNA; 50 nM, 24 h).

For all groups, serum-containing medium was discarded, and cells were washed three times with sterile PBS. After adding 1 mL of sterile PBS, cells were scraped with a cell scraper, collected into 1.5 ml EP tubes, and stored at -20°C.

To verify the physiological relevance of the findings, complementary experiments were performed using human primary monocyte-derived macrophages. Peripheral blood mononuclear cells were isolated from healthy donors using Ficoll density gradient centrifugation. CD14⁺ monocytes were purified by magnetic bead separation (Miltenyi Biotec) and cultured in RPMI-1640 medium containing 50 ng/mL M-CSF for 7 days to induce macrophage differ-

entiation. These primary macrophages were then induced to form foam cells as described above and transfected with the corresponding siRNAs.

Main reagent configuration

(1) 1x electrophoresis buffer: 5x electrophoresis buffer was taken (100 ml), and the volume was adjusted to 500 ml with distilled water. The mixture was mixed thoroughly, stored at room temperature. This buffer can be reused 3 times. (2) 5x electrophoresis buffer: Tris alkali (7.55 g), glycine (47 g), and SDS (2.5 g) were weighed. Distilled water was added to adjust the mixture to a final volume of 500 ml with distilled water, followed by stirring with a glass rod until complete dissolution was achieved. The buffer was stored at room temperature (RT). (3) 1x transfer buffer: 300 ml of 1x transfer buffer and 600 ml of methanol solution were taken with a measuring cylinder, made up to 3000 ml volume with distilled water, mixed thoroughly, and stored at 4°C. One preparation can be recycled 3-4 times. (4) 10x transfer buffer: 60.6 g of Tris alkali and 288 g of glycine were weighed, made up to 2000 ml volume with distilled water, stirred with a glass rod until fully dissolved, and stored at room temperature. (5) TBST buffer: 100 ml of 20x TBS solution was taken with a measuring cylinder, 1900 ml of distilled water and 2 ml of 20% Tween20 were added with a pipette. After mixing thoroughly, the solution was stored at 4°C. (6) 10% APS solution: 0.3 g of APS was weighed on a balance, and 3 ml of double distilled water was added with a pipette to make up the volume. After shaking thoroughly and dissolving, it was stored at 4°C for 7 days. (7) 10% SDS solution: 10 g of SDS was weighed on a balance, made up to 100 ml with double distilled water, stirred thoroughly, and stored at room temperature. (8) 5% skim milk sealing solution: 3 g of skim milk was weighed on a balance, 60 ml of TBST buffer was added, stirred with a glass rod until fully dissolved, and used immediately. (9) Protein lysate: 2 ml of RIPA lysate (strong) was taken with a pipette, 20 ul of proteinase inhibitor was added, mixed thoroughly, and stored at -20°C for immediate use. (10) PBS buffer: A package of PBS powder (ZLI-9062 type) was taken, made up to 2000 ml with double distilled water, stirred with a glass rod until fully dissolved, and stored at room temperature. (11) EDTA antigen repair

solution: 4 ml of 50x EDTA repair solution was taken with a pipette, put into 196 ml of PBS buffer, mixed thoroughly, and used immediately. (12) DAB colorant: 25 μ l of DAB stock solution and 500 μ l of DAB substrate were taken with a pipette. The preparation was done in the light, shaken thoroughly to mix, and used immediately. (13) 3% H_2O_2 solution: 5 ml of 30% H_2O_2 stock solution was taken, made up to 50 ml with double distilled water, stirred with a glass rod until fully mixed, kept in the dark, and stored at 4°C. (14) 0.5% Pseudamine sky blue: 0.25 g of pseudamine sky blue powder was weighed on a balance, made up to 50 ml with distilled water, stirred with a glass rod until fully dissolved, and used immediately. (15) 1% Osmic Acid Fixation Solution: An osmic acid ampoule was thoroughly cleaned, wrapped with filter paper, and crushed immediately after placement into the master solution bottle. Pure water was added and the bottle was sealed to mix thoroughly. The mixture was stored in a sealed, light-protected refrigerator at 4°C. For use, the 1% osmic acid working solution was prepared by mixing an equal volume of master solution with 0.2 M PBS (pH $\approx$ 7.4) before application.

Methods

Cell recovery: (1) Thawing: All required equipment was first sterilized using an autoclave and placed in a laminar flow hood for UV disinfection for 45 minutes. The water bath temperature was set to 37°C in advance. When the temperature reached 37°C, the cell cryopreservation tubes were removed from the liquid nitrogen tank, held using disposable gloves, and immediately immersed in the 37°C water bath. The tubes were gently shaken continuously during this process to accelerate thawing (approximately 2 minutes, following the “slow-freezing, rapid-melting” principle). (2) Homogenization: The frozen tube was taken out of the water bath, the mouth of the tube was wiped with an alcohol cotton ball, and placed on the ultra-clean table. Cells were sucked out with a 1 mL pipette and put into a 15 mL centrifuge tube. 1640 culture medium with more than 10 times the original volume was added and mixed thoroughly. (3) Centrifugation: Centrifugation was performed at 1000 rpm for 5 minutes. (4) Discard supernatant, resuspend and inoculate: The supernatant was discarded, pre-prepared

medium (1640 medium with 12% FBS) was added to resuspend the cells. A hemocytometer was used to adjust the cell density. The cells were inoculated into T25 culture flasks, with the flask edges clearly labeled with cell name, generation, recovery time, and name of the rescuer. The flasks were placed in a CO₂ incubator (condition: 37°C, 5% CO₂) [6-8].

Cell subculture: (1) The T25 culture bottle was taken out and the cell morphology and density were observed under the microscope to determine the cell growth status and whether the cells needed to be subcultured or changed. (2) After confirming healthy cell growth and absence of contamination, the T25 culture flask was removed. It was disinfected using an alcohol spray bottle and placed in a laminar flow cabinet for handling. The cells were gently dispersed using a pipette, were transferred into sterile 15 mL centrifuge tubes and centrifuged at low speed (1000 rpm for 5 minutes). (3) The supernatant was aspirated with a pipette, the culture medium was added, and the cells were gently resuspended. The suspension was transferred into two or three new T25 culture bottles according to the cell density [9-12]. (4) After marking the cell name, generation, time of subculture, and name of the subculture on the edge of the culture bottle, cells were placed into the incubator at 37°C and 5% CO₂ for cultivation.

Cell cryopreservation: (1) The morphology and growth of cells in culture bottles were observed under an inverted microscope to determine whether the cells could be subcultured and cryopreserved. (2) The required cryopreservation solution was prepared. (3) The cells were transferred from the culture flask to a sterile 15 mL centrifuge tube and then centrifuged at 1000 rpm for 5 minutes, and the supernatant was discarded. 1 mL of cell cryopreservation medium was added and mixed thoroughly. The mixture was transferred into a designated cryovial with labels including cell name, cryopreservation date, cryopreservation generation, cryopreservation conditions, and the name of the person performing the cryopreservation. The labeled cryovial was placed in a pre-cooled program-controlled freezer, allowing it to cool overnight at -80°C. After cooling, the cryovial was transferred to liquid nitrogen for permanent storage [13-15].

Construction of long chain non-coding RNA siRNA: The long chain non-coding RNA siRNA was provided by RiboBio as a chemically synthesized double-stranded small molecule RNA [16].

Cell experiment grouping and treatment: The cells were grouped as follows: (1) Cell grouping: A: THP-1 + PMA = PMA group; B: THP-1 + PMA + Ox-LDL = Ox-LDL group; C: THP-1 + PMA + Ox-LDL + siRNA-NC = si-NC group; D: THP-1 + PMA+Ox-LDL + siRNA-lncRNA = si-lncRNA group; E: THP-1 + PMA + Ox-LDL + siRNA-TCF7 = si-TCF7 group; F: THP-1 + PMA + Ox-LDL + siRNA-AURKA = si-AURKA group.

(2) Macrophage induction: THP-1 cells with a counted density of about 1×10^6 cells/mL were inoculated into a six-well plate. PMA was added at a concentration of 160 nmol/mL to induce all six groups of cells into macrophages. After 48 h of incubation, the cells became adherent and polygonal with multiple processes under the microscope, indicating that PMA induction was successful.

(3) Induction of foam cells: After successful induction of macrophages, the cells were gently washed with sterile PBS and Ox-LDL with a concentration of 80 nmol/mL was added. After 24 h of incubation, Oil Red O staining was used to identify the cells, which were stained dark red or orange, and deep red or orange particles visible in the cytoplasm, indicating the formation of foam cells [17-20].

(4) Silencing of long chain non-coding RNA, TCF7, and AURKA genes: 1) siRNA easily adhered to the inner wall of a 1.5 mL Ep tube. Before the lid was opened, low-speed instantaneous centrifugation was performed. 250 μ L of RNase-free water was added to the siRNA lyophilized powder to prepare a stock solution of 20 μ M. The siRNA was then aliquoted and stored at -20°C to avoid repeated freezing and thawing. 2) After successful induction of foam cells, the optimal concentrations of 50 nM siRNA-lncRNA, siRNA-TCF7, and siRNA-AURKA were transfected. ① Dilution of siRNA: 1.25 μ L of 20 μ M siRNA stock solution (V3) was added to 30 μ L of riboFECTTM CP Buffer (V2), and gently mixed by pipetting. ② Preparation of the transfection complex: 3 μ L of riboFECTTM CP Reagent (V4) was added to V2. The mixture was gently

mixed by pipetting and incubated at room temperature for 10 minutes to prepare the transfection complex. Shaking was avoided and the mixture was kept at room temperature for no more than 24 hours. ③ Transfection: The riboFECTTM CP transfection complex was added to an appropriate volume of complete medium (V1) and gently shaken to mix. 2 mL of the mixture (composed of V1: 1863 μ L, V2: 120 μ L, V3: 5 μ L, and V4: 12 μ L) was added into per well respectively. Cells in the si-NC, si-lncRNA, si-TCF7, and si-AURKA groups were transfected with 50 nM respectively. After gently shaking the six-well plate, it was incubated at 37°C in an incubator with 5% CO_2 concentration for 24 hours. ④ Detection of transfection effect: siRNA was incorporated into the RISC complex to target and cleave the mRNA of the target gene. RNA levels of the target gene measurement directly reflected the siRNA silencing efficiency of siRNA. The specific inhibition efficiency was calculated using qRT-PCR. After 24-72 hours of siRNA transfection, the mRNA expression levels of long non-coding RNAs, TCF7, and AURKA genes were significantly reduced, indicating successful transfection. ⑤ Treatment with pathway inhibitors: To verify the causal relationship of the PI3K/AKT/mTOR pathway in foam cell formation, Ox-LDL-induced foam cells were treated with the PI3K inhibitor LY294002 (20 μ M), the AKT inhibitor MK-2206 (5 μ M), and the mTOR inhibitor rapamycin (100 nM) respectively, either alone or in combination with the corresponding siRNA for 24 hours. Subsequently, Oil Red O staining, western blot, and cholesterol quantification analysis were performed.

