Up-regulated microRNA-185-3p inhibits the development of hyperlipidemia in rats

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Research

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Abstract

Objective

MicroRNA (miR)-185-3p roles have been probed in multiple cancers, while the underlying function of miR-185-3p in hyperlipidemia remained obscure. This research was conducted to unravel miR-185-3p function in hyperlipidemia development via modulating mastermind-like 1 (MAML1).

Methods

The hyperlipidemia rat model was constructed. 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 biochemical indices, inflammatory factors, oxidative stress, 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 levels were depleted while MAML1 expression was elevated in HH rats. MiR-185-3p overexpression or MAML1 silencing reduced levels of inflammatory factors in serum, mitigated oxidative stress and biochemical response, relieved lipid accumulation and cellular morphology in liver tissues; while up-regulated MAML1 reversed the effects of augmented MAML1 in hyperlipidemia rats. MiR-185-3p targeted MAML1.

Conclusion

Up-regulated miR-185-3p represses hyperlipidemia development via modulating MAML1. 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 and reduced intake of fatty foods, remaining a healthy diet and appropriate physical activities [2]. Specifically, statins are the effective drugs to lower elevated LDL-C, in addition, some substitute drugs for statins like ezetimibe, fibrates, niacin, and dietary supplements also contribute to repressing 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, 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 pre-clinical 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 mimic [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]. Nevertheless, the researches for probing miR-185-3p function in hyperlipidemia development remained extremely rare. It was predicted through the bioinformatics website that miR-185-3p had binding sites with Mastermind-like 1 (MAML1). As the major transcriptional co-activator of Notch signaling pathway, MAML1 exerts a pleiotropic role in physiological and pathological signaling networks [9]. MAML1 has been unveiled to be associated with resting heart rate, acting as a putative risk factor for cardiovascular diseases [10]. Moreover, it has been found that mouse myocardial ischemia-reperfusion injury displays high levels of MAML1 [11]. 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 aim 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 weighing 260 ± 9 g were purchased from Nanjing Medical University (Nanjing, China). The rats were kept in captivity at 22-24°C and relative humidity (40-60%) with a light-dark cycle every 12 hours. Rats were allowed to eat and drink freely and to acclimatize to the new environment for 1 w prior to the experiment.

Hyperlipidemia rat model

After 1-w acclimatization with normal chow, all animals were randomly classified into normal control groups and high-fat diet (HH) model groups. After 4-w modeling, when the concentrations of serum total cholesterol (TC) and triglycerides of the rats in hyperlipidemia rat were saliently higher than those in normal rats, the successful modeling was confirmed [12].

Animal grouping

After the successful modeling, the HH rats were randomly classified into: mimic NC group (injected with high-expressed miR-185-3p negative control [NC] vectors), miR-185-3p mimic group (injected with high-expressed miR-185-3p vectors), short hairpin RNA (sh)-NC group (injected with low-expressed MAML1 NC vectors), sh-MAML1 group (injected with low-expressed MAML1 vectors), overexpression (oe)-NC group (injected with high-expressed MAML1 NC vectors), oe-MAML1 group (injected with high-expressed MAML1 vectors), miR-185-3p mimic + oe-NC group (injected with high-expressed miR-185-3p and MAML1 blank control vectors), miR-185-3p mimic + oe-MAML1 (injected with high-expressed miR-185-3p and high-expressed MAML1 vectors). After 4-w modeling, rats were subjected to tail vein injections of 100 μL (1.95×10^{12} vg/mL) miR-185-3p mimic, sh-MAML1, and oe-MAML1 adenoviral vectors (AAV9) for 10 days. There were six rats per group. At the end of the 12-w study, after anesthetization by sodium pentobarbital, all rats were fasted for 12 h, and then severed from the head and neck. Blood was collected from the retro-orbital venous plexus. After 15-min centrifugation at 3000 rpm, serum was stored at -20°C . After rapid separation and weighing to 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 [12].

Biochemical analysis

After the animal treatment, the concentrations of serum TC, triglyceride (TG), HDL-C and LDL-C were assessed through a spectrophotometer (UV-6100s, Mapada, Shanghai, China) concerning the kit instructions (F002, F001-1-1, A112-1-1, A113-1-1 Nanjing Jiancheng Bioengineering Institute, Nanjing, China). TG and TC levels were measured at 510 nm, HDL-C and LDL-C at 546 nm. Other evaluation parameters were calculated as below: body fat ratio = the whole fat weight (g)/body weight (g) $\times 100\%$; liver index = wet liver weight (g)/body weight (g) $\times 100\%$ [13].

