Biochemical and Biophysical Research Communications 458 (2015) 890–895

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

CTRP9 enhances carotid plaque stability by reducing pro-inflammatory cytokines in macrophages

Jun Li [a, b, 1], Peng Zhang [a, b, 1], Tingting Li [a, b], Yawei Liu [a, b], Qing Zhu [a, b], Tongshuai Chen [a, b], Tianjiao Liu [a, b], Chengmin Huang [a, b], Jianning Zhang [a, b], Yun Zhang [a, b], Yuan Guo [a, b, *]

[a] Department of Cardiology, Qilu Hospital, Shandong University, Jinan, 250012, Shandong, China
[b] The Key Laboratory of Cardiovascular Remodeling and Function Research, Chinese Ministry of Education and Chinese Ministry of Health, Shandong University, Jinan, 250012, Shandong, China

ARTICLE INFO

Article history:
Received 9 February 2015
Available online 19 February 2015

Keywords:
CTRP9
Anti-inflammation
Atherosclerosis
Plaque stabilization

ABSTRACT

The aim of this study was to investigate whether C1q/TNF-related protein 9 (CTRP9) could stabilize the mature plaques by targeting macrophages in the apolipoprotein E knockout (ApoE KO) mice model. *In vivo*, the mice were subjected to high-fat diet and constrictive collars on the right carotid artery for eight weeks, a lentiviral vectors expressing CTRP9 (LV-CTRP9) or green fluorescence protein (LV-eGFP) as a control was intravenously injected into ApoE KO mice. Delivery of LV-CTRP9 resulted in low contents of macrophages and lipids, and high contents of collagen and vascular smooth muscle cells in the carotid mature plaques. In addition, CTRP9 also decreased pro-inflammatory cytokines in mature plaques. *In vitro*, RAW264.7 macrophages were pretreated with or without LV-CTRP9 transfection, and then stimulated with oxLDL (50 μg/mL). We found that the expression levels of tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein 1 (MCP-1) in the LV-CTRP9 group were significantly lower than those in the LV-eGFP group after exposure to oxLDL. The present data indicate that CTRP9 over-expression enhances the plaque stability in ApoE KO mice by reducing pro-inflammatory cytokines in macrophages. Our study suggests that the therapeutic approaches to enhance CTRP9 production could be valuable for plaque stabilization.

1. Introduction

Atherosclerosis is the main cause of cardiovascular disease and stroke [1]. The rupture of vulnerable plaques followed by thrombus formation leads to myocardial infarction, stroke, and sudden death [2]. Therefore, plaque vulnerability has become a hot debate at home and abroad. During the atherosclerosis pathological process, macrophages contribute to size-independent changes in plaque morphology, notably necrotic core formation and fibrous cap thinning, that characterize the “vulnerable” plaque [3,4]. Since macrophages and foam cells secret a variety of inflammatory cytokines, which attract more monocytes to infiltrate into the sub-endothelial space, propagate inflammatory response and subsequently advance atherosclerotic plaques [5]. It is essential to investigate the mediators of inflammation in macrophages.

CTRP9 is a novel adipocyte-derived cytokine, which belongs to the C1q family and has the highest homology to adiponectin [6]. It is well known that adiponectin is an anti-inflammatory and anti-atherosclerotic cytokine, and is associated with plaque vulnerability [7–10]. Similar with adiponectin, Recent studies show that CTRP9 is associated with cardiovascular disease. Delivery of CTRP9 significantly attenuates vascular smooth muscle cell proliferation and neointimal formation [11], and induces vascular relaxation

Abbreviations: ApoE KO mice, apolipoprotein E knockout mice; CTRP9, C1q/TNF-related protein 9; LV-CTRP9, lentiviral vector expressing CTRP9; LV-eGFP, lentiviral vector expressing green fluorescence protein; PBS, phosphate-buffered saline; oxLDL, oxidized low-density lipoprotein; VSMC, Vascular Smooth Muscle Cell; AMPK, AMP-activated protein kinase; eNOS, endothelial Nitric Oxide Synthase; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NEFA, nonesterified fatty acids; GLU, glucose; TNF-α, tumor necrosis factor-α; MCP-1, monocyte chemoattratctant protein-1.