Identification of foam cell model: (1) The medium was removed, and the cells were rinsed with pre-chilled PBS three times. (2) The cells were fixed with ORO (Oil Red O) Fixative for 25 minutes. (3) The cells were gently washed twice with PBS and immersed in 60% isopropyl alcohol for 5 minutes. (4) The isopropanol was discarded and freshly prepared ORO Stain (A:B = 3:2) was added for 15 minutes. (5) The staining solution was removed and an appropriate amount of Mayer hematoxylin staining solution was added to counterstain the cell nuclei for 1 minute. (6) ORO Buffer was added to rinse the cells for 1 minute, distilled water was added to cover the cells, and the cells were observed and photographed under the microscope.

Table 1. Upstream and downstream sequences of each target gene

Target Gene	Forward Primer (F)	Reverse Primer (R)
Long noncoding RNA	GTCATCTACACAGTCAACCTCTAC	TCCAGACGCCAACATAGACC
TCF7	TCGGATAACTGTTTCAGGAGGAG	TCGGATTCTGGCATCTGTAGG
AURKA	GCGAGTTGGTCAAGATCATCC	TTCTCCTGCTCGTAGATGTCC
β -actin	CATCATGAAGTGTGACGTGG	TCGTCATACTCCTGCTTGCT

Note: F: Forward primer (upstream primer); R: Reverse primer (downstream primer).

Cell protein extraction: After removing the culture medium from the petri dish, the cells were collected and subjected to low-temperature centrifugation (4°C, 6000 rpm, 10 minutes). The supernatant was discarded, and an appropriate amount of lysis buffer (250 μ l per 1 million cells) and phosphatase inhibitor were added to the cell pellet. The mixture was placed on ice for 30 minutes to ensure complete lysis. After low-temperature centrifugation, the supernatant was collected and its concentration was measured using a UV spectrophotometer. 5x loading buffer was added at a 1:4 ratio, and the mixture was incubated in a 100°C water bath for 10 minutes to fully denature the protein. Finally, the mixture was aliquoted and stored at -80°C for future use [21-23].

Detection of target gene mRNA levels by quantitative real-time PCR (qRT-PCR): (1) After removing the culture medium from the culture dish and collecting the cells, total RNA was extracted from the cells using the TRIzol reagent method. (2) The purity and concentration of the extracted total RNA were determined using a UV spectrophotometer. Qualified total RNA was reverse transcribed into complementary DNA (cDNA) using a reverse transcription kit. (3) The PCR reaction system was prepared with SYBR® Select Master Mix and corresponding primers, and the cycle threshold (Ct) values were measured using the 7500 Fast Real-Time PCR System. The relative transcription level of the target gene was calculated using the $2^{-\Delta\Delta Ct}$ method. (4) The PCR conditions were set as follows: initial UDG digestion at 50°C for 2 min; pre-denaturation at 95°C for 2 min; followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min. Melting curve analysis was performed as follows: 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s. β -actin was used as the internal reference gene, with three technical replicates set up for each sample group. The primers used in this experiment

were synthesized by Shanghai Biological Co., Ltd. in accordance with primer design principles. The upstream and downstream sequences of each target gene are listed in **Table 1** [21-25].

Detection of target protein expression levels by Western blot blot: First, protein samples were prepared as follows: cells were lysed in ice-cold lysis buffer containing protease and phosphatase inhibitors. The lysate was centrifuged at 4°C (12,000 rpm, 10 minutes) to collect the supernatant, and protein concentration was quantified using the BCA method. Samples were then mixed with 5x loading buffer and denatured by heating. Subsequent steps included SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) with the following workflow: gel preparation, sample loading, and electrophoresis at low voltage (80 V) for stacking gel followed by high voltage (120 V) for separating gel. After membrane transfer, the PVDF membrane was activated with methanol (1 minute) prior to assembly with the gel in a transfer cassette. The membrane was blocked with 5% skim milk or BSA for 1-2 hours at room temperature, then incubated with primary antibodies either overnight at 4°C or for 2-3 hours at room temperature, followed by incubation with secondary antibodies for 1-2 hours at room temperature. Between each incubation step, the membrane was rinsed three times with TBST (5 minutes per wash). Chemiluminescence was detected using an ECL substrate and a chemiluminescence imaging system. Finally, grayscale analysis was performed using image analysis software to calculate the relative expression of target proteins normalized to the endogenous reference protein (e.g., β -actin).

To validate the kinase activity of AURKA, phosphorylation-specific antibodies were used to detect the phosphorylation levels of AURKA substrates (e.g., p-Aurora A Thr288, p-CDC25B

Ser353). Additionally, an AURKA kinase activity detection kit (CycLex, Japan) was employed for in vitro kinase activity analysis in accordance with the manufacturer's instructions [26-28].

Detection of lncRNA, TCF7, and AURKA localization and fluorescence intensity by immunofluorescence: (1) Cells were seeded on coverslips in six-well plates, washed three times with pre-chilled PBS (5 minutes per wash). (2) Cells were fixed with 4% paraformaldehyde for 15 minutes, washed three times with pre-chilled PBS (5 minutes per wash), and permeabilized with 0.5% Triton X-100 for 15 minutes at room temperature. (3) Cells were blocked with 10% goat serum for 30 minutes at room temperature, then incubated overnight at 4°C with primary antibodies against TCF7 and AURKA (1:100) and an in situ hybridization probe for lncRNA (1:100; note: lncRNA cannot be detected by antibodies, requiring in situ hybridization). (4) Cells were washed three times with PBS (5 minutes per wash), incubated with fluorescently labeled secondary antibodies (1:200) for 90 minutes at room temperature, and washed three times with PBS. (5) Coverslips were carefully removed with tweezers, inverted onto glass slides (cell-side down), and cell nuclei were stained with DAPI for 10 minutes. Excess DAPI was removed, and coverslips were sealed with nail polish. Finally, images were captured using a confocal laser scanning microscope. (Note: Blue fluorescence: cell nuclei; red fluorescence: lncRNA and AURKA; green fluorescence: TCF7).

Cholesterol quantification analysis and Dil-oxLDL uptake experiment: The contents of intracellular free cholesterol (FC) and cholesterol esters (CE) were determined using the Amplex Red Cholesterol Assay Kit (Invitrogen) (colorimetric method; note: Amplex Red is not compatible with high-performance liquid chromatography (HPLC). After cell lysis, lipids were extracted, dried under a nitrogen stream, and resuspended in isopropanol. For HPLC-based quantification (if used), separation was performed on a C18 column with a detection wavelength of 210 nm.

For the Dil-oxLDL uptake assay, cells were treated with Dil-oxLDL (10 µg/mL) for 4 hours, washed three times with PBS, digested with trypsin, and resuspended in PBS. Fluorescence intensity was detected using a flow cytometer

(BD FACSCanto) with excitation/emission wavelengths set at 549/565 nm. Data analysis was performed using FlowJo software [29-32].

Functional endpoint detection: Cholesterol efflux assay: Cells were labeled with [³H]-cholesterol (tritiated cholesterol) for 24 hours, then treated with ApoA-I (10 µg/mL) for 4 hours. Culture medium and cells were collected separately, and radioactivity was detected using a liquid scintillation counter to calculate the cholesterol efflux rate.

Inflammatory factor detection: The levels of IL-1β and TNF-α in culture supernatants were determined using ELISA kits (R&D Systems) according to the manufacturer's protocols.

Cell migration assay: A Transwell chamber (8 µm pore size) was used. MCP-1 (monocyte chemoattractant protein-1) (50 ng/mL) was added to the lower chamber as a chemoattractant, and cells resuspended in serum-free medium were seeded into the upper chamber. After 6 hours of incubation, non-migrated cells on the upper surface of the membrane were removed, and migrated cells on the lower surface were fixed, stained, and counted under a microscope [33, 34].

Statistical analysis

SPSS 26.0 software was used for statistical analysis. Measured data were expressed as mean ± standard deviation (± SD). Comparisons between two groups were performed using the t-test, while comparisons among multiple groups were analyzed using one-way analysis of variance (ANOVA). Categorical data were analyzed using chi-square tests. A P-value < 0.05 was considered significant. Graphs were generated using GraphPad Prism 9.0 software.

Results

Establishment and verification of the foam cell model by Oil Red O staining

After 24 hours of PMA induction, THP-1 cells differentiated into irregularly shaped, multi-lobed macrophages. Subsequent induction with Ox-LDL for 24 hours followed by Oil Red O staining revealed dark red or orange-red lipid granules in the cytoplasm, confirming the successful establishment of the foam cell model (**Figure 1**).