Enzyme-linked immunosorbent assay (ELISA)

After 20-min centrifugation at 1000 g, the supernatant was obtained and then immediately assayed. ELISA kits were adopted to examine the concentrations of inflammatory factors (all from R&D Systems, Minneapolis, MN, USA) tumor necrosis factor (TNF)- α (DY410), interleukin (IL)-1 β (MLB00C), and IL-6 (DY406) [14].

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 analysed using the ImageJ software (Microsoft Corporation, WA, USA) [13].

Determination of oxidative stress indicators

Frozen samples were subjected to homogenization in phosphate-buffered saline, and the supernatant of tissue homogenate was used to estimate oxidative stress indicators. Malondialdehyde (MDA) was

detected according to the red products generated from the reaction between MDA and thiobarbituric acid, and was quantified using a spectrophotometer. A standard curve was constructed based on a reference standard to quantify MDA levels. The activities of superoxide dismutase (SOD) (A001-3-1) and catalase (CAT) (A007-1-1, Nanjing Jiancheng Bioengineering Institute) in liver tissue were tested with regard to the kit instruction [15].

Hematoxylin-eosin (HE) staining

Rat liver tissue was fixed with 4% paraformaldehyde solution, dehydrated with gradient, embedded with paraffin, and sectioned into 4 μm slices. After staining with hematoxylin and eosin (Servicebio, Wuhan, China), the slices were observed under a light microscope (Olympus) [13].

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 luciferase gene in the pmirGLO dual luciferase miRNA target expression vector (Promega, WI, USA). For the dual luciferase reporter gene assay, HEK293T cells were seeded into 96-well plates, and subjected to co-transfection with 0.5 μg MAML1-WT, MAML1-MUT, mimic NC or miR-185-3p mimic plasmids using the Lipofectamine 2000 reagent (Invitrogen, CA, USA). 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 [16].

RNA immunoprecipitation (RIP) assay

The EZ-Magna RIP™ RNA Binding Protein Immunoprecipitation Kit (Millipore) was applied for RNA immunoprecipitation assay. Cells were lysed in RIP lysis buffer. Afterward, pre-incubated 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) [17].

RT-qPCR

The Takara RNAiso Reagent Kit (Takara, 9108, Shiga, Japan) was used for total RNA extraction; and the TaqMan MicroRNA Kit (4440886, Applied Biosystems, CA, USA) was adopted for miRNA extraction. The cDNA reverse transcription was conducted by adopting the PrimeScript RT Reagent Kit (Takara, RR047Q), and the miRNA was reversely transcribed into cDNA using the miScript Reverse Transcription Kit (QIAGEN, 218061, Hilden, Germany). The miScript SYBR Green PCR Kit (QIAGEN, 218075, Germany) was used for real-time qPCR assessment. Gene expression was examined by the $2^{-\Delta\Delta\text{Ct}}$ method. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 were set as endogenous controls for mRNA and miRNA normalization. A thorough description of the primer sequences was indicated in Table 1 [18].

Table 1
Primer sequences used for RT-qPCR

Genes	Primer sequences (5'– 3')
miR-185-3p	F: GGGGCTGGCTTTCCTCTG
	R: GTGGAGTCGGCAATTGCAC
U6	F: GCTTCGGCAGAGCACATATACTAA
	R: AACGCTTCACGAATTTGCGT
MAML1	F: ACAAGTCCCAAGGGTGTCAG
	R: TCACACAGCTGTTCCCAGAC
GAPDH	F: GTCAACGGATTTGGTCTGTATT
	R: AGTCTTCTGGGTGGCAGTGAT
Note: F, forward; R, reverse; miR-185-3p, microRNA-185-3p; MAML1, mastermind-like 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase	

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 sulphate 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; ab65090, 1:1000, Abcam, Cambridge, USA) (GAPDH; 2118, 1:1000, Cell Signaling, MA, USA). After sufficient washing, secondary antibodies were added. The target protein expression was detected by enhanced chemiluminescence and normalized to GAPDH expression; whereas the signal detection was conducted using the enhanced chemiluminescence (Bio-Rad, Hercules, CA, USA) and assessed with the ImageJ software (1.51K) [19].