* Corresponding author. Department of Cardiology, Qilu Hospital, Shandong University, No. 44 Wenhua Xi Road, Jinan 250012, Shandong Province, China.
E-mail address: guoyuan9092@163.com (Y. Guo).

[1] Jun Li & Peng Zhang contributed equally to the work.

http://dx.doi.org/10.1016/j.bbrc.2015.02.054
0006-291X/

[12]. Furthermore, serum CTRP9 concentrations are associated with favorable glucose utilization and arterial stiffness in patients with type 2 diabetes [13,14]. All the information indicates that CTRP9 may have effect on atherosclerosis. However, the role of CTRP9 in carotid plaque stability remains unclear.

Therefore, the aims of our study are (1) to detect whether CTRP9 can stabilize atherosclerotic plaque in ApoE KO mice; (2) to elucidate the mechanisms responsible for the anti-atherosclerosis effects of CTRP9.

2. Materials and methods

2.1. Reagents

Raw264.7 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA). Cell culture medium was purchased from Hyclone (Utah, USA). oxLDL was purchased from Yiyuan biotechnology (Guangzhou, China). MOMA-2 antibody was purchased from AbD (Oxford, UK). CTRP9 specific antibody was purchased from Aviscera Bioscience (CA, USA), TNF-α specific antibody was purchased from CST (MA, USA) and MCP-1 specific antibody was purchased from Novus (CO, USA). α-SMA antibody and MMP9 antibody were purchased from Abcam (MA, USA).

2.2. Animal model of atherosclerosis

8-week-old male ApoE KO mice were obtained from Peking University, and mice were maintained under conditions of standard lighting (12:12 h light–dark cycle), temperature (21°C–23 °C) and humidity (50%–60%) with food and water freely available. All mice received a high-fat diet (0.25% cholesterol and 15% cocoa butter) until sacrifice. A constrictive collar (0.30 mm inner diameter, 0.50 mm outer diameter, and 2 mm long) was placed around right carotid artery to induce plaque formation. Eight weeks after surgery, the LV-CTRP9 or LV-eGFP was injected into the right jugular vein respectively, the titers averaged 2×10^7 TU/mL, and total volume was 100 μL. Equal volume of PBS was used as a vehicle control. Another four weeks of high-fat diet was maintained. Mice sera were collected thirty days after lentivirus injection for further analysis. Tissues were collected, snap frozen in liquid nitrogen, and kept at −80 °C. All animal protocols were approved by Animal Care Committee of Shandong University (Jinan, China).

2.3. Tissue harvesting and quantification of atherosclerosis

The right carotid arteries were removed and perfusion-fixed with 4% paraformaldehyde overnight and then embedded in optimal cutting temperature compound. The cryosections were cut in 5-μm thick for further histological and morphological staining. Plaque contents, including the lipid core, macrophages, collagen, and smooth muscle cells were identified respectively by Oil Red O staining, MOMA-2, Sirius red and α-actin. Sections were then taken for the histological analysis, which were performed and analyzed by two additional operators who were blinded as to the lesion classification. The positive component areas of the sections were quantitated by Image Pro-Plus software. The vulnerable index was calculated by the formula as follows: the relative positive staining areas of (macrophages % + lipid %)/the relative positive staining areas of (α-SMCs % + collagen %).