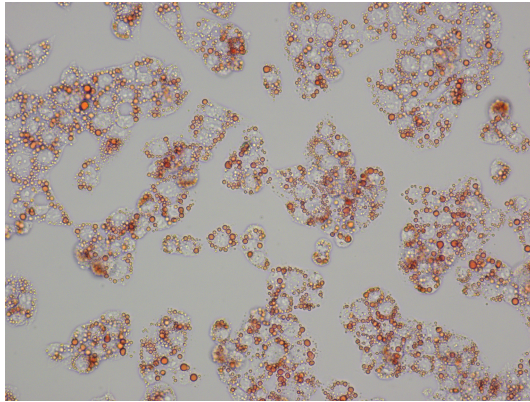


Figure 1. Model of foam cell identification by Oil Red O staining (ORO, 400×). Oil Red O staining of foam cell models (400× magnification). THP-1 cells were induced with PMA to differentiate into macrophages, followed by Ox-LDL stimulation to form foam cells. Cytoplasmic lipid droplets were stained dark red or orange-red, confirming successful foam cell establishment.

Validation of si-LncRNA, si-TCF7, and si-AURKA transfection by western blot and RT-PCR

Western blot and RT-qPCR results showed that, compared with the PMA group, Ox-LDL-induced macrophages exhibited significantly increased intracellular levels of LncRNA, TCF7 protein, and AURKA mRNA ($P < 0.05$). In contrast, transfection with si-LncRNA, si-TCF7, or si-AURKA led to a marked reduction in these biomarkers relative to both the Ox-LDL and si-NC groups ($P < 0.05$), confirming the successful silencing of LncRNA, TCF7, and AURKA expression (**Figure 2**).

Reduced lipid deposition in foam cells after silencing of LncRNA, TCF7, or AURKA

THP-1 cells were induced into macrophages by PMA, and the macrophages were then allowed to phagocytose Ox-LDL. Oil Red O staining showed dark red staining in the Ox-LDL, si-NC, si-LncRNA, si-TCF7, and si-AURKA groups, with orange-red or yellowish lipid granules visible in the cytoplasm. Compared to the Ox-LDL and si-NC groups, cells in the si-LncRNA, si-TCF7, and si-AURKA groups exhibited increased cell size, reduced intracellular lipid accumulation, and lighter staining intensity. Statistical analysis confirmed that these differences were statistically significant ($P < 0.05$), indicating that LncRNA, TCF7, and AURKA promoted the formation of macrophage-derived foam cells (**Figure 3**).

Inhibition of LncRNA, TCF7, or AURKA attenuates the PI3K/AKT/mTOR pathway in foam cells

Densitometric analysis of western blot bands showed that, compared to the PMA group, the Ox-LDL and si-NC groups had significantly elevated intracellular protein levels of PI3K, p-PI3K, AKT, p-AKT, mTOR, and p-mTOR ($P < 0.05$). Conversely, transfection with si-LncRNA, si-TCF7, or si-AURKA significantly reduced the levels of these proteins ($P < 0.05$), indicating that silencing LncRNA, TCF7, or AURKA effectively inhibits the activity of the PI3K/AKT/mTOR signaling pathway (**Figures 4 and 5**).

Detection of LncRNA, TCF7, AURKA, P62, and LC3 expression in foam cells by immunofluorescence single and double labeling

Immunofluorescence results showed that, compared to the PMA group, the Ox-LDL and si-NC groups exhibited increased intracellular P62 expression and decreased LC3 expression. After transfection with si-LncRNA, si-TCF7, or si-AURKA, foam cells showed reduced P62 expression and increased LC3 expression relative to the Ox-LDL and si-NC groups. These findings indicate that silencing LncRNA, TCF7, or AURKA can enhance autophagy in macrophage-derived foam cells (**Table 2**).

Detection of Beclin1, P62, and LC3 expression in macrophage foam cells by western blot and RT-PCR

Western blot and RT-PCR results demonstrated that, compared to the PMA group, foam cells had increased P62 expression and decreased LC3/Beclin1 levels. Transfection with si-LncRNA, si-TCF7, or si-AURKA significantly reduced P62 expression and enhanced LC3/Beclin1 levels in foam cells relative to the Ox-LDL and si-NC groups, with significant differences ($P < 0.05$). These results further confirm that inhibiting LncRNA, TCF7, or AURKA can promote autophagy in macrophage-derived foam cells (**Figure 6**).

Effects of gene silencing on foam cell lipid deposition, signaling pathway activity, and autophagy

Lipid deposition in the Ox-LDL and si-NC groups was significantly higher than that of the PMA

LncRNA-TCF7-AURKA axis in macrophage foam cells and coronary heart disease

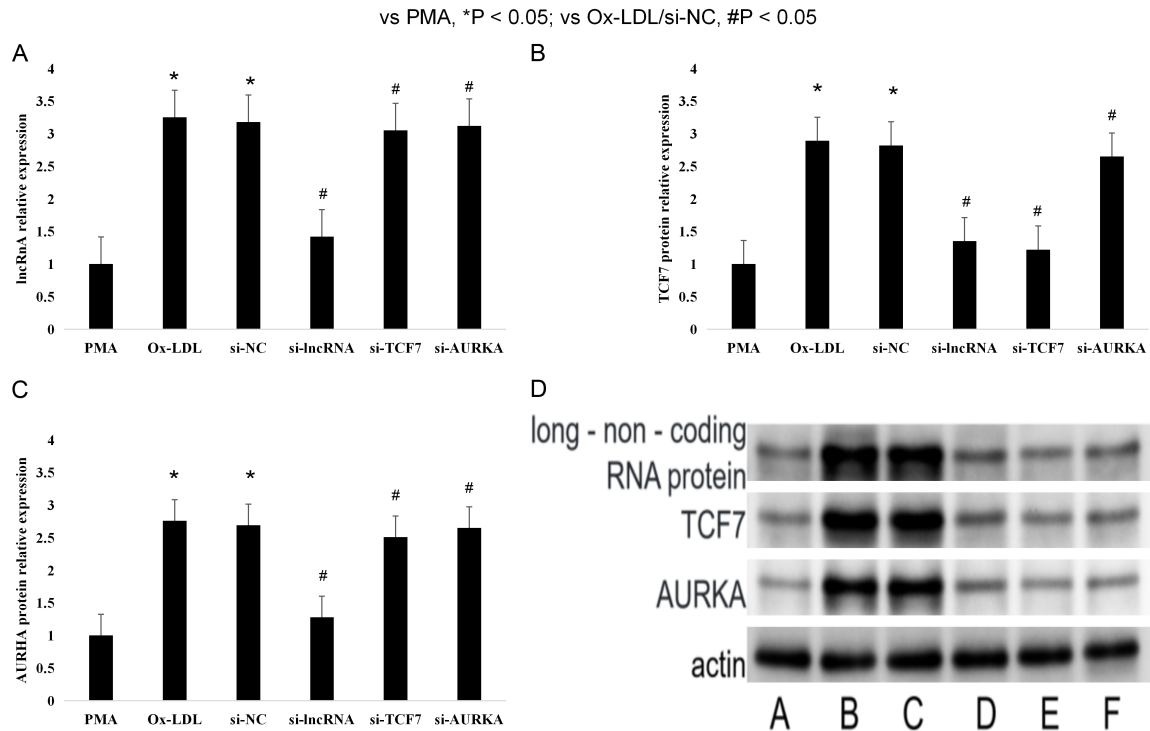


Figure 2. Validation of si-lncRNA, si-TCF7 and si-AURKA silencing efficiency. A-C: Quantitative analysis of relative RNA/protein expression of lncRNA, TCF7 and AURKA. D: Representative raw western blot bands. β -actin served as internal reference. Data are presented as mean $\pm$ SD (n = 3). *P < 0.05 versus PMA group; #P < 0.05 versus Ox-LDL/si-NC group.

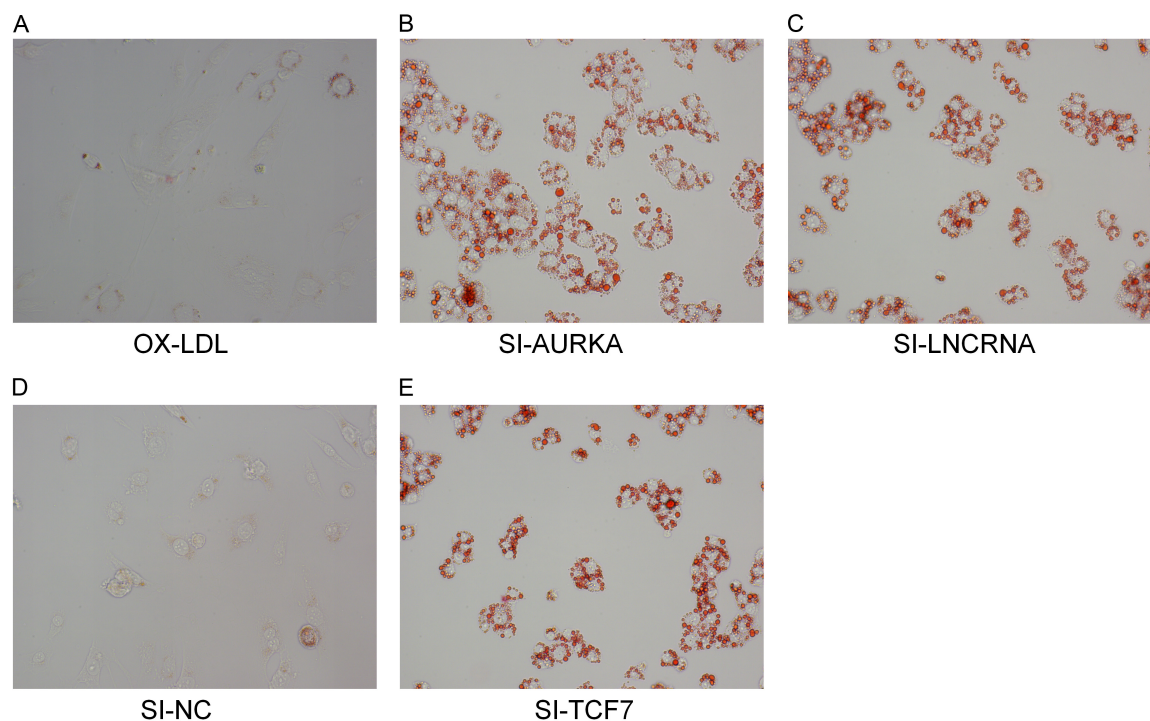


Figure 3. Oil Red O staining analysis of intracellular lipid deposition in macrophage-derived foam cells (400 $\times$ magnification). Representative images show lipid-laden foam cells from PMA, Ox-LDL, si-NC, si-lncRNA, si-TCF7 and si-AURKA groups. Diminished red-orange staining indicates reduced intracellular lipid accumulation following gene silencing.

