Knockdown of mTOR by lentivirus-mediated RNA interference suppresses atherosclerosis and stabilizes plaques via a decrease of macrophages by autophagy in apolipoprotein E-deficient mice

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Abstract. Atherosclerotic plaque destabilization and rupture leads to acute coronary syndromes which cause serious damage to human health worldwide. However, there is currently a lack of efficient therapeutic methods. Mammalian target of rapamycin (mTOR) has been suggested to be involved in the development of atherosclerotic plaques and serves as a therapeutic target. The present study was performed to determine whether RNA interference (RNAi) of mTOR *in vivo* by LV-mediated small hairpin RNA (shRNA) was capable of inhibiting the progression of atherosclerotic plaques. LV-mediated shRNA against mTOR (LV-shmTOR) was designed and obtained. Male apolipoprotein E-deficient mice were fed a high-fat diet and a constrictive collar was placed around the right carotid arteries of these mice to induce plaque formation. Eight weeks after surgery, mice were randomly divided into the mTOR RNA interference (LV-shmTOR) group, receiving treatment with LV-mTOR-shRNA; the LV-shCON group, receiving treatment with LV-non-specific-shRNA; and the control group, receiving treatment with phosphate-buffered saline. Following transfection, the mice were sacrificed to evaluate the effects of mTOR expression silencing on atherosclerosis. Transfection of LV-mTOR-shRNA markedly inhibited the mRNA and protein expression levels. Knockdown of mTOR ameliorated dysregulated blood lipid metabolism and stabilized aortic atherosclerotic plaques by decreasing the plaque area and increasing the fibrous cap and cap-to-core ratio. Furthermore, macrophages were decreased by silencing mTOR in atherosclerotic plaques. In addition, western blot analysis revealed that the knockdown of mTOR increased autophagy-related protein 13 (Atg13) dephosphorylation and light chain 3-I/light chain 3-II (LC3-I/LC3-II) ratios, both of which were associated with a high activity of autophagy, suggesting an increase of autophagy in atherosclerotic plaques. Moreover, genes including matrix metalloproteinase 2, monocyte chemoattractant protein 1 and tissue factor, which promote plaque instability, were downregulated by silencing mTOR. These results demonstrate that LV-mediated mTOR silencing by RNAi treatment induces macrophage autophagy and is a potential strategy for the treatment of atherosclerotic plaques.

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Abbreviations: mTOR, mammalian target of rapamycin; shRNA, small hairpin RNA; RNAi, RNA interference; LV, lentivirus; MMPs, matrix metalloproteinases; MCP-1, monocyte chemoattractant protein 1; TF, tissue factor

Key words: mammalian target of rapamycin, small hairpin RNA, atherosclerotic plaque, macrophage, autophagy

Introduction

Cardiovascular diseases such as myocardial infarction and stroke, which cause serious damage to human health, are mainly caused by the destabilization and rupture of atherosclerotic plaques (1,2). However, there is currently a lack of efficient therapeutic methods for the treatment of atherosclerotic plaque stability. The hallmarks of rupture-prone plaques are a large lipid core, a thin fibrous cap and loss of collagen caused by leukocyte infiltration and a decrease of smooth muscle cells (SMCs) (3,4). The stability of the thickness and structure of the fibrous cap are crucial for plaque stability (5). Therefore, reduction of acute coronary events through stabilization of atherosclerotic plaques has become a new treatment target.

Macrophages are largely accumulated in atherosclerotic plaques, which is crucial in the occurrence of atherosclerosis as well as in the process of atherosclerotic plaque rupture (6). Macrophages express inflammatory factors including matrix

metalloproteinases (MMPs) (7), monocyte chemoattractant protein 1 (MCP-1) (8) and tissue factor (TF) (9), which promote the destabilization and rupture of atherosclerotic plaques. Therefore, macrophage removal has been considered to be beneficial for plaque stability (10).

