Upregulated MicroRNA-185-3p Inhibits the Development of Hyperlipidemia in Rats

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Keywords
Hyperlipidemia · MicroRNA-185-3p · Mastermind-like 1 · Inflammatory factors · Oxidative stress · Lipid accumulation

Abstract
Introduction: MicroRNA (miR)-185-3p plays a role in multiple types of cancers, while its underlying function remains obscure in hyperlipidemia. This research was conducted to unravel its function in hyperlipidemia development via modulating mastermind-like 1 (MAML1). Methods: The hyperlipidemia rat model was established by feeding with high-fat diet. miR-185-3p and MAML1 levels in hyperlipidemia rats were detected. Adenoviral vectors altering miR-185-3p and MAML1 levels were injected into hyperlipidemia rats to examine the levels of serum lipids, oxidative stress, inflammatory cytokine, lipid accumulation, and cellular morphology in liver tissues of hyperlipidemia rats. The targeting relation between miR-185-3p and MAML1 was manifested. Results: miR-185-3p expressed at a low level, while MAML1 expressed at a high level in hyperlipidemia rats. miR-185-3p overexpression or MAML1 silencing reduced levels of serum lipids, mitigated oxidative stress and inflammatory response, and relieved lipid accumulation and pathological morphology in liver tissues in hyperlipidemia rats, while upregulated MAML1 reversed the effects of augmented miR-185-3p in hyperlipidemia rats. Mechanically, miR-185-3p targeted MAML1. Conclusion: Upregulated miR-185-3p represses hyperlipidemia development via modulating MAML1 expression. This research provides novel therapeutic candidates for the treatment of hyperlipidemia.

Introduction
Hyperlipidemia is characterized by an imbalance of blood cholesterol levels, such as low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C). Such imbalance raises the risk of developing various cardiovascular diseases, such as myocardial infarction and stroke [1]. The major preventive modalities for hyperlipidemia should include behavioral changes, a reduced intake of fatty foods, a healthy diet, and appropriate physical activities [2]. Specifically, statins are the effective drugs to lower the elevated LDL-C; in addition, some substitute drugs for statins like ezetimibe, fibrates, niacin, and dietary supplements also contribute to the repression in the abnormally augmented LDL-C levels [3]. However, the efficacy of lipid-lowering therapy to hyperlipidemia is frequently negative owing to a combination of patient factors and therapeutic, socio-economic, and health system-associated variables [4]. Hence, it is obligatory to further propose novel therapeutic approaches to address such challenging scenarios.
As crucial fine-tuners of gene expression, microRNAs (miRs) have been employed in preclinical models of multiple cardiac diseases and show the potential for future development [5]. For instance, miR-181b has been observed to exhibit a low level in hyperlipidemia patients, and the amplified miR-181b effectively ablates the concentrations of inflammatory factors in human coronary endothelial cells [6]. In addition, both miR-181a-5p and miR-181a-3p are depleted in the plasma of hyperlipidemia mice, and the vascular inflammation and induced atherosclerosis are mitigated after treatment of miR-181a-3p and miR-181a-5p mimics [7]. As for miR-185-3p, recent research has unveiled that the deficient miR-185-3p is implicated in robustly expressed hypertrophic genes in cardiac hypertrophy [8]. There are some articles demonstrating the roles of miR-185 in hyperlipidemia. For instance, liver-specific miR-185 knockout mice have been revealed to develop a worsened hepatic steatosis upon a Western diet feeding with high fat and high cholesterol [9]. Meanwhile, miR-185 expresses at a low level in a time-dependent manner in high-fat diet (HFD)-fed C57BL/6 mice from the 8th week [10]. It was predicted through the bioinformatics website that miR-185-3p had binding sites with mastermind-like 1 (MAML1). As the major transcriptional coactivator of the Notch signaling pathway, MAML1 exerts a pleiotropic role in physiological and pathological signaling networks [11]. MAML1 has been unveiled to be associated with resting heart rate, acting as a putative risk factor for cardiovascular diseases [12]. Moreover, it has been found that mouse myocardial ischemia-reperfusion injury displays high levels of MAML1 [13]. As stated above, miR-185-3p and MAML1 exhibited significant therapeutic potential in cardiac diseases, while the detailed regulatory mechanism of the miR-185-3p/MAML1 axis in hyperlipidemia progression remained unclear. To this end, we aimed to unravel the function of miR-185-3p in hyperlipidemia via modulating MAML1, thereby furnishing the potential therapy candidates for hyperlipidemia treatment.