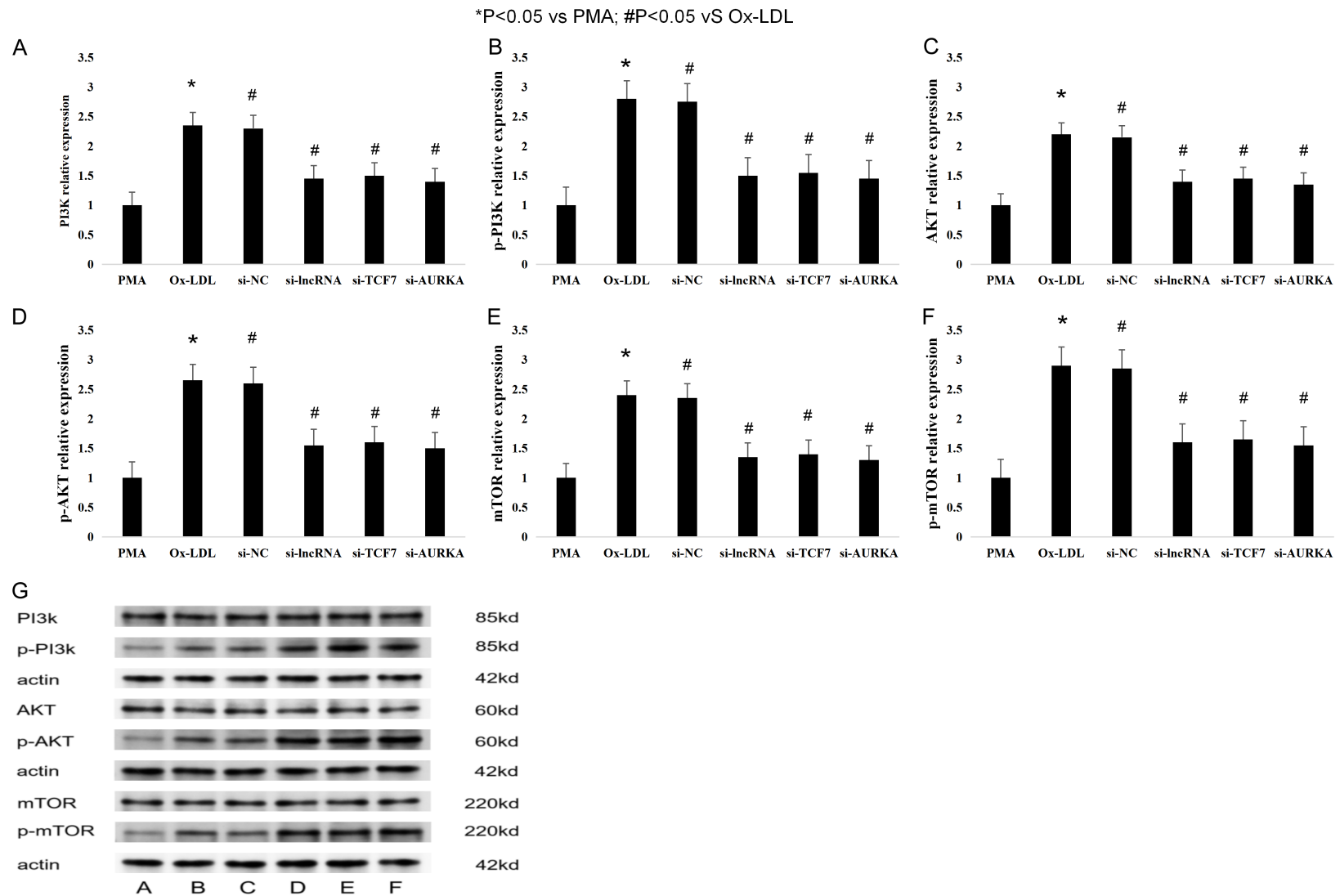


Figure 4. Basal expression of PI3K/AKT/mTOR-signaling-related proteins in foam cells before gene silencing. A-F: Quantification of relative protein levels of PI3K, p-PI3K, AKT, p-AKT, mTOR and p-mTOR. G: Representative raw western blot images. β -actin was used as internal loading control. Data are presented as mean $\pm$ SD (n = 3). *P < 0.05 versus PMA group.

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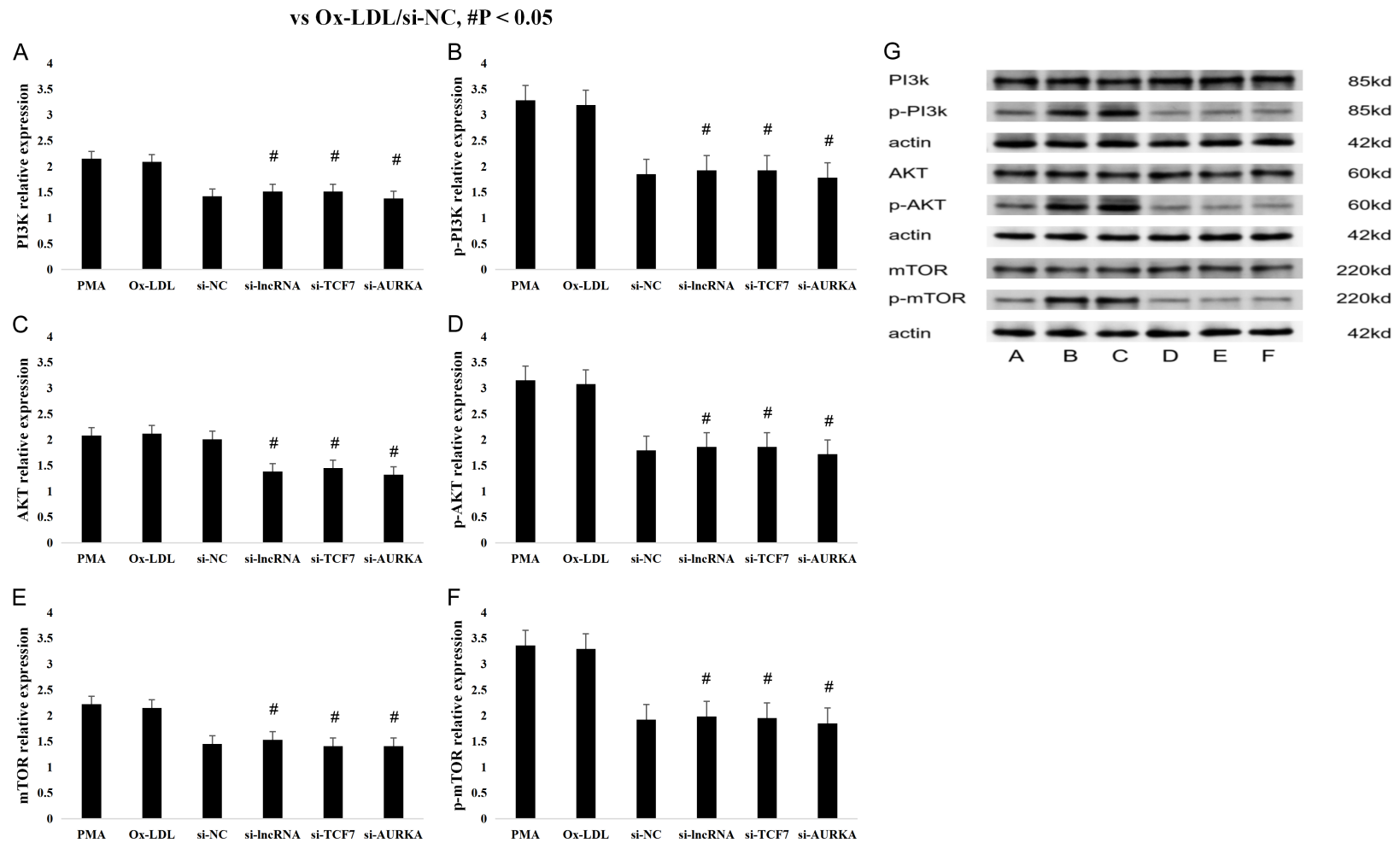
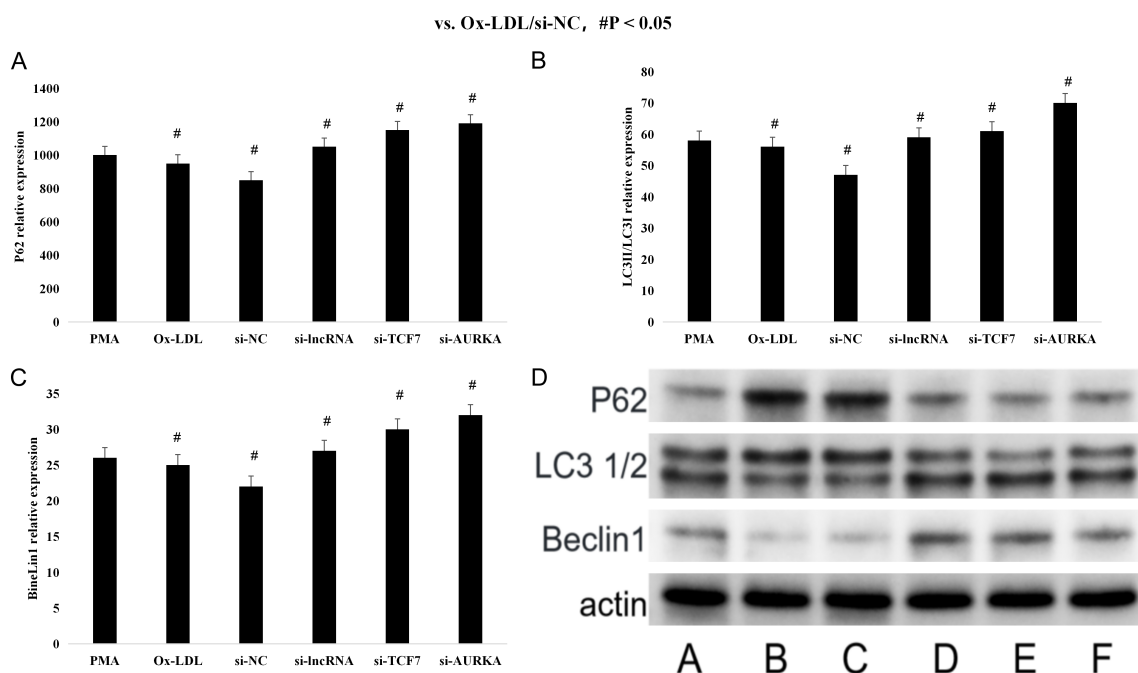