Statistical analysis

All data were processed using SPSS 21.0 statistical software (IBM, Armonk, NY, USA). The measurement data were represented as mean $\pm$ standard deviation. Independent samples t-test was adopted for two groups comparisons; while one-way analysis of variance (ANOVA) and Tukey's post hoc test were adopted for multiple group comparisons. $P < 0.05$ was an indicator statistical significance.

Results

Construction of the HH rat model

The HH model was established to induce hyperlipidemia in rats. It came out that serum contents of TC, TG and LDL-C were augmented while HDL-C levels were decreased in HH rats (Fig. 1A). The liver weight,

total fat, body weight, liver index and body fat ratio were also elevated in HH rats (Fig. 1B). ELISA results indicated that the contents of inflammatory factors IL-1 β , IL-6 and TNF- α were augmented in liver tissues of HH rats (Fig. 1C). As reflected in Oil Red O staining, HH 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 HH rats (Fig. 1E). HE staining disclosed severe liver damages and increased fat vacuoles (Fig. 1F). These outcomes suggested that the HH rat model was successfully constructed.

Upregulation of miR-185-3p suppresses hyperlipidemia in rats

It has been reported that miR-185-3p is silenced in the heart tissue of transverse aortic constriction rats [8]. However, miR-185-3p was poorly studied in hyperlipidemia in rats. To unravel miR-185-3p level in the liver tissues of HH rats, miR-185-3p levels in the liver tissues of Control rats and HH rats was assessed by RT-qPCR, which disclosed that miR-185-3p was depleted in the liver tissues of HH rats (Fig. 2A). The HH 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 up-regulated in the liver tissues of HH rats after being treated with miR-185-3p mimic (Fig. 2B). After the overexpression of miR-185-3p, it was suggested that, in HH rats, the concentrations of TC, TG, LDL-C in serum were reduced while HDL-C was elevated (Fig. 2C); the liver weight, total fat, body weight, liver index, and body fat ratio were all reduced (Fig. 2D); the inflammatory factors IL-1 β , IL-6 and TNF- α were depleted as shown in ELISA (Fig. 2E); the lipid accumulation was decelerated as suggested in Oil red O staining (Fig. 2F); the contents of MDA were decreased while the activities of SOD and CAT were accelerated (Fig. 2G). Moreover, the HE staining showed mitigated liver injury and fewer fat vacuoles in HH rats with miR-185-3p mimic (Fig. 2H). These discoveries uncovered that the up-regulated miR-185-3p contributed to inhibiting hyperlipidemia in rats.

Down-regulated MAML1 represses hyperlipidemia while up-regulation of MAML1 deteriorates hyperlipidemia in rats

To unravel the expression and impacts of MAML1 in hyperlipidemia rats, the adenoviral vectors carrying sh-NC and sh-MAML1, oe-NC adenoviral vector and oe-MAML1 were injected into to the tail vein of hyperlipidemia rats. The outcomes of RT-qPCR and Western blot analysis suggested that MAML1 exhibited a high level in HH rats (Fig. 3A, B). And the MAML1 expression was silenced in the HH rats being treated with sh-MAML1 yet elevated in HH rats being treated with oe-MAML1 (Fig. 3C, D). Affected by the silenced MAML1, it was disclosed that the levels of TC, TG, LDL-C serum, and liver weight, total fat, body weight, liver index and body fat ratio were decreased while HDL-C was elevated in HH rats, while the opposite expression was observed in rats displaying up-regulated MAML1 (Fig. 3E, F); IL-1 β , IL-6 and TNF- α in liver tissues was depleted in HH rats after treatment of sh-MAML1, while amplified in HH rats with oe-MAML1 (Fig. 3G); the lipid accumulation was significantly decelerated in rats that treated with sh-MAML1 but accelerated in rats treated with oe-MAML1 (Fig. 3H); MDA content in liver tissue was significantly