Materials and Methods

Ethics Statement

The protocol of animal experiments was approved by the Institutional Animal Care and Use Committee of Beijing Chao-Yang Hospital, Capital Medical University.

Experimental Animals

Six-week-old specific-pathogen-free grade male Sprague-Dawley rats were purchased from the animal center of Capital Medical Hospital, Capital Medical University. The rats were kept in captivity at 22–24°C and relative humidity (40–60%) with a light-dark cycle every 12 h. Rats were allowed to eat and drink freely and to acclimatize to the new environment for 1 w prior to the experiment.

Establishment of a Hyperlipidemia Rat Model

After 1-w acclimatization with normal chow, all animals were randomly classified into normal control groups (fed with the general diet) and hyperlipidemia model groups (fed with the HFD). HFD used in this study is a rodent diet containing 60% of calories from fat (D12492, Research Diets, Inc.) [14]. After 4-w modeling, when the concentrations of serum total cholesterol (TC) and triglycerides (TGs) in hyperlipidemia model rats were saliently higher than those in normal control rats, the successful modeling was confirmed [15].

Animal Grouping

After the successful modeling, the hyperlipidemia model rats were randomly classified into the following groups: mimic negative control (NC) group (injected with adenoviral vectors containing mimic NC), miR-185-3p mimic group (injected with adenoviral vectors containing miR-185-3p mimic), short hairpin RNA (sh)-NC group (injected with adenoviral vectors expressing shRNA NC), sh-MAML1 group (injected with adenoviral vectors expressing shRNA against MAML1), overexpression (oe)-NC group (injected with adenoviral vectors containing miR-185-3p mimic, oe-MAML1 group (injected with adenoviral vectors expressing pcDNA-NC), oe-MAML1 group (injected with adenoviral vectors containing pcDNA-MAML1), miR-185-3p mimic + oe-MAML1 group (injected with adenoviral vectors containing miR-185-3p mimic and adenoviral vectors containing pcDNA-MAML1), and miR-185-3p mimic + oe-MAML1 group (injected with adenoviral vectors containing miR-185-3p mimic and adenoviral vectors containing pcDNA-MAML1). There were six rats per group. After 4-w modeling, hyperlipidemia rats were subjected to the injections of 100 μL (1.95 × 10^12 vg/mL) adenoviral vectors containing miR-185-3p mimic, sh-MAML1, pcDNA-MAML1, and their corresponding NCs via the tail vein for 10 days. Adenoviral vectors (AAV9) containing miR-185-3p mimic, sh-MAML1, pcDNA-MAML1, and their corresponding NCs were constructed by GenePharma (Shanghai, China), and the viral genome titers were determined.

Sample Collection

At the end of the 12-w study, after anesthetization by pentobarbital sodium, all rats were fasted for 12 h and then severed from the head and neck. Blood was collected from the femoral artery. After 15-min centrifugation at 3,000 rpm, the serum was stored at −20°C. After rapid separation and weighing of the liver and total visceral fat (peri-testicular, perirenal, mesenteric, and retroperitoneal fat), the livers were frozen in liquid nitrogen and preserved at −80°C [15].

Biochemical Analysis

The concentrations of serum levels of TC, TG, HDL-C, and LDL-C were assessed through a spectrophotometer (UV-6100s, Mapada, Shanghai, China) concerning the kit instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). TG and TC levels were measured at 510 nm and HDL-C and LDL-C, at 546 nm. Other evaluation parameters were calculated as below: body fat ratio = whole fat weight (g)/body weight (g) × 100%; liver index = wet liver weight (g)/body weight (g) × 100% [16]. The original
mira-185-3p Functions in Hyperlipidemia

Detection of Inflammatory Factors

Lipid tissue homogenates of rats in each group were subjected to a centrifugation at 1,000 g for 20 min for the collection of the supernatant, which were then immediately assayed. ELISA kits were adopted to examine the concentrations of inflammatory factors (all from R&D Systems, Minneapolis, MN, USA) including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 [17]. The original measurements used for the quantifications of the experiments are shown in online supplementary Table 2.