Previous studies reported that everolimus, which is a rapamycin derivative and an inhibitor of mammalian target of rapamycin (mTOR), selectively eliminated macrophages in atherosclerotic plaques, suggesting that mTOR is important in the destabilization and rupture of atherosclerotic plaques (11). mTOR is a member of the phosphoinositol kinase-related kinase (PIKK) family which mediates gene translation in the presence of nutrients including insulin, mitogens and growth factors involved in cell growth, differentiation, migration and survival (12,13). Inhibition of mTOR has been shown to have anti-cell proliferative effects *in vitro* and *in vivo* (14), leading to cell apoptosis (15,16) and autophagic cell death (17,18). Thus, mTOR has become a new target for the treatment of diseases such as cancer (19). mTOR also affects inflammatory cell activity and cytokine release (20). Inhibition of mTOR by sirolimus or everolimus suppresses atherosclerosis and shows a decrease in macrophages via the induction of autophagy (11,21). Autophagy is considered to be a conserved pathway for the destruction of damaged or unwanted intracellular material, including proteins and entire organelles contributing to cell homeostasis, and is closely associated with various diseases (22,23). Given this, targeting mTOR for macrophage autophagy is a promising strategy for the treatment of atherosclerotic plaque stability.

Molecular-targeted therapy is also considered to have potential applications in disease treatment. RNA interference (RNAi) is a simple and effective method for blocking gene expression and has great advantages over traditional methods for cardiovascular disease therapy. In the present study, we used small hairpin RNA (shRNA) lentivirus (LV)-mediated-targeted mTOR in a mouse model that specifically inhibited mTOR expression *in vivo*. Results showed that the transfection of LV-mediated mTOR shRNA effectively inhibited mTOR mRNA and protein expression in the carotid artery of the mouse model. Silencing of mTOR ameliorated the dysregulated blood lipid metabolism caused by a high-fat diet (HFD) including the downregulation of total cholesterol, triglycerides and LDL cholesterol (LDL-C) and the upregulation of HDL cholesterol (HDL-C) in the atherosclerotic mouse model. Silencing of mTOR increased plaque stability by decreasing the plaque area and increasing the fibrous cap. In addition, silencing of mTOR expression led to a decrease of macrophages throughout the atherosclerotic plaque. Furthermore, inhibition of mTOR induced macrophage autophagy by promoting autophagy-related protein 13 (Atg13) dephosphorylation and upregulating light chain 3-I/light chain 3-II (LC3-I/LC3-II). MMP-2, MCP-1 and TF, all of which were proposed to promote plaque destabilization, were also decreased by the silencing of mTOR. These results demonstrated that the suppression of mTOR in an atherosclerotic mouse model by LV-mediated RNAi inhibited atherosclerosis and stabilized plaques via a decrease of macrophages by autophagy. The profound effects and underlying mechanism should be further investigated. However, to the best of our knowledge, the present study identified a new method for the stabilization of atherosclerotic plaques.

Materials and methods

Animals. Male apolipoprotein E-deficient mice (3 weeks old, weighing 25-30 g) were obtained from Changzhou Cavens Laboratory Animal Co., Ltd. (Changzhou, China). Mice were raised under standard conditions of room temperature, dark-light cycles and humidity and fed on HFD (20% fat, 20% sugar and 1.25% cholesterol) with free access to water. Experiments were conducted under a protocol approved by the Institutional Animal Care and Use Committee of the Xi'an Jiaotong University. All efforts were made to minimize suffering.

Construction and production of LV shRNA vectors. LV shRNA vectors were constructed as described previously (24). Briefly, DNA fragments containing GACAGCACA as the loop for shRNA and shRNA sequences against mTOR were synthesized and cloned into human U6 promoter-containing pBluescript SK(+) plasmid (pU6). shRNA fragments (shmTOR) were then subcloned into the plasmid pRRL (Irvin S.Y. Chen, UCLA) with a human PSMA promoter, with restriction sites for *Bam*HI and *Hin*dIII (Takara, Tokyo, Japan). Non-specific shRNA sequences (shCON) cloned into the vectors were obtained as the control (25). A total of 20 μg of lentiviral vector carrying shRNA, 15 μg of packaging vectors pCMVR (Beijing Zhongyuan Ltd., Beijing, China), 2 μg of pCMV-VSVG (Sidansai Stem Cell Technology Co., Ltd., Shanghai, China) and 100 μl Lipofectamine® 2000 (Invitrogen, Carlsbad, CA, USA) were mixed and incubated with 293T cells at 37˚C, 5% CO_2 for 48 h. The cell supernatants were collected and concentrated using a 0.45 μm filter (Amicon Ultra-15 100K; Millipore, Billerica, MA, USA). The viral titer was determined by p24 ELISA kit (Cell Biolabs, Inc., San Diego, CA, USA) and recombinant virus was stored at -80˚C until use.