Figure 5. Changes in PI3K/AKT/mTOR pathway protein levels after silencing lncRNA, TCF7 or AURKA in foam cells. A-F: Quantitative analysis for PI3K, p-PI3K, AKT, p-AKT, mTOR and p-mTOR. G: Representative raw Western blot bands. β -actin served as internal reference. Data are presented as mean $\pm$ SD (n = 3). #P < 0.05 versus Ox-LDL/si-NC group.

Table 2. Immunofluorescence intensity values of long non-coding RNA, TCF7, AURKA, P62 and LC3

Group	Average Fluorescence Intensity of P62 (Mean $\pm$ SD)	P-value (Compared with PMA Group)	Average Fluorescence Intensity of LC3 (Mean $\pm$ SD)	P-value (Compared with PMA Group)
PMA Group	20.2 $\pm$ 2.8	-	80.5 $\pm$ 4.6	-
Ox-LDL Group	50.6 $\pm$ 3.7	< 0.001	30.3 $\pm$ 2.9	< 0.001
si-NC Group	48.4 $\pm$ 3.2	< 0.001	32.2 $\pm$ 3.8	< 0.001
si-lncRNA Group	30.1 $\pm$ 1.9	0.021	60.3 $\pm$ 3.7	0.027
si-TCF7 Group	32.3 $\pm$ 2.6	0.029	58.2 $\pm$ 3.1	0.038
si-AURKA Group	28.5 $\pm$ 1.8	0.012	62.4 $\pm$ 4.5	0.019


Figure 6. Expression profiles of autophagy-associated proteins P62, LC3 and Beclin1 in foam cells. A-C: Quantitative statistics of relative expression of P62, LC3-II/LC3-I and Beclin1. D: Representative western blot raw images. β -actin was used as internal control. Data are presented as mean $\pm$ SD ($n = 3$). #P < 0.05 versus Ox-LDL/si-NC group.

group, accompanied by a marked increase in the phosphorylation of PI3K-AKT-mTOR pathway proteins, upregulation of the autophagy-related protein P62, and downregulation of LC3-II/LC3-I ratio (ratio of lipidated to cytosolic form of LC3) and Beclin1 expression. These results indicate that Ox-LDL-induced foam cell formation is associated with increased lipid deposition, activated PI3K-AKT-mTOR signaling pathway, and inhibited autophagy. Compared to the Ox-LDL and si-NC groups, transfection with si-lncRNA, si-TCF7, or si-AURKA significantly reduced lipid deposition, decreased the phosphorylation of pathway proteins and P62 expression, and increased LC3-II/LC3-I ratio

and Beclin1 expression. These findings further confirm that silencing lncRNA, TCF7, or AURKA can inhibit lipid deposition in foam cells, reduce PI3K-AKT-mTOR pathway activity, and promote autophagy (**Figure 7**).

Validation of the lncRNA-TCF7-AURKA axis in primary macrophages

In primary monocyte-derived macrophages, Oil Red O staining showed a significant reduction in lipid deposition after silencing lncRNA, TCF7, or AURKA ($P < 0.01$), which was consistent with the trend observed in THP-1 cells (**Figure 8A**; **Table 3**). Cholesterol quantification revealed

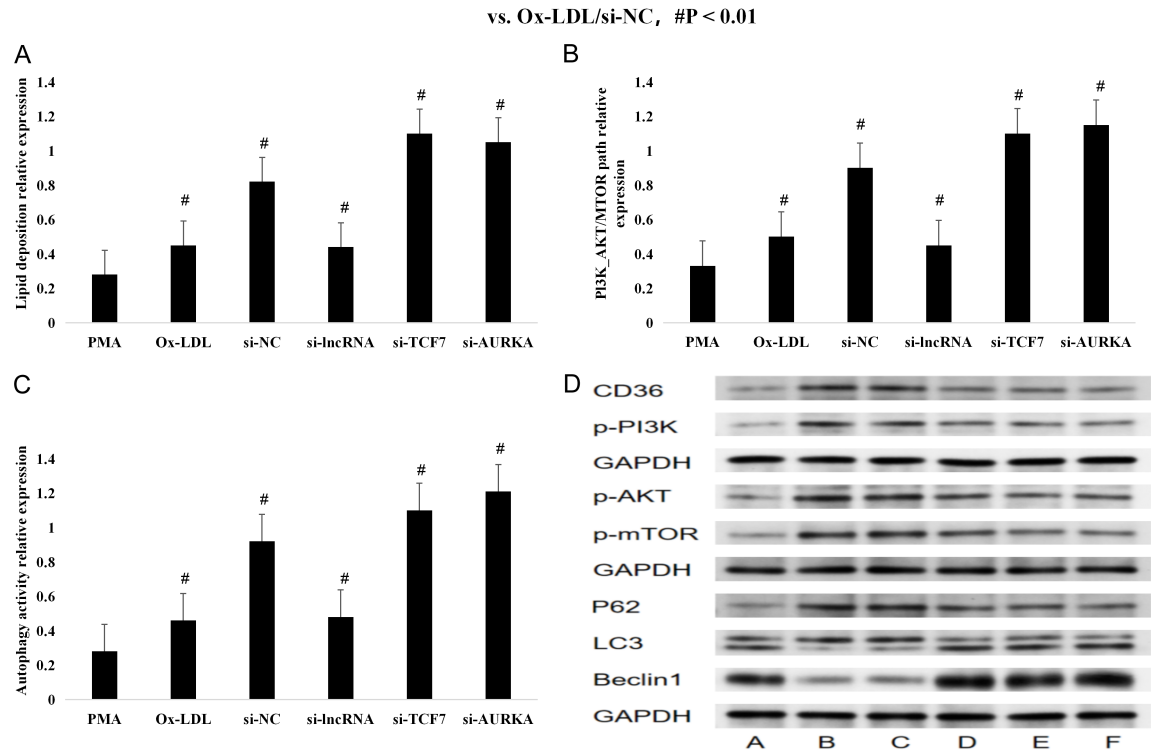


Figure 7. Effects of lncRNA/TCF7/AURKA silencing on lipid deposition, PI3K/AKT/mTOR signaling and autophagy in foam cells. A-C: Violin plots showing relative levels of lipid deposition, pathway activation and autophagy activity. D: Western blot quantification and representative raw bands for CD36, p-PI3K, p-AKT, p-mTOR, P62, LC3 and Beclin1. GAPDH served as internal reference. Data are presented as mean $\pm$ SD (n = 3). #P < 0.01 versus Ox-LDL/si-NC group.

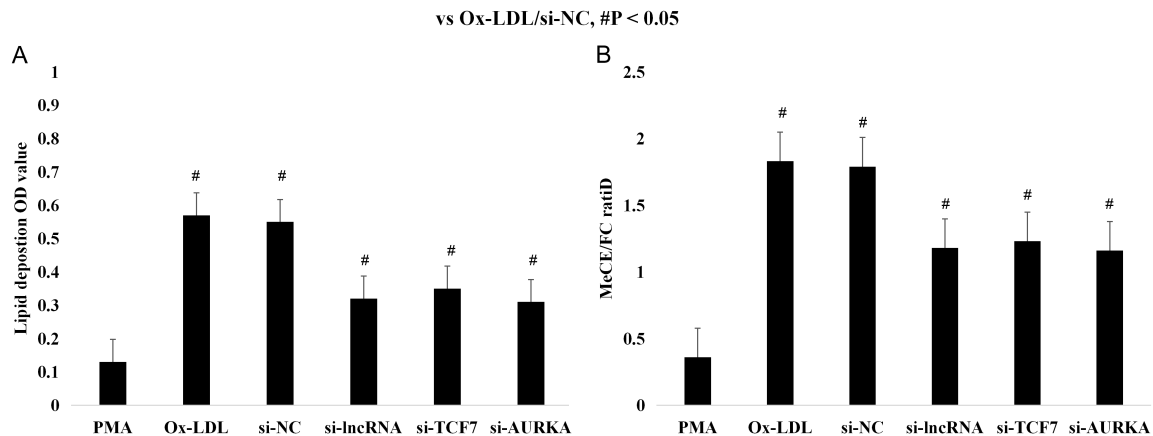


Figure 8. Detection of lipid-metabolism-related indicators in primary monocyte-derived macrophages. A: Quantification of lipid deposition measured by Oil Red O OD value. B: CE/FC (cholesterol ester/free cholesterol) ratio determined by HPLC. Data are presented as mean $\pm$ SD (n = 3). *P < 0.05 versus PMA group; #P < 0.05 versus Ox-LDL/si-NC group.

that the cholesterol ester/free cholesterol (CE/FC) ratio decreased by approximately 35% (Figure 8B; Table 4), suggesting that the lncRNA-TCF7-AURKA axis still exerts a regulatory role under physiologic conditions.