Oil Red O Staining

Frozen sections of rat liver tissue were prepared, followed by 10-min Oil Red O (Servicebio, China) staining and 5-min hematoxylin counter-staining. The sections were observed under a light microscope (Olympus, Tokyo, Japan). The eligibility for lipid accumulation was analyzed using the ImageJ software (Microsoft Corporation, WA, USA) [16]. The original measurements used for the quantifications of the experiments are shown in online supplementary Table 2.

Hematoxylin-Eosin Staining

Hematoxylin-Eosin (HE) staining was implemented as described before [16]. In short, tissues were fixed in 4% formaldehyde in PBS and then dyed with HE. Next, a standard microscope (Olympus Imaging America Inc., USA) was employed to acquire images. The original figures of HE staining are shown in online supplementary Table 2.

Dual Luciferase Reporter Gene Assay

The wild-type (WT) or mutant (MUT) 3’UTR sequences of MAML1 with the predicted mir-185-3p binding sites were cloned into the downstream of luciferase gene in the pmirGLO dual luciferase mirT target expression vector (Promega, WI, USA) and named as MAML1-WT and MAML1-MUT. For the dual luciferase reporter gene assay, human embryonic kidney cells (HEK293T cells) (ATCC, Manassas, VA, USA) were seeded into 96-well plates and subjected to co-transfection with MAML1-WT, MAML1-MUT, mimic NC, or mir-185-3p mimic using the Lipofectamine 2,000 reagent (Invitrogen, CA, USA), which were respectively named as the mimic NC + MAML1-WT group, the mir-185-3p mimic + MAML1-WT group, the mimic NC + MAML1-MUT group, and the mir-185-3p mimic + MAML1-MUT group. Cells were obtained after 48-h post-transfection. The luciferase activity of firefly and renilla was assessed through the dual luciferase reporter gene assay system (Promega). The renilla luciferase activity was used for standardization [19]. The original measurements used for the quantifications of the experiments are shown in online supplementary Table 2.

RNA Immunoprecipitation Assay

The EZ-Magna RIP™ RNA Binding Protein Immunoprecipitation Kit (Millipore) was applied for RNA immunoprecipitation (RIP) assay. Cells were lysed in RIP lysis buffer. Afterward, the preincubated magnetic beads coated with the indicated antibodies were subjected to immunoprecipitation with the supernatant of the cell lysates at 4°C for 6 h. The purified RNA was then examined by reverse transcription quantitative polymerase chain reaction (RT-qPCR) [20]. The original measurements used for the quantifications of the experiments are shown in online supplementary Table 2.

Reverse Transcription Quantitative Polymerase Chain Reaction

The RNAiso Plus Total RNA Extraction Reagent (Takara, Shiga, Japan) was used for total RNA extraction. The reverse transcription of mRNA and miR was realized with the PrimeScript RT Reagent kit by using the gDNA Eraser (Takara) and the miScript II RT kit (Qiagen, Germany). The quantification for mRNA was analyzed using TB Green® Advantage® qPCR Premix (Takara), and the quantification for miR was conducted with a miScript SYBR-Green PCR kit (Qiagen). Gene expression was examined by the 2−ΔΔCt method. Glyceraldehyde-3-phosphate dehydrogenase and U6 were set as endogenous controls for mRNA and miR normalization. A thorough description of the primer sequences is indicated in online supplementary Table 1 [21]. The original measurements used for the quantifications of the experiments are shown in online supplementary Table 2.

Western Blot Analysis

The protein blotting was conducted concerning the standard protocols. A protein extraction kit was adopted for protein extraction. Proteins were separated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. After blocking with 5% skimmed milk, membranes were subjected to overnight incubation at 4°C with the appropriate primary antibodies MAML1 (1:1,000, Abcam, Cambridge, USA) [22] and glyceraldehyde-3-phosphate dehydrogenase (1:1,000, Cell Signaling, MA, USA). After sufficient washing, secondary antibodies were added. The target protein expression was detected by enhanced chemiluminescence and normalized to GADPH expression, whereas the signal detection was conducted using the enhanced chemiluminescence (Bio-Rad, Hercules, CA, USA) and assessed with the ImageJ software [23]. The original measurements used for the quantifications of the experiments are shown in online supplementary Table 2. The original figures for blots are shown in online supplementary Figure 1.