Carotid collar placement and LV transfection. Atherosclerotic lesions were induced according to a previously reported study (26). In brief, a restraint perivascular silica collar (0.3 mm in internal diameter and 3 mm in length) was placed around the left common carotid artery to induce the formation of atherosclerotic plaque. Sodium pentobarbital (40 mg/kg; no. 1; Biochem & Pharm, Shanghai, China) was used to anesthetize mice by subcutaneous injection. The common carotid arteries were dissected without causing damage to the carotid bodies and the vagus nerves. Collars were placed around the right common carotid arteries and their axial edges were approximated by placement of 3 circumferential silk ties. Then, the entry wound was closed and the mice were recovered under normal conditions for subsequent experiments. Eight weeks after surgery, the collars were removed and mice were transfected with LV through the right common carotid arteries. Mice were randomly divided into the control group, where mice received 200 μl phosphate-buffered saline (PBS) injection; the LV-shCON group, where mice received 200 μl recombinant LV ($5x10^{10}$ plaque-forming units of virus) expressing no-specific shRNA; and the LV-shmTOR group, where mice received 200 μl ($5x10^{10}$ pfu) recombinant LV expressing mTOR shRNA. Another injection was performed after 2 weeks and mice were euthanized for analysis 2 weeks after the second injection.

Quantitative real-time PCR. Two weeks after the second injection, mice were euthanized and total RNA was extracted from the right common carotid arteries using TRIzol reagent (Invitrogen) following the manufacturer's protocol. Up to 5 μg of the total RNA was reverse-transcribed into cDNA using M-MLV reverse transcriptase (Clontech, Palo Alto, CA, USA). The cDNAs were used as templates for quantitative real-time PCR. The PCR primers for mTOR were 5'-ctgggactcaaatgtg tgcagttc-3' (forward) and 5'-gaacaatagggtgaatgatccggg-3' (reverse); while those for β-actin were 5'-caacttgatgtatgtat gaaggctttggt-3' (forward) and 5'-acttttattggtctcaagtcagtg-tacag-3' (reverse). The qRT-PCR system contained 5 μl SsoFast™ EvaGreen® Supermix (Bio-Rad), 1 μl of cDNA (diluted in 1:50) and 2 μl of each of the forward and reverse primers (1 μM) to a final volume of 10 μl. The PCR procedure was as follows: 94˚C for 4 min; 94˚C for 20 sec, 55˚C for 30 sec and 72˚C for 20 sec; 2 sec for plate reading for 35 cycles; and melt curve from 65 to 95˚C. β-actin was used as the control for normalizing gene expression. Three independent experiments were performed. All the values obtained were normalized to mouse β-actin. The data obtained were calculated by $2^{-\Delta\Delta Ct}$ and treated for statistical analysis as described previously (27), followed by an unpaired sample t-test.

Western blotting. Two weeks after the second injection, mice were euthanized and tissues from the right common carotid arteries were frozen in liquid nitrogen and treated with ice-cold lysis buffer, prior to being homogenized and centrifuged. The supernatant was collected and protein concentration was determined by the Bradford method. A total of 30 μg of protein was fractionated by 12% SDS-PAGE electrophoresis and transferred to a nitrocellulose membrane (Amersham, Little Chalfont, UK). The membrane was treated using the following procedure: shaking and blocking at room temperature with 2% non-fat dry milk in PBS for 1 h, followed by incubation in primary antibody blocking solution at 4˚C overnight. Subsequently, the membrane was incubated in HRP-labeled secondary antibodies diluted at 1:3,000 in the blocking buffer for 4 h. 4-Chloro-1-naphthol (4-CN) was used as an HRP substrate for visualizing the target protein.

Detection of blood lipid. Mice were sacrificed by pentobarbital overdose (200 mg/kg) and blood was collected from mouse orbital veins. Blood samples were centrifuged and serum was collected. Levels of total cholesterol, triglyceride, LDL-C and HDL-C in serum samples were measured according to kit standard procedures (Shanghai Hushang Biotechnology Co. Ltd., Shanghai, China) by an Automatic Biochemistry Analyzer (Hitachi, Tokyo, Japan).