Cholesterol quantification and oxLDL uptake analysis

HPLC results showed that after silencing lncRNA, TCF7, or AURKA, the CE content de-

Table 3. Lipid deposition by Oil Red O staining (OD value, 490 nm)

Group	OD value (Mean $\pm$ SD)	P value vs PMA	P value vs Ox-LDL
PMA	0.12 $\pm$ 0.02	-	< 0.001
Ox-LDL	0.58 $\pm$ 0.05	< 0.001	-
si-NC	0.56 $\pm$ 0.04	< 0.001	0.521
si-lncRNA	0.34 $\pm$ 0.03	0.002	< 0.001
si-TCF7	0.36 $\pm$ 0.04	0.003	< 0.001
si-AURKA	0.33 $\pm$ 0.03	0.001	< 0.001

Table 4. Cholesterol ester/free cholesterol ratio (HPLC)

Group	CE/FC ratio (Mean $\pm$ SD)	P value vs PMA	P value vs Ox-LDL
PMA	0.35 $\pm$ 0.04	-	< 0.001
Ox-LDL	1.85 $\pm$ 0.12	< 0.001	-
si-NC	1.82 $\pm$ 0.10	< 0.001	0.614
si-lncRNA	1.20 $\pm$ 0.08	0.002	< 0.001
si-TCF7	1.25 $\pm$ 0.09	0.003	< 0.001
si-AURKA	1.18 $\pm$ 0.07	0.001	< 0.001

creased by 42%, 38%, and 40%, respectively ($P < 0.01$, **Figure 9A**). The Dil-Ox-LDL uptake experiment showed that the average fluorescence intensity in the siRNA-treated groups decreased by approximately 30% ($P < 0.01$, **Figure 9B**), indicating a reduced ability of foam cells to take up lipids. Consistently, fluorescence microscopic observation further visually confirmed the reduction of Dil-oxLDL-derived fluorescent signals in gene-silenced foam cells (**Figure 9C**).

Verification of AURKA kinase activity

Western blot results showed that after silencing AURKA, the level of p-Aurora A (Thr288) decreased by approximately 50% (**Figure 10A**). *In vitro* kinase experiments demonstrated that AURKA activity in the si-AURKA group decreased by approximately 45% ($P < 0.01$, **Figure 10B**), confirming that the knockdown effect was functional. Representative raw western blot bands for p-Aurora A (Thr288) and OD450 readouts of kinase activity are presented in **Figure 10C**.

Pathway inhibitor rescue experiment

Treatment with LY294002, MK-2206, or rapamycin significantly reduced lipid deposition ($P < 0.01$, **Figure 11A**; **Table 5**) and downregulated the CE/FC ratio (**Figure 11B**; **Table 6**). Combined treatment with si-lncRNA further reduced lipid deposition, suggesting that lncRNA regulates foam cell formation through the PI3K/AKT/mTOR pathway.

Functional endpoint analysis

Cholesterol efflux experiments showed that after silencing lncRNA, TCF7, or AURKA, the ApoA-I-mediated cholesterol efflux rate increased by 32%, 28%, and 30%, respectively ($P < 0.01$, **Figure 12A**). ELISA results showed that the levels of IL-1 β (**Figure 12C**) and TNF- α (**Figure 12D**) were significantly decreased ($P < 0.05$). Transwell experiments demonstrated that cell migration ability decreased by approximately 25% (**Figure 12B**), and representative crystal-violet-stained migrated cell images are displayed in **Figure 12E**.

Discussions

This study successfully induced THP-1 cells to differentiate into macrophages using PMA, followed by ox-LDL-induced foam cell formation. Oil Red O staining was employed to visually validate the establishment of the model. This methodology has been repeatedly applied in previous similar studies with consistent outcomes, demonstrating its stability and reliability, which laid a solid foundation for the present research. Oil Red O staining not only visually reveals lipid accumulation in foam cells but also confirms successful model establishment from a morphological perspective, which is crucial for subsequent investigations. After model establishment, western blot and RT-PCR analyses showed that the levels of long non-coding RNA (lncRNA), TCF7 protein, AURKA protein, and their corresponding mRNAs were significantly increased following ox-LDL induction. Although lncRNA does not encode proteins, it plays critical regulatory roles in various cellular processes. Its elevated expression suggests its involvement in complex gene regulatory networks during foam cell formation. As a transcription factor that binds to specific DNA sequences to regulate gene expression, TCF7 may influence lipid metabolism-related genes through its increased expression. AURKA, a protein kinase essential for cell cycle regulation and mitosis, is upregulated upon ox-LDL induction, indicating its potential participation in cellular proliferation and metabolic changes dur-

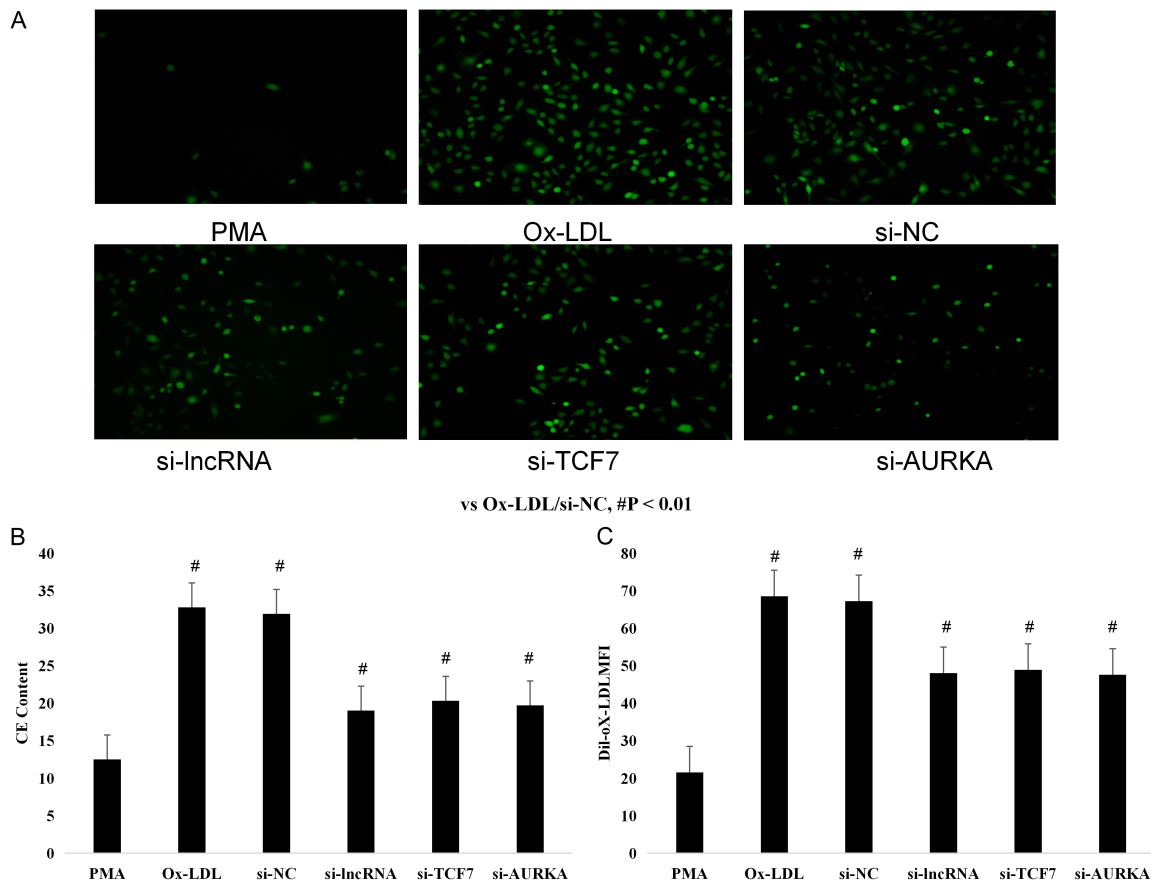


Figure 9. Analysis of cellular cholesteryl ester content and Dil-oxLDL uptake in foam cells. A: Quantification of intracellular cholesterol ester (CE) content determined by HPLC. B: Mean fluorescence intensity (MFI) of cellular Dil-oxLDL uptake measured via flow cytometry. C: Representative fluorescence microscopy images showing Dil-oxLDL fluorescent signals in different groups. Data are presented as mean $\pm$ SD ($n = 3$). #P < 0.01 versus Ox-LDL/si-NC group.

ing foam cell development. Transfection with the corresponding siRNA, significantly reduced the expression of these three factors, further confirming their association with foam cell formation. Previous studies had shown that changes in the expression of related signaling molecules in cells are closely linked to their pathophysiological states. This study further clarifies the distinct expression patterns of these three factors during the transformation of macrophages into foam cells, providing new clues for a deeper understanding of the molecular mechanisms underlying foam cell formation [35, 36].