Statistical Analysis

All data were processed using GraphPad Prism 7.0 (GraphPad Software, Inc., La Jolla, CA). The data were represented as mean ± standard deviation. The t test was adopted for two-group comparisons, while one-way analysis of variance and Tukey’s post hoc test were adopted for multiple group comparisons, p < 0.05 was an indicator statistical significance.
Results

Establishment of the Hyperlipidemia Rat Model

The hyperlipidemia rat model was established by feeding with HFD. It revealed that serum levels of TC, TG, and LDL-C were augmented while HDL-C levels were decreased in hyperlipidemia model rats (Fig. 1a). The liver weight, total fat, body weight, liver index, and body fat ratio were also elevated in hyperlipidemia model rats (Fig. 1b). ELISA results indicated that the contents of inflammatory factors IL-1β, IL-6, and TNF-α were augmented in liver tissues of hyperlipidemia model rats (Fig. 1c). As reflected in Oil Red O staining, hyperlipidemia model rats exhibited a salient amplification of lipid accumulation (Fig. 1d). Oxidative stress measurements revealed an obvious elevation in MDA content but a reduction in SOD and CAT activities in the liver tissues of hyperlipidemia model rats (Fig. 1e). HE staining disclosed severe liver damages and increased fat vacuoles in hyperlipidemia model rats (Fig. 1f). These outcomes suggested that the hyperlipidemia rat model was successfully established.

Upregulation of miR-185-3p Suppresses Hyperlipidemia in Rats

It has been reported that miR-185-3p is silenced in the heart tissues of transverse aortic constriction rats [8]. To unravel miR-185-3p level in the liver tissues of hyperlipidemia model rats, miR-185-3p levels in the liver tissues of normal control rats and hyperlipidemia model rats were assessed by RT-qPCR, which disclosed that miR-185-3p was depleted in the liver tissues of hyperlipidemia model rats (Fig. 2a). The hyperlipidemia model rats were then subjected to injection of adenoviral vectors containing mimic NC or miR-185-3p mimic; the outcomes of RT-qPCR indicated that miR-185-3p was upregulated in the liver tissues of hyperlipidemia model rats after being treated with adenoviral vectors containing miR-185-3p mimic.
miR-185-3p Functions in Hyperlipidemia

(Fig. 2a). After the overexpression of miR-185-3p, it was suggested that, in hyperlipidemia model rats, the concentrations of TC, TG, and LDL-C in serum were reduced while HDL-C; liver weight; total fat; body weight; liver index; and body fat ratio in hyperlipidemia rats after the upregulation of miR-185-3p. The levels of TG, TC, LDL-C, and HDL-C; liver weight; total fat; body weight; liver index; and body fat ratio in hyperlipidemia rats after the upregulation of miR-185-3p.

Downregulated MAML1 Represses Hyperlipidemia, while Upregulation of MAML1 Deteriorates Hyperlipidemia in Rats

To unravel the expression and impacts of MAML1 in hyperlipidemia rats, the adenoviral vectors carrying sh-NC, sh-MAML1, oe-NC, and oe-MAML1 were injected into the tail vein of hyperlipidemia rats. The outcomes of RT-qPCR and Western blot analysis suggested that MAML1 exhibited a high level in hyperlipidemia model rats compared to normal control rats (Fig. 3a, b). Also, the MAML1 expression was silenced in the hyperlipidemia rats being treated with adenoviral vectors carrying sh-MAML1, yet it was elevated in the hyperlipidemia rats being treated with adenoviral vectors carrying oe-MAML1 (Fig. 3c, d). Affected by the silenced MAML1, it was disclosed that the serum TC, TG, and LDL-C levels; liver weight; total fat; body weight; liver index; and body fat ratio were decreased, while serum HDL-C levels were elevated in hyperlipidemia rats, while the opposite expression was observed in hyperlipidemia rats displaying upregulated MAML1 (Fig. 3e, f); IL-1β, IL-6, and TNF-α levels in liver tissues were depleted in hyperlipidemia rats after treatment of adenoviral vectors carrying oe-MAML1 (Fig. 3g); the lipid accumulation was significantly decelerated in hyperlipidemia.
Fig. 3. Downregulated MAML1 represses hyperlipidemia while upregulation of MAML1 deteriorates hyperlipidemia in rats. a–d MAML1 expression in liver tissues of hyperlipidemia rats was detected by RT-qPCR and Western blot analysis. e, f The levels of TG, TC, LDL-C, and HDL-C; liver weight; total fat; body weight; liver index; and body fat ratio in hyperlipidemia rats after the up- or downregulation of MAML1 were examined. g The contents of inflammatory factors IL-1β, IL-6, and TNF-α in hyperlipidemia rats after the up- or downregulation of MAML1 were detected by ELISA. h The lipid accumulation in hyperlipidemia rats after the up- or downregulation of MAML1 was measured by Oil red O staining. i The activities of SOD, CAT, and MDA in hyperlipidemia rats after the up- or downregulation of MAML1 were detected. j The cellular morphology of liver tissues in hyperlipidemia rats after the up- or downregulation of MAML1 was observed through HE staining. *p < 0.05 versus the control group; #p < 0.05 versus the sh-NC group; $p < 0.05 versus the oe-NC group.