reduced while SOD and CAT activities were promoted in rats with down-regulated MAML1, whereas rats with oe-MAML1 displayed opposite levels of these oxidative stress factors (Fig. 3I). HE staining showed relieved liver damage and fat vacuoles in the sh-MAML1 group, but aggravated liver injury and increased fat vacuolation were observed in oe-MAML1 group (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 bound to MAML1 through the database RNA22 (Fig. 4A). The dual luciferase reporter gene assay was conducted to further confirm the targeting relationship between miR-185-3p and MAML1, and it turned out that miR-185-3p impaired the luciferase activity after co-transfection with MAML1-WT and miR-185-3p mimic (Fig. 4B). MAML1 levels in rats treated with mimic NC or miR-185-3p mimic were assessed by RT-qPCR and Western blot, which suggested that miR-185-3p amplification resulted in depletion of MAML1 (Fig. 4C, D). The RIP assay indicated that miR-185-3p and MAML1 were preferentially enriched on the Ago2 antibody (Fig. 4E). These findings evidenced that miR-185-3p targeted MAML1.

Up-regulation of MAML1 abrogates the inhibitory impacts of miR-185-3p overexpression on hyperlipidemia in rats

To observe the effect of up-regulation of MAML1 expression on hyperlipidemia in rats, adenoviral vectors containing miR-185-3p mimic + oe-NC or miR-185-3p mimic + oe-MAML1 were injected into HH rats. It was uncovered that in rats being treated with miR-185-3p mimic + oe-MAML1, the TC, TG, LDL-C and HDL-C concentrations were elevated; while HDL-C contents were reduced; the liver weight, total fat, body weight, liver index and body fat ratio were elevated; IL-1 β , 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 (Fig. 5A-F). These results unveiled 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 induces for cardiovascular disease [20]. 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. In line with our findings, Xu *et al.* 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 [8]. To further probe miR-185-3p function in hyperlipidemia, we then up-regulated 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 in serum, mitigating oxidative stress and lipid accumulation, and relieving liver injury. The similar repressive influences of miR-185-3p on the inflammatory response have also been confirmed by Ma *et al.*, who have elucidated that the up-regulated miR-185-3p contributes to decelerating the progression of malignant inflammatory bowel disease via modulating the inflammation-related pathway [21]. 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 efficacy in relieving oxidative stress and renal function [22]. 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 [23].

Thereafter, it was predicted that miR-185-3p had binding sites with MAML1 through the bioinformatics websites. Here in the current study, miR-185-3p was confirmed to display a high level in hyperlipidemia rats. Consistently, the robustly expressed MAML1 has also been validated in the myocardium with ischemia-reperfusion injury [11] and in the heart, spleen, pancreas and leukocytes in peripheral blood [10]. To unravel MAML1 function in hyperlipidemia development, MAML1 was up- or -down-regulated 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 levels, mitigating oxidative stress and lipid accumulation, and attenuating liver injury. Partly consistent to our finding, Kratsios *et al.* have illustrated that MAML1 accumulation leads to various phenotypic defects after myocardial infarction [24]. As for MAML1 effects on the inflammatory response, MAML1 depletion contributes to mitigating the inflammatory osteoclastogenesis induced by inflammatory cytokine TNF- α [25]. Moreover, in chronic inflammatory disease, the silenced MAML1 induces the inactivation of Notch signaling, thus protecting against inflammatory osteolysis [26]. Jin *et al.* have demonstrated that MAML1-deficient mouse embryonic fibroblasts display impaired TNF- α activities [27]. In addition, our study further validated that MAML1 deficiency could restrain liver injury in hyperlipidemia rats. As similarly reported by Zheng *et al.*, MAML1 knockdown effectively ameliorates liver fibrosis in rats [28].

Taken together, this research manifests that miR-185-3p is silenced 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 down-regulating 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, and more study samples and grouping samples are required in future work, thus diminishing the potential data error in experimental results.

Declarations

Funding

None

Ethics approval and consent to participate

Animal experiments were conformed to the Guide to the Management and Use of Laboratory Animals issued by the National Institutes of Health. 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

The authors declare no competing interests.

Consent for publication

Not applicable

Availability of data and material

Not applicable

Authors' contributions

Hua Zhao contributed to study design and manuscript editing; Yanbing Li contributed to experimental studies; Hua Zhao and Yanbing Li contributed to data analysis.