Oil Red O staining results demonstrated reduced lipid deposition in foam cells after silencing lncRNA, TCF7, and AURKA, accompanied by cell enlargement and weaker staining intensity. This finding visually indicates that these three factors significantly affect lipid

accumulation in foam cells. From a cell biology perspective, foam cell formation primarily results from the extensive uptake and intracellular storage of lipids by macrophages. These three factors may regulate lipid content in foam cells by influencing macrophage-mediated lipid uptake and metabolism. Previous studies on lipid metabolic regulators have achieved significant progress, and the present research corroborates these findings, further confirming the crucial role of these factors in lipid regulation. In the development of diseases such as atherosclerosis, lipid deposition is a key pathologic process. LncRNA, TCF7, and AURKA may exert this regulatory effect by modulating macrophage lipid uptake - for example, by influencing the expression or activity of lipid transport proteins to alter the lipid absorption capacity of macrophages - or by participating in lipid metabolic pathways such as fatty acid synthesis and oxidation. This discovery provides new insights

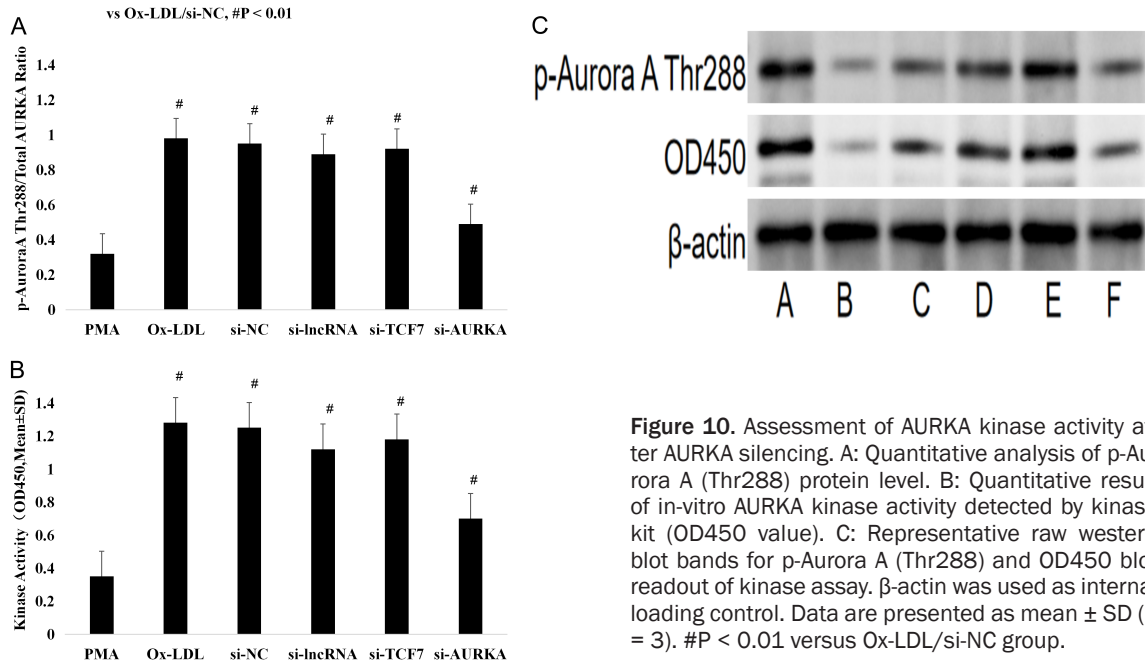


Figure 10. Assessment of AURKA kinase activity after AURKA silencing. A: Quantitative analysis of p-Aurora A (Thr288) protein level. B: Quantitative result of in-vitro AURKA kinase activity detected by kinase kit (OD450 value). C: Representative raw western blot bands for p-Aurora A (Thr288) and OD450 blot readout of kinase assay. β-actin was used as internal loading control. Data are presented as mean ± SD (n = 3). #P < 0.01 versus Ox-LDL/si-NC group.

into understanding the mechanisms of diseases like atherosclerosis, facilitating the comprehension of disease progression at the molecular level. Additionally, this study verified the function of the lncRNA-TCF7-AURKA axis in primary macrophages, eliminating the cell line-specific effects of THP-1 cells and enhancing the physiological relevance of the results [37-39].

Western blot results demonstrated that ox-LDL induction significantly increased the protein levels associated with the PI3K-AKT-mTOR pathway. As a crucial intracellular signaling cascade, the PI3K-AKT-mTOR pathway plays a pivotal role in various physiological and pathological processes, including cell growth, metabolism, and survival. As an oxidized form of LDL, ox-LDL activates this pathway through binding to receptors on the cell surface, triggering a series of signaling cascades that elevate the concentrations of pathway-related proteins. The inhibition of long non-coding RNA (lncRNA), TCF7, and AURKA - downstream of this pathway - suggests that these three factors may act as upstream regulators of the PI3K-AKT-mTOR pathway. Specifically, lncRNA is likely to influence the transcription and translation of pathway-related genes by interacting with DNA, RNA, or proteins; TCF7, as a transcription factor, directly regulates the expression of key

pathway genes; and AURKA may modulate the activity of pathway proteins through phosphorylation. These three factors may promote foam cell formation by activating the PI3K-AKT-mTOR pathway, thereby affecting intracellular lipid metabolism and inflammatory responses. Activation of the PI3K-AKT-mTOR pathway enhances lipid uptake and synthesis while suppressing lipolysis, leading to lipid accumulation and facilitating foam cell generation. Furthermore, this pathway is closely associated with inflammatory responses. Its activation may stimulate the release of inflammatory factors, thereby exacerbating the progression of diseases such as atherosclerosis. This discovery provides a theoretical basis for developing therapeutic strategies targeting this pathway and its related regulatory factors. Future research could focus on developing drugs that target these three factors or the PI3K-AKT-mTOR pathway to regulate foam cell formation and lipid metabolism, ultimately achieving the treatment of related disorders [40].

Experimental results from single-target and double-target silencing assays, combined with western blotting, RT-PCR, and other methods, demonstrate that silencing long non-coding RNAs (lncRNAs), TCF7, and AURKA enhances autophagy in macrophage-derived foam cells. As a vital cellular self-protective mecha-

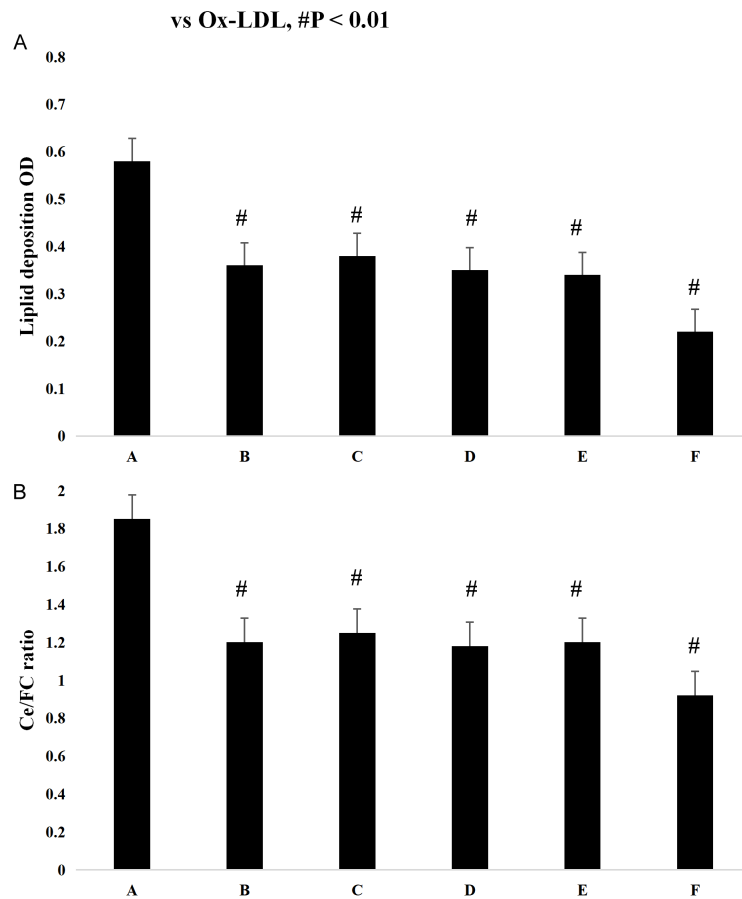


Figure 11. Analysis of lipid deposition and cholesteryl ester/free cholesterol ratio. A: Bar graph showing lipid deposition (Oil Red O OD value) in foam cells after treatment with PI3K/AKT/mTOR pathway inhibitors (LY294002, MK-2206, Rapamycin) alone or in combination with si-lncRNA. Data are presented as mean $\pm$ SD (n = 3). Compared to the Ox-LDL group, #P < 0.01. B: Bar graph showing the CE/FC ratio in foam cells after inhibitor treatment detected by HPLC. Data are presented as mean $\pm$ SD (n = 3). Compared to the Ox-LDL group, #P < 0.01.

Table 5. Lipid deposition (Oil Red O OD value)

Group	OD value (Mean $\pm$ SD)	P value vs Ox-LDL
Ox-LDL	0.58 $\pm$ 0.05	-
+LY294002	0.36 $\pm$ 0.04	< 0.001
+MK-2206	0.38 $\pm$ 0.03	< 0.001
+Rapamycin	0.35 $\pm$ 0.04	< 0.001
+si-lncRNA	0.34 $\pm$ 0.03	< 0.001
+si-lncRNA + LY294002	0.22 $\pm$ 0.02	< 0.001

nism, autophagy eliminates excess lipids and damaged organelles to maintain cellular homeostasis. In foam cells - where lipid accumulation and organelle damage are prominent - proper autophagic function is crucial for clearing these harmful substances. Previous studies

have established that impaired autophagy is closely associated with the development of atherosclerosis and other related diseases. When autophagy is compromised, intracellular lipids and damaged organelles accumulate uncontrollably, exacerbating cellular damage and disease progression. This study revealed that lncRNAs, TCF7, and AURKA may promote foam cell formation by inhibiting autophagy, thereby inducing intracellular lipid buildup. These three factors potentially suppress autophagy through interactions with autophagy-related genes or proteins, disrupting processes such as autophagosome formation and lysosomal fusion. Targeting their expression could therefore become a promising strategy to enhance autophagy and prevent atherosclerosis. Future therapeutic approaches may involve gene therapy or pharmacological interventions to regulate these factors, ultimately improving the efficiency of autophagy in foam cells, reducing lipid accumulation, and achieving the goals of disease prevention and treatment. Notably, this study is the first to integrate lncRNA, TCF7, and AURKA kinase activity into the same regulatory axis. Through inhibitor rescue experiments, it confirmed the causal role of the PI3K/AKT/mTOR pathway in this regulatory network, expanding the research dimension beyond the previous focus solely on expression levels [41].