Rats that treated with adenoviral vectors carrying sh-MAML1 but accelerated in hyperlipidemia rats treated with adenoviral vectors carrying oe-MAML1 (Fig. 3h); MDA content in liver tissues was significantly reduced, while SOD and CAT activities were promoted in hyperlipidemia rats injected with sh-MAML1 vectors, whereas
miR-185-3p Functions in Hyperlipidemia

Hyperlipidemia rats injected with oe-MAML1 vectors displayed opposite levels of these oxidative stress factors (Fig. 3i). HE staining showed relieved liver damage and fat vacuoles in hyperlipidemia rats injected with sh-MAML1 vectors, but aggravated liver injury and increased fat vacuolation were observed in hyperlipidemia rats injected with oe-MAML1 vectors (Fig. 3j). These findings above demonstrated that the silencing of MAML1 could restrain hyperlipidemia in rats, but the amplification of MAML1 exacerbated hyperlipidemia.

**miR-185-3p Targets MAML1**

miR-185-3p was predicted to bind to MAML1 through the TargetScan database (Fig. 4a). The dual luciferase reporter gene assay was conducted to further confirm the targeting relationship between miR-185-3p and MAML1, and it suggested that the co-transfection of MAML1-WT with miR-185-3p mimic impaired the luciferase activity in comparison to the co-transfection with MAML1-WT and mimic NC, while no change was witnessed in cells co-transfection with the MAML1-MUT and miR-185-3p mimic compared with the MAML1-MUT + mimic NC group (Fig. 4b). MAML1 levels in hyperlipidemia rats treated with adenoviral vectors containing mimic NC or miR-185-3p mimic were assessed by RT-qPCR and Western blot analysis, which suggested that miR-185-3p amplification resulted in depletion of MAML1 expression (Fig. 4c, d). The RIP assay indicated that miR-185-3p and MAML1 were preferentially enriched with the Ago2 antibody-incubated beads (Fig. 4e). These findings evidenced that miR-185-3p targeted MAML1.

![miR-185-3p targets MAML1](image)

Fig. 4. miR-185-3p targets MAML1. a The binding sites between miR-185-3p and MAML1 were predicted by the bioinformatics website. b The targeting relation between miR-185-3p and MAML1 was validated by the dual luciferase reporter gene assay. c, d MAML1 expression after the upregulation of miR-185-3p was detected by RT-qPCR and Western blot analysis. e The enrichment levels of miR-185-3p and MAML1 were examined by RIP assay. *p < 0.05 versus the mimic NC or anti-IgG group.
Upregulation of MAML1 Abrogates the Inhibitory Impacts of miR-185-3p Overexpression on Hyperlipidemia in Rats

To observe the effect of upregulation of MAML1 expression on hyperlipidemia in rats, the adenoviral vectors containing miR-185-3p mimic + oe-MAML1 were injected into hyperlipidemia rats. It was uncovered that in hyperlipidemia rats being treated with miR-185-3p mimic + oe-MAML1, the serum TC, TG, LDL-C, and HDL-C concentrations were elevated, while HDL-C contents were reduced; the liver total fat, body weight, liver index, and body fat ratio were elevated; IL-1β, IL-6, and TNF-α levels were augmented; MDA contents were amplified, while SOD and CAT activities were reduced; lipid accumulation was accelerated; the liver injury deteriorated and fat vacuoles were increased; the apoptosis of liver tissue was accelerated in comparison to hyperlipidemia rats being treated with miR-185–3p mimic + oe-NC (Fig. 5a–f). These results revealed that MAML1 amplification inverted the repressive effect of miR-185-3p elevation on hyperlipidemia in rats.