Hematoxylin and eosin (H&E) staining. Mouse hearts were perfused with PBS and then 4% paraformaldehyde for 30 min under physiological pressure. The carotid artery was isolated and fixed with 4% paraformaldehyde for 12 h, embedded in paraffin and cut into 5-μm serial sections. Corresponding sections were stained with hematoxylin (Sigma, St. Louis, MO, USA) for 10 min at room temperature. The sections were then washed with running water. Subsequently, the sections were washed with Scott promote blue liquid for 1 min, 1% hydrochloric acid alcohol differentiation liquid for 20 sec and Scott promote blue liquid for 1 min. Sections were then stained with eosin (0.5%; Merck, Whitehouse Station, NJ, USA) for 30 sec. The sections were washed with running water and sealed for observation. For the lesion area analysis and calculation (plaque area, fibrous cap, cap-to-core ratio, and intima-media thickness), the sections were observed by Image-Pro Plus 5.0 software (Media Cybernetics, Inc., Bethesda, MD, USA).

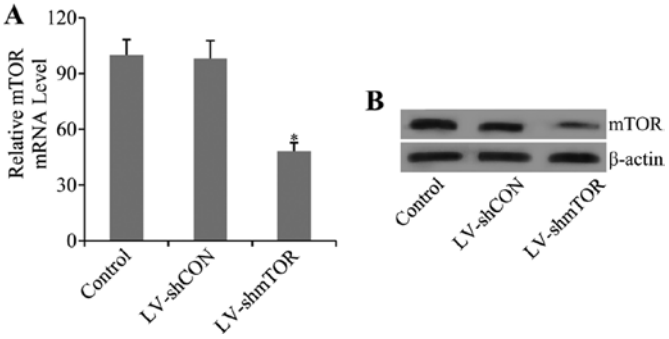

Figure 1. Effect of lentivirus (LV)-mediated RNA interference (RNAi) on the expression of mammalian target of rapamycin (mTOR). (A) Real-time PCR was performed to analyze the mRNA level of mTOR in carotid artery tissues. (B) Western blotting was used for the detection of the protein expression level of mTOR. β-actin served as a loading control. Control group mice received PBS treatment; LV-shCON group mice received LV-mediated non-specific shRNA treatment; LV-shmTOR group mice received LV-mediated mTOR shRNA treatment. *P<0.05 vs. control or LV-shCON denotes significant difference.

Statistical analysis. Assays were performed in triplicate and data were presented as mean ± SEM. Differences between groups were analyzed by the Student's t-test. P<0.05 was considered statistically significant. Statistical analyses were performed using SPSS v11.5 (SPSS Inc., Chicago, IL, USA).

Results

Efficient knockdown of mTOR by LV-mediated shRNA. To examine whether transfection of LV-mediated shRNA of mTOR inhibited the expression of mTOR, real-time PCR (qRT-PCR) and western blot analysis were performed. Results showed that the mRNA level of mTOR was markedly decreased (48.36±4.32) in LV-shmTOR-transfected mice (P<0.05), while control LV-shCON transfection had no effect on mTOR mRNA expression (Fig. 1A). Furthermore, the results were confirmed by western blot analysis. As shown in the results, the protein expression level of mTOR was also significantly decreased in carotid artery tissues of LV-shmTOR-infected mice compared with the mice infected with LV-shCON or uninfected control mice (Fig. 1B).

Knockdown of mTOR ameliorated dysregulated blood lipid metabolism. To evaluate the effect of mTOR knockdown on the mice of atherosclerosis, serum levels of total cholesterol, triglyceride, LDL-C and HDL-C was determined. Knocking down of mTOR led to a significant decrease in the total cholesterol, LDL-C and triglycerides in LV-shmTOR-infected mice compared to the control, whereas LV-shCON had no effect. Otherwise, knockdown of mTOR increased HDL-C level in