2.4. Immunohistochemical analyses

Immunohistochemical analyses involved the use of primary antibodies for MOMA-2 (diluted 1:200), α-SMA (diluted 1:200), TNF-α (diluted 1:200), MCP-1 (diluted 1:100) and MMP9 (diluted 1:200). Briefly, cryosections were rehydrated in distilled water for 10 min and blocked with 3% H_2O_2 for 10 min, washed with PBS 3times and 5 min per time, and cultured in 5% bovine serum albumin (BSA) for 30 min at 37 °C. Tissue sections were incubated with primary antibodies overnight at 4 °C and appropriate biotinylated secondary antibodies for 30 min at 37 °C. Afterward, a DAB staining was used to visualize the secondary antibody. To detect cell nuclei, sections were rinsed in water and counterstained with Hematoxylin. Data were analyzed by Image Pro-Plus software.

2.5. Cell culture and lentiviral transfection

RAW264.7 cells were cultured in DMEM medium supplemented with 10% fetal calf serum at 37 °C in 5% CO_2. For overexpression of CTRP9, the coding sequences of CTRP9 (NM_183175) were cloned into the lentiviral vector GV287 (Ubi-MCS-3FLAG-SV40-EGFP) (GeneChem, Shanghai, China). To establish stable expression of CTRP9 in RAW264.7 macrophage cell line, recombinant LV-CTRP9 or recombinant LV-eGFP was used to transfect RAW264.7 cells with Polybrene at a final concentration of 6 μg/mL. After 72 h, the transfection efficiency was detected by fluorescence microscope, and then cells were treated with oxLDL (50 μg/mL) for another 24 h for further experiments.

2.6. Western blots

Proteins were separated by SDS-polyacrylamide gel electrophoresis and then transferred onto PVDF membranes in an indicated time, incubated with blocking solution in 2 h (5% nonfat milk in TBS). The blocked membranes were separately incubated with the specific antibodies overnight at 4 °C, including TNF-α (diluted 1:1000), MCP-1 (diluted 1:1000), washed three times with TBS

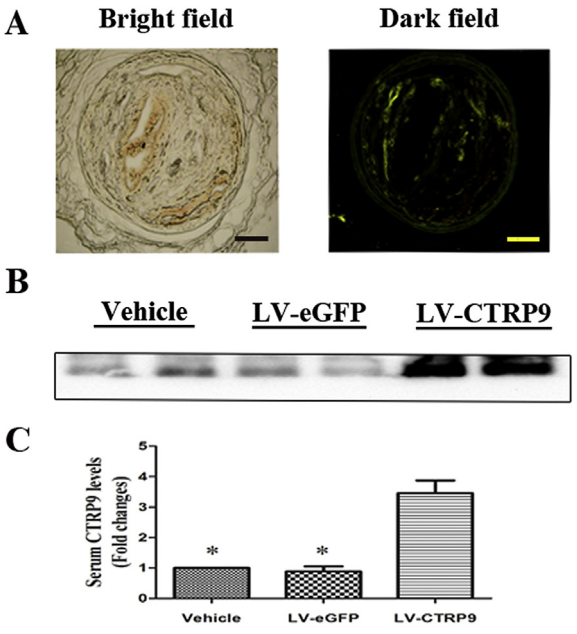

Fig. 1. The CTRP9 level is increased in the LV-CTRP9 group. (A) The transfection efficiency of LV-CTRP9 in plaques. Bright field and dark field of carotid plaque were shown by fluorescence microscope. Bar = 100 μm. (B) The serum CTRP9 levels after lentivirus injection for thirty days. LV-CTRP9 or LV-eGFP was injected into the jugular vein of ApoE KO mice (2×10^7 TU/mL), PBS was injected as a vehicle control. The serum CTRP9 levels were analyzed by western blots. Each lane was loaded with 20 μl undiluted mouse serum. (C) Quantitative analysis of CTRP9 in each group as in (B) (*$p < 0.05$ *vs.* LV-CTRP9 group). Data are presented as means ± SEM, n = 6 in each group.

buffer containing 0.1% Tween, 10 min each. The secondary antibody conjugated to horseradish peroxidase (1:5000 dilutions with 1% nonfat milk in TBS) against to the primary antibody was added, incubated, and washed as described steps above at room temperature with shaking. An ECL Western blotting detection kit (Millipore) was used for detection. Relative protein levels were quantified by using the Image J.