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Figures

Figure 1

Construction of the HH rat model. A/B, the levels of TG, TC, LDL-C, HDL-C, liver weight, total fat, body weight, liver index and body fat ratio were examined by Biochemical analysis; C, the contents of inflammatory factors IL-1 β , IL-6 and TNF- α were detected by ELISA; D, the lipid accumulation was measured by Oil red O staining; E, the activities of SOD, CAT, MDA were detected; F, the cellular morphology of liver tissues was observed through HE staining. The measurement data were represented as mean $\pm$ standard deviation. Independent samples t-test was used to compare data between two groups. * $P < 0.05$ vs. the control group.

Figure 2

Upregulation of miR-185-3p suppresses hyperlipidemia in rats. A/B, miR-185-3p expression in liver tissues of HH rats were detected by RT-qPCR; C/D, the levels of TG, TC, LDL-C, HDL-C, liver weight, total fat, body weight, liver index and body fat ratio in HH rats after the up-regulation of miR-185-3p were examined by biochemical analysis; E, the contents of inflammatory factors IL-1 β , IL-6 and TNF- α in HH rats after the up-regulation of miR-185-3p were detected by ELISA; F, the lipid accumulation in HH rats after the up-regulation of miR-185-3p was measured by Oil red O staining; G, the activities of SOD, CAT, MDA in HH rats after the up-regulation of miR-185-3p were detected; H, the cellular morphology of liver tissues in HH rats after the up-regulation of miR-185-3p was observed through HE staining. The measurement data were represented as mean $\pm$ standard deviation. Independent samples t-test was used to compare data between two groups. # $P < 0.05$ vs. the Control group; * $P < 0.05$ vs. the mimic NC group.

Figure 3

Down-regulated MAML1 represses hyperlipidemia while up-regulation of MAML1 deteriorates hyperlipidemia in rats. A-D, MAML1 expression in liver tissues of HH rats was detected by RT-qPCR and Western blot analysis; E/F, the levels of TG, TC, LDL-C, HDL-C, liver weight, total fat, body weight, liver index and body fat ratio in HH rats after the up- or down-regulation of MAML1 were examined by biochemical analysis; G, the contents of inflammatory factors IL-1 β , IL-6 and TNF- α in HH rats after the up- or down-regulation of MAML1 were detected by ELISA; H, the lipid accumulation in HH rats after the up- or down-regulation of MAML1 was measured by Oil red O staining; I, the activities of SOD, CAT, MDA in HH rats after the up- or down-regulation of MAML1 were detected; J, the cellular morphology of liver tissues in HH rats after the up- or down-regulation of MAML1 was observed through HE staining. The measurement data were represented as mean $\pm$ standard deviation. Independent samples t-test was used to compare data between two groups; one-way ANOVA and Tukey's post hoc test were used to compare data among multiple groups; # $P < 0.05$ vs the Control group; * $P < 0.05$ vs. the sh-NC group; \$ $P < 0.05$ vs. the oe-NC group.

Figure 4

MiR-185-3p targets MAML1. A, the binding sites between miR-185-3p and MAML1 were predicted by the bioinformatics website RNA22; 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 up-regulation 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. The measurement data were represented as mean $\pm$ standard deviation. Independent samples t-test was used to compare data between two groups; * $P < 0.05$ vs. the mimic NC group.

Figure 5

Up-regulation of MAML1 reverses the inhibitory effect of miR-185-3p overexpression on hyperlipidemia in rats. A/B, the levels of TG, TC, LDL-C, HDL-C, liver weight, total fat, body weight, liver index and body fat ratio in HH rats after being treated with miR-185-3p mimic + oe-MAML1 were examined by biochemical analysis; C, the contents of inflammatory factors IL-1 β , IL-6 and TNF- α in HH rats after being treated with miR-185-3p mimic + oe-MAML1 were detected by ELISA; D, the lipid accumulation in HH rats after being treated with miR-185-3p mimic + oe-MAML1 was measured by Oil red O staining; E, the activities of SOD, CAT, MDA in HH rats after being treated with miR-185-3p mimic + oe-MAML1 were detected; F, the cellular morphology of liver tissues in HH rats after being treated with miR-185-3p mimic + oe-MAML1 was observed through HE staining. The measurement data were represented as mean $\pm$ standard deviation. Independent samples t-test was used to compare data between two groups; * $P < 0.05$ vs. the miR-185-3p mimic + oe-NC group.