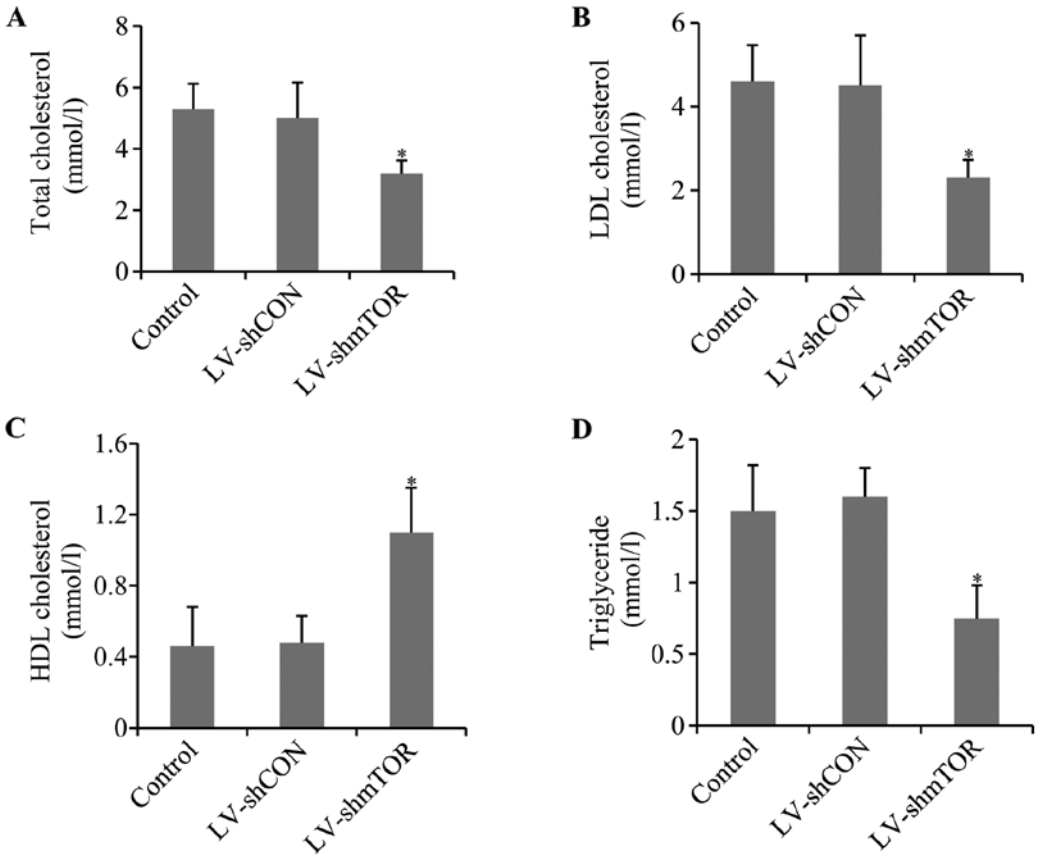

Figure 2. Effect of LV-mediated RNA interference (RNAi) on blood lipid. Serum levels of (A) total cholesterol, (B) LDL cholesterol, (C) HDL cholesterol in mice and (D) triglyceride in three groups of mice. At least three independent experiments were performed and the data are presented as the mean ± SEM. *P<0.05 vs. control or LV-shCON denotes statistically significant difference.

LV-shmTOR-infected mice, compared with mice infected with LV-shCON or uninfected control mice (P<0.05) (Fig. 2).

Knockdown of mTOR stabilized aortic atherosclerotic plaque. To evaluate the role of mTOR on plaque stability, plaque area, fibrous cap, cap-to-core ratio and intima-media thickness were analyzed by H&E staining. LV-shmTOR-infected mice demonstrated a significant decrease in the plaque area compared with control or LV-shCON-infected mice (0.11±0.08 vs. 0.15±0.12 or 0.16±0.10 mm^2; P<0.05) (Fig. 3A). Fibrous cap in LV-shmTOR-infected mice (18.93±3.02 μm) showed a marked increase (P<0.05), compared with that in control mice (8.9±5.75 μm) or LV-shCON infected mice (9.2±5.45 μm) (Fig. 3B). The cap-to-core ratio was also increased in LV-shmTOR-infected mice (0.17±0.028, P<0.05), compared to the control mice (0.11±0.025) or LV-shCON-infected mice (0.09±0.032) (Fig. 3C). However, the intima-media thickness showed no significant alterations in the three groups (Fig. 3D).

Knockdown of mTOR decreased macrophages within atherosclerotic plaques. To evaluate the effect of LV-mediated RNAi on macrophages within atherosclerotic plaques, western blotting was performed using macrophage marker CD14 antibody. Results showed that CD14 was decreased by the knockdown of mTOR in LV-shmTOR-infected mice, compared to the control mice and LV-shCON-infected mice (Fig. 4), suggesting that macrophages were decreased in mTOR siRNA-transfected mice. The results suggested that macrophages could be decreased by silencing mTOR throughout the atherosclerotic plaque.