2.7. Statistics

SPSS v13.0 statistical software package was used for data analysis. Data are presented as means ± SEM. Group differences were analyzed by Student's unpaired t test or one-way analysis of variance. p value <0.05 was considered statistically significant.

3. Results

3.1. Serum level of CTRP9 is significantly elevated in LV-CTRP9 group

To investigate the relationship between CTRP9 and the plaque stability, an atherosclerosis mouse model was constructed. ApoE KO mice were subjected to surgery on the right carotid with a constrictive collar, and then treated intravenously with LV-CTRP9 (tilter = 2×10^7 TU/mL), LV-eGFP (tilter = 2×10^7 TU/mL) or PBS. The transfection efficiency of LV-eGFP on mouse carotid plaque was detected by fluorescence with infusion protein GFP (green light) (Fig. 1A). Mice infected with LV-CTRP9 for thirty days showed a 3.9 or 3.5-fold increase in serum level of CTRP9, compared with the other two groups ($p < 0.05$ *vs.* LV-CTRP9 group), but there was no significant difference between the LV-eGFP group and the vehicle group (Fig. 1B and C). These results suggested that the serum level of CTRP9 was significantly elevated by LV-CTRP9 transfection in mouse atherosclerosis model.

3.2. CTRP9 decreases serum glucose level

Serum biochemical index of mice were measured at the end of twenty weeks. The LV-CTRP9 group displayed markedly reduced glucose levels compared with the LV-eGFP group or the vehicle group (Table S1). However, no significant differences were found in serum lipid levels among the three groups. These data suggest that CTRP9 has decreased the serum glucose level of mice with a high-fat diet, but showed no significant effects on lipid levels (Table S1).

3.3. CTRP9 enhances the carotid plaque stability

To examine the role of CTRP9 in the stabilization of atherosclerotic plaque, the relative contents of the carotid plaque components were assessed by staining and immunohistochemistry. The relative contents of lipids and macrophages were lower in the LV-