Through functional endpoint experiments, this study linked the foam cell phenotype to cholesterol efflux, inflammatory factor release, and migratory capacity, providing a functional basis for the subsequent development of therapeutic strategies targeting the lncRNA-TCF7-AURKA

Table 6. CE/FC ratio (HPLC)

Group	CE/FC ratio (Mean $\pm$ SD)	P value vs Ox-LDL
Ox-LDL	1.85 $\pm$ 0.12	-
+LY294002	1.20 $\pm$ 0.08	< 0.001
+MK-2206	1.25 $\pm$ 0.09	< 0.001
+Rapamycin	1.18 $\pm$ 0.07	< 0.001
+si-lncRNA	1.20 $\pm$ 0.08	< 0.001
+si-lncRNA + LY294002	0.92 $\pm$ 0.06	< 0.001

axis. While this study has elucidated the critical roles of lncRNAs, TCF7, AURKA, and their related mechanisms in foam cell formation, it still has limitations. Current research relies primarily on *in vitro* experiments, which - although capable of simulating cellular physiological processes to a certain extent - differ significantly from *in vivo* conditions. In living organisms, these factors are regulated by multiple cell types, cytokines, hormones, and other elements, and the underlying mechanisms may be far more complex. Future efforts should focus on advancing animal studies and clinical trials. Animal models enable comprehensive investigation of the roles of these factors in disease progression (e.g., atherosclerosis) by monitoring blood lipid levels and vascular lesions, thereby providing deeper insights into their biological mechanisms. Clinical research can directly observe the manifestations of human diseases and the therapeutic effects of targeting these factors, laying a solid foundation for the development of diagnostic biomarkers and therapeutic targets. Additionally, future studies could explore pharmacologic or gene therapy approaches to precisely modulate the expression of these factors for the effective prevention and treatment of atherosclerosis and related disorders. Therapeutic strategies might include developing specific drugs to inhibit or activate these factors, while gene therapies could directly modify genetic sequences through technologies such as gene editing, enabling precision medicine. These advancements hold promise for delivering transformative solutions for the management of atherosclerosis and other cardiovascular diseases.

Conclusion

This study was the first to systematically clarify that the lncRNA-TCF7-AURKA axis promotes lipid deposition and inflammatory responses, inhibits macrophage autophagy, and acceler-

ates foam cell formation by activating the PI3K/AKT/mTOR signaling pathway. In both THP-1 and primary macrophages, silencing this axis can significantly reduce lipid accumulation, enhance cholesterol efflux, decrease the release of inflammatory factors, and inhibit cell migration - confirming its physiologic and functional relevance. This study provides new multi-molecular combined intervention targets for atherosclerosis and coronary heart disease.

Acknowledgements

The Role and Mechanism of the TCF7/AURKA Axis in the Pathogenesis of Coronary Heart Disease via Regulating Macrophage Foam Cell Formation (24JRRJ001).

Disclosure of conflict of interest

None.

Abbreviations

lncRNA, Long non-coding RNA; TCF7, T-cell factor 7; AURKA, Aurora kinase A; MDMs, Monocyte-derived macrophages; HPLC, High-performance liquid chromatography; Dil-oxLDL, Dil-labeled oxidized low-density lipoprotein; PI3K, Phosphatidylinositol 3-kinase; AKT, Protein kinase B; mTOR, Mammalian target of rapamycin; CE, Cholesterol ester; FC, Free cholesterol; LC3, Microtubule-associated protein 1 light chain 3; P62, Sequestosome 1; ApoA-I, Apolipoprotein A-I; IL-1 β , Interleukin-1 beta; TNF- α , Tumor necrosis factor-alpha; ORO, Oil Red O; M-CSF, Macrophage colony-stimulating factor; PBS, Phosphate-buffered saline; siRNA, Small interfering RNA; qRT-PCR, Quantitative real-time polymerase chain reaction; ELISA, Enzyme-linked immunosorbent assay; OD, Optical density; MFI, Mean fluorescence intensity; SD, Standard deviation; CAD, Coronary artery disease; Ox-LDL, Oxidized low-density lipoprotein; ATVB, Arteriosclerosis Thrombosis and Vascular Biology; NM, Nitrogen mustard; NMAR, Nitrogen mustard-induced atherosclerosis; si-NC, Negative control siRNA; LY294002, PI3K inhibitor; MK-2206, AKT inhibitor; Rapamycin, mTOR inhibitor; PMA, Phorbol 12-myristate 13-acetate; NLRP3, NOD-like receptor family pyrin domain containing 3; RISC, RNA-induced

LncRNA-TCF7-AURKA axis in macrophage foam cells and coronary heart disease

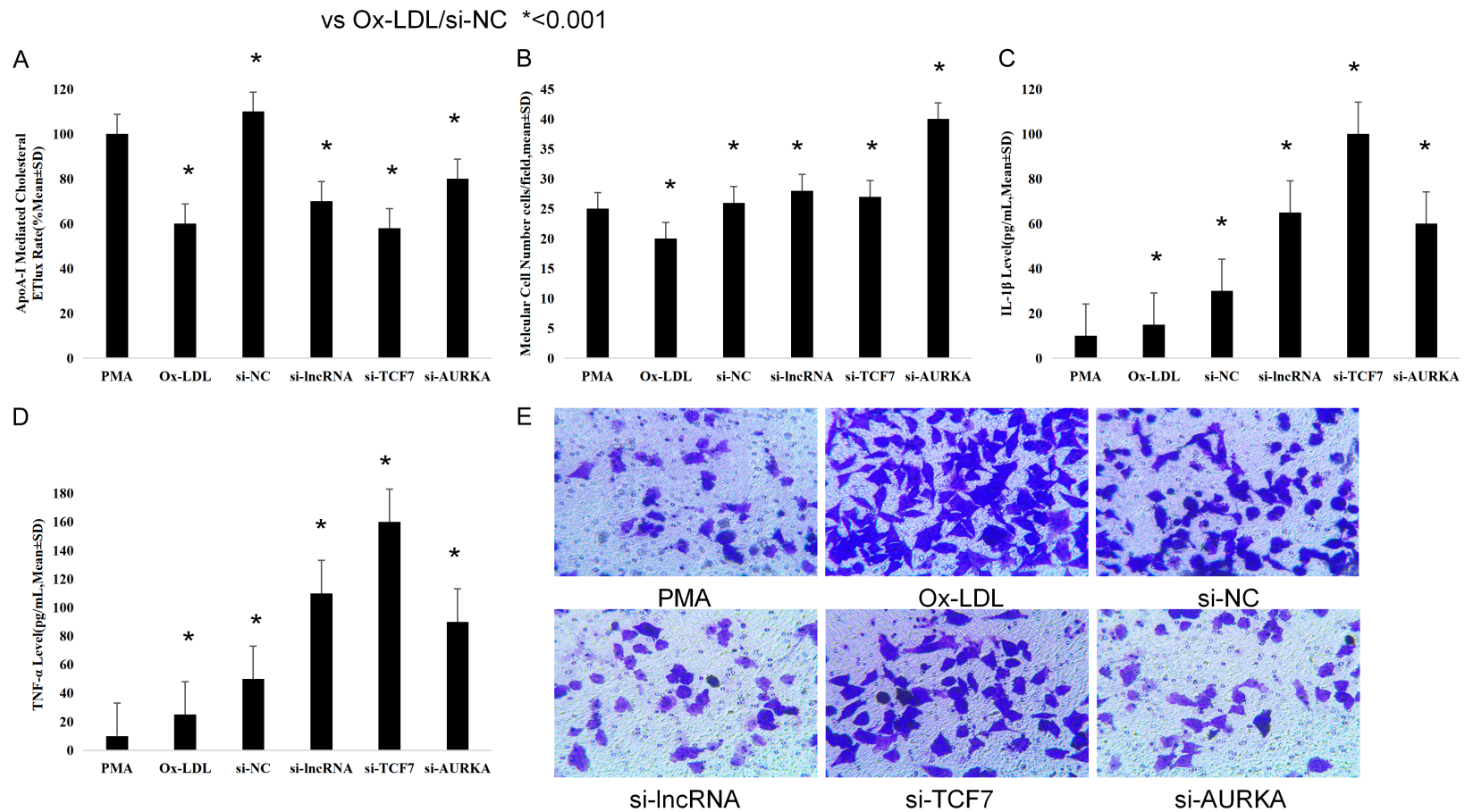


Figure 12. Functional phenotypes of foam cells following lncRNA-TCF7-AURKA axis silencing: cholesterol efflux, inflammatory cytokine secretion and cell migration. A: ApoA-I-mediated cholesterol efflux rate. B: Quantification of migrated cell number per field under microscope. C: Secreted IL-1 β level in cell supernatant detected by ELISA. D: Secreted TNF- α level in cell supernatant detected by ELISA. E: Representative crystal-violet-stained Transwell images of migrated foam cells (200 $\times$ magnification). Data are presented as mean $\pm$ SD (n = 3). *P < 0.01 or #P < 0.05 versus Ox-LDL/si-NC group.

silencing complex; P62/LC3-II/LC3-I, P62/Microtubule-associated protein 1 light chain 3-II/Microtubule-associated protein 1 light chain 3-I; Beclin1, Beclin 1; MCP-1, Monocyte chemoattractant protein-1; CAD, Coronary artery disease; CHOP, C/EBP homologous protein; UPR, Unfolded protein response; Rho, Ras homolog gene family member.

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