Knockdown of mTOR promoted macrophage autophagy. To explore the underlying mechanism of mTOR in macrophage decrease, the protein expression level of Atg13, which is involved in macrophage autophagy, was analyzed (28). Dephosphorylated Atg13 was reported to induce autophagy. The results showed that silencing mTOR promoted Atg13 dephosphorylation, suggesting that macrophage autophagy activity was increased. LC3 protein is the marker of autophagosome formation and consists of the isoforms LC3-II and LC3-I. An increased LC3-I/LC3-II ratio indicates the accumulation of autophagosomes (29). In mTOR siRNA-transfected mice, LC3-II was upregulated while LC3-I was downregulated, which led to an increase in the LC3-I/LC3-II ratio (Fig. 5), further confirming that autophagy was induced.

Knockdown of mTOR suppressed the expression of genes promoting plaque destabilization. Inflammatory factors including MCP-1, MMPs and TF have been reported to promote plaque rupture. To investigate the effect of inhibition of mTOR on the expression of MCP-1, MMPs and TF, western blot analysis was performed. Results showed that the inhibition of mTOR significantly suppressed the protein expression of MCP-1, MMP-2 and TF (Fig. 6).

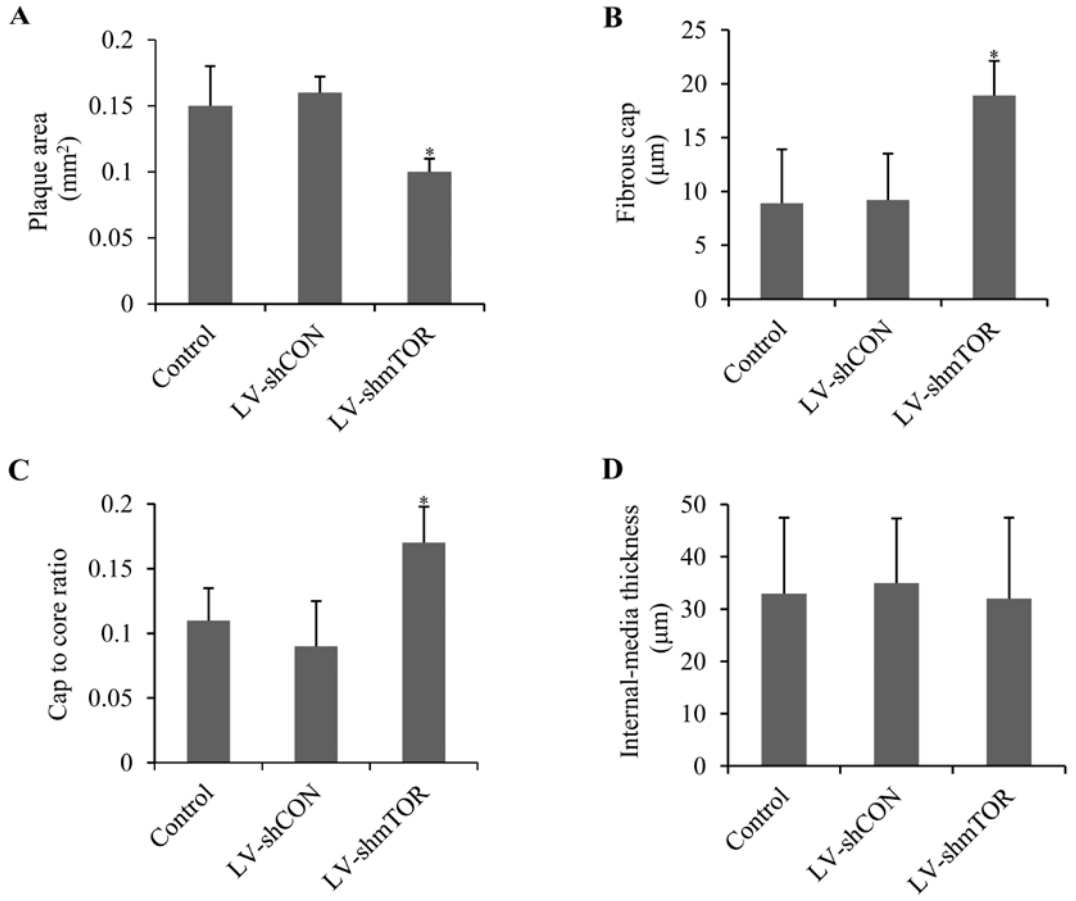

Figure 3. Effect of LV-mediated RNA interference (RNAi) on morphological characteristics of the carotid plaque. Quantitative analysis of (A) plaque area, (B) fibrous cap, (C) cap-to-core ratio and (D) internal-media thickness in three groups of mice. At least three independent experiments were performed and the data are presented as the mean ± SEM. *P<0.05 vs. control or LV-shCON denotes statistically significant difference.

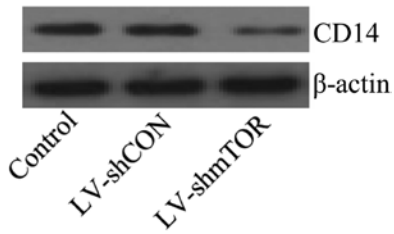

Figure 4. Effect of LV-mediated RNA interference (RNAi) on macrophages in three groups of mice. The macrophage marker CD14 antibody was used for the determination of the macrophages by western blotting. β-actin was used as a loading control.

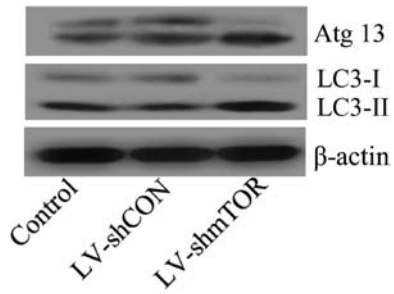

Figure 5. Effect of LV-mediated RNA interference (RNAi) on macrophages autophagy. Western blot analysis of autophagy-related protein 13 (Atg13) and light chain 3 (LC3) protein expression in carotid arteries of three groups of mice. β-actin served as a loading control.

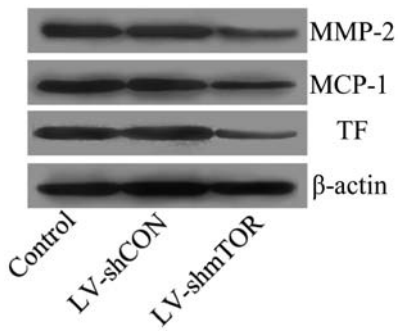