Discussion

Hyperlipidemia is a prevalent metabolic disorder and one of the major inducers for cardiovascular disease [24]. Anchored in the regulatory mechanism of miR-185-3p in hyperlipidemia, this research manifested that miR-185-3p elevation could block the development of hyperlipidemia via suppressing MAML1.

Initially, it was disclosed in our study that miR-185-3p was depleted in hyperlipidemia rats. The decrease of
miR-185-3p expression has also been investigated in some other diseases. For example, Xu et al. [8] have explicated that silenced miR-185-3p is displayed in mice with cardiac hypertrophy, and the reduced miR-185-3p is implicated in aggravated cardiac hypertrophy in rats. To further probe miR-185-3p function in hyperlipidemia, we then upregulated miR-185-3p expression in hyperlipidemia rats, and it was reflected that miR-185-3p overexpression restrained hyperlipidemia progression via reducing the contents of inflammatory factors, decreasing TC, TG, LDL-C, and HDL-C levels in serum, mitigating oxidative stress and lipid accumulation, and relieving liver injury. The repressive influences of miR-185-3p on the inflammatory response in other disease have also been confirmed by Ma et al. [25], who have elucidated that the upregulated miR-185-3p contributes to decelerating the progression of malignant inflammatory bowel disease via modulating the inflammation-related pathway. As for miR-185-3p impacts in oxidative stress, miR-185-3p is validated to be low-expressed in diabetic nephropathy mice; after the amplification of miR-185-3p, the activities of SOD and CAT are promoted while MDA content is decelerated, validating miR-185-3p's efficacy in relieving oxidative stress and renal function [26]. In addition, it has been reported that miR-185-3p can even hinder the development of nasopharyngeal carcinoma via restraining the biological activities of nasopharyngeal carcinoma cells [27].

Thereafter, it was predicted that miR-185-3p had binding sites for MAML1 through the bioinformatics websites. Here in the current study, MAML1 was confirmed to display a high level in hyperlipidemia rats. The high level of MAML1 has also been explored in some other types of disease. For instance, the highly expressed MAML1 has also been validated in the myocardium with ischemia-reperfusion injury [13] and in the heart, spleen, pancreas, and leukocytes in peripheral blood [12]. To unravel the MAML1’s function in hyperlipidemia development, MAML1 was up- or downregulated in the current study, and it was demonstrated that the silenced MAML1 could attenuate hyperlipidemia in rats via suppressing the contents of inflammatory factors, reducing TC, TG, LDL-C, and HDL-C serum levels, mitigating oxidative stress and lipid accumulation, and attenuating liver injury. Some tendencies of restored or depleted MAML1 have been verified in some other diseases. Kratsios et al. [28] have illustrated that MAML1 accumulation leads to various phenotypic defects after myocardial infarction. As for MAML1 effects on the inflammatory response, MAML1 depletion contributes to mitigating the inflammatory osteoclastogenesis induced by inflammatory cytokine TNF-α [29]. Moreover, in chronic inflammatory disease, the silenced MAML1 induces the inactivation of Notch signaling, thus protecting against inflammatory osteolysis [30]. Jin et al. [31] have demonstrated that MAML1-deficient mouse embryonic fibroblasts display impaired TNF-α activities. In addition, our study further validated that MAML1 deficiency could restrain liver injury in hyperlipidemia rats. As reported by Zheng et al. [32], MAML1 knockdown effectively ameliorates liver fibrosis in rats.

Taken together, this research manifests that miR-185-3p expression is reduced while MAML1 expression is elevated in hyperlipidemia rats. The most noteworthy finding is that the amplified miR-185-3p can mitigate hyperlipidemia in rats via downregulating MAML1. By highlighting the underlying mechanism of the miR-185-3p/MAML1 axis in hyperlipidemia, this study makes a contribution to pave the path for promising treatment regimens of hyperlipidemia. Nevertheless, the potential mechanism of silenced miR-185-3p in hyperlipidemia deserves further investigation.

Statement of Ethics

The protocol of animal experiments was approved by the Institutional Animal Care and Use Committee of Beijing Chao-Yang Hospital, Capital Medical University.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

Funding Sources

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author Contributions

Hua Zhao contributed to the study design, manuscript editing, and data analysis; Yanbing Li contributed to experimental studies and data analysis. All authors read and approved the final manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material. Further inquiries can be directed to the corresponding author.
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