Figure 6. Effect of LV-mediated RNA interference (RNAi) on the protein levels of related inflammatory factors. Western blot analysis was performed to determine the protein levels of matrix metalloproteinase 2 (MMP-2), monocyte chemoattractant protein 1 (MCP-1) and tissue factor (TF), in carotid arteries of three groups of mice. β-actin served as a loading control.

Discussion

In the present study, we demonstrated that knockdown of mTOR by LV-mediated RNAi in atherosclerotic plaques showed beneficial effects for maintaining atherosclerotic plaque stability. Thus, targeting mTOR by RNAi for atherosclerosis therapy is a promising method that should be further investigated.

The dysregulation of lipid metabolism has been proposed to be one of the major causes contributing to the processes

underlying the formation and development of atherosclerosis. High levels of cholesterol, triglycerides and LDL-C aggravate atherosclerotic progression, while high levels of HDL-C are able to reduce the risk of atherosclerotic progression (30). It has also been reported that inhibition of mTOR restricts lipid accumulation in macrophages and facilitates macrophage cholesterol efflux, while promoting reverse cholesterol transport (31-33). Results of the present study demonstrate that silencing of mTOR *in vivo* by RNAi alleviated the dysregulated lipid metabolism by decreasing the level of cholesterol, triglyceride and LDL-C, and increasing the level of HDL-C in the atherosclerotic mouse model.

Accordingly, suppression of mTOR alleviated lipid metabolism and prevented macrophage accumulation (31-33). It has been demonstrated that administration of the mTOR inhibitor everolimus leads to inhibition of protein synthesis and results in the selective elimination of macrophages in atherosclerotic plaques by autophagy-mediated cell death (11,34,35). Furthermore, *in vitro* studies demonstrated that mTOR gene silencing by mTOR-specific siRNA induced macrophage cell death. Consistent with previous studies (11), we found that knockdown of mTOR in an atherosclerotic mouse model by RNAi resulted in a decrease of macrophages. As macrophages have been suggested to contribute to plaque destabilization (10), these results suggest that targeting mTOR for inhibition of macrophages is a promising for the treatment of atherosclerotic plaque stability.

However, the mechanism of mTOR-mediated macrophage death remains elusive. As mTOR regulates gene translation, inhibition of mTOR by everolimus showed a strong inhibition on protein synthesis by dephosphorylation of p70S6 kinase and 4E-BP1, as well as hyperphosphorylation of eIF2α and eEF2, which were the protein effectors (34). Thus, suppression of the protein synthesis by mTOR inhibition may be responsible for the induction of macrophage death. Findings of studies have suggested that mTOR regulates proteins involved in autophagy including Atg13 (28). Accordingly, the inhibition of mTOR rapidly dephosphorylated Atg13, which then formed a complex with Atg1 and led to the induction of autophagy. The LC3 protein is the marker of autophagosome formation and an increase of the LC3-I/LC-II ratio indicated the accumulation of autophagosome (29). In the present study, we found that inhibition of mTOR *in vivo* by RNAi led to dephosphorylation of Atg13, suggesting that autophagy activity was increased. In addition, the LC3-I protein level was decreased whereas the LC3-II protein level was increased by silencing mTOR, which confirmed the accumulation of autophagosomes. Thus, in accordance with previous studies (28), our findings suggest that the inhibition of mTOR led to autophagy by dephosphorylation of Atg13. However, in addition to Atg13, mTOR regulates other autophagy-specific genes such as Atg8 and Atg14 (36,37). Therefore, the precise mechanism of mTOR inhibition-mediated autophagy needs further clarification.

Enhanced inflammation activity was also a hallmark of plaque rupture. As macrophages were decreased subsequent to mTOR inhibition, we detected the effects on macrophage-secreted inflammatory factors, including MMP-2, MCP-1 and TF, which have been suggested to be responsible for atherosclerotic plaque destabilization and rupture and are considered a clinical detection marker (8,38). One of the causes of plaque rupture was the fibrous cap becoming thin (39). The fibrous cap is mainly composed of extracellular matrix and the decrease of extracellular matrix was suggested to be associated with fibrous cap thinning (40). Increasing evidences have reported that MMPs are hyper-expressed by various cells including macrophages upon various cytokines affection in atherosclerosis, which largely degrades the extracellular matrix (41). Studies have demonstrated that MMP-2 was significantly upregulated in unstable plaques (7), suggesting that MMP-2-mediated degradation of the extracellular matrix was an important risk factor for unstable plaques. MCP-1 is a type of chemokine that mainly recruits monocytes (42) and is highly expressed in macrophage-enriched plaques, suggesting critical roles in the processes of atherosclerosis and plaque instability (43). More recently, it has been demonstrated that silencing of MCP-1 prevents vulnerable plaque disruption (44). TF was the regulator of coagulation and hemostasis, and is mainly secreted by macrophages, which activate the coagulation system when the fibrous cap is broken, resulting in thrombosis (9,45). Thus, stability of the fibrous cap is critical for plaque stability. The fibrous cap is capable of preventing blood entering the arterial lumen from tissues under the fibrous cap (46). Results of the present study show that the knockdown of mTOR promotes plaque stability by decreasing the plaque area and increasing the fibrous cap. Knockdown of mTOR also suppressed MMP-2, MCP-1 and TF protein expression levels, which was beneficial for plaque stability.

The method of RNAi which efficiently and selectively silences target gene expression has been applied in experimental studies for the treatment of various diseases (47,48). In the present study, we successfully inhibited mTOR expression *in vivo* in an atherosclerotic mouse model by RNAi mediated by LV. Inhibition of mTOR expression showed protective effects on the stability of atherosclerotic plaques via the removal of macrophages induced by autophagy. In addition, the genes that promoted plaque instability, including MMP-2, MCP-1 and TF, were downregulated. These results indicate that mTOR is a promising target for the treatment of atherosclerotic plaque destabilization and rupture. However, the underlying mechanism of mTOR in regulating plaque stability remains to be clarified. The RNAi-mediated silencing of gene expression with specificity for target genes has advantages over traditional drug inhibitors. Thus, the investigation of mTOR *in vitro* and *in vivo* models with regard to RNAi should be conducted.

Acknowledgements

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