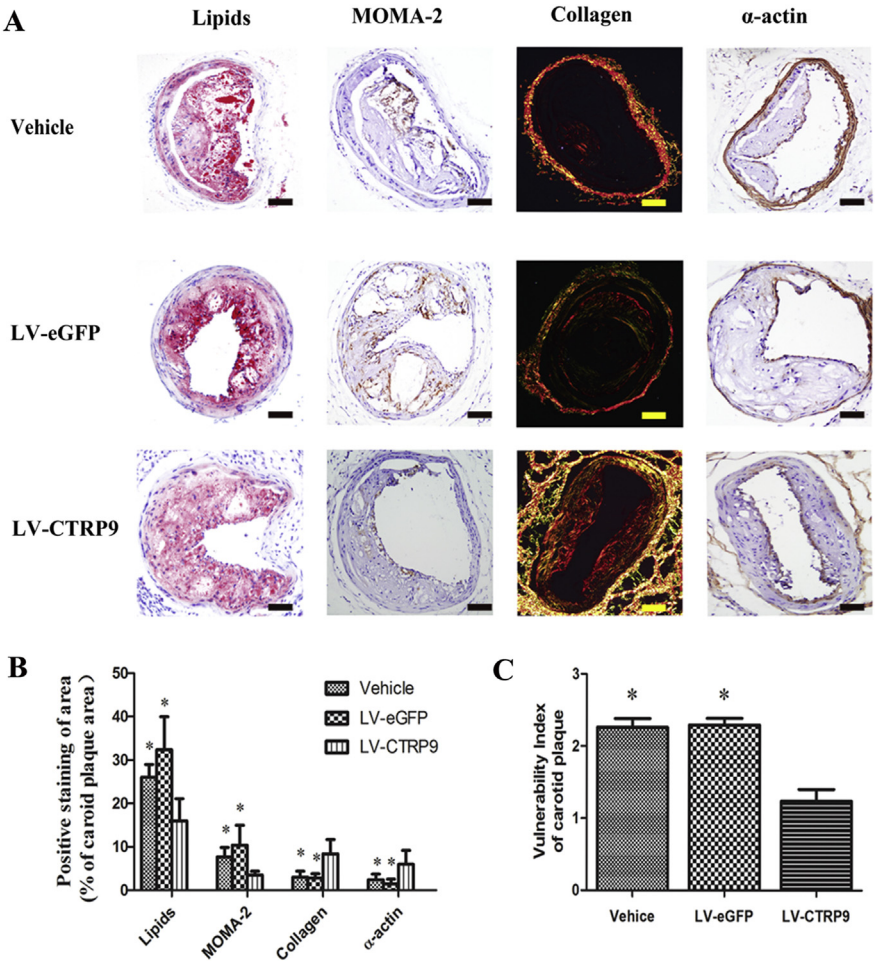

Fig. 2. CTRP9 alters the relative contents of the carotid plaque components. (A) Representative immunohistochemical staining of lipids (oil red O), macrophages (monocyte/macrophage marker; MOMA-2), collagen (Sirius red), and vascular smooth muscle cells (VSMCs; α-actin) of the carotid plaques. Bar = 100 μm. (B) Quantitative analysis of carotid components from each group as in (A) (*$p < 0.05$ *vs.* LV-CTRP9 group). (C) Quantitative analysis of plaque vulnerability index of the carotid plaques (*$p < 0.05$ *vs.* LV-CTRP9 group). Data are presented as means ± SEM, n = 5 in each group.

CTRP9 group than in the LV-eGFP group or the vehicle group ($p < 0.05$). In contrast, the relative contents of collagen and smooth muscle cells were higher in the LV-CTRP9 group than the other two groups ($p < 0.05$). However, there was no difference of the relative contents between the LV-eGFP group and the vehicle group (Fig. 2A and B). The vulnerability index of the LV-CTRP9 group was lower than the LV-eGFP group or vehicle group ($p < 0.05$) (Fig. 2C). Again the vulnerability index between the LV-eGFP group and vehicle group has no significant difference. In addition, the expression of MMP9 was also detected in the carotid plaque, but no significant difference was shown among the three groups (Fig. 3A and C). These results demonstrated that delivery of CTRP9 could enhance the carotid plaque stability by altering the components of atherosclerotic plaque.

3.4. CTRP9 reduces pro-inflammatory cytokines secretion in the carotid plaque

Pro-inflammatory cytokines are important in the process of atherosclerosis. To determine whether CTRP9 has the effect on pro-inflammatory cytokines expression in the carotid plaques, TNF-α and MCP-1 were analyzed by immunohistochemistry. As shown in Fig. 3B and D, the expression levels of TNF-α and MCP-1 in the LV-CTRP9 group were apparently lower than those in other two groups ($p < 0.05$). However, there was no significantly difference between the LV-eGFP group and the vehicle group ($p > 0.05$). These data suggested that CTRP9 could reduce the pro-inflammatory cytokines expression such as TNF-α and MCP-1 in local plaque lesions.

3.5. CTRP9 is detected in both the cell lysates and the culture medium of RAW264.7 macrophages after LV-CTRP9 transfection

To investigate the role of CTRP9 *in vitro*, the LV-CTRP9 or LV-eGFP was transfected into RAW264.7 macrophage cells, respectively. The transfection efficiency was measured by fluorescence with infusion protein GFP (green light). We found that when MOI was at 60, almost all cells were transfected successfully (Fig. 4A). Western blots were then performed to examine the protein expression level of CTRP9. Results show that CTRP9 exists both in the cell lysates and the culture medium, but there is no CTRP9 expression in the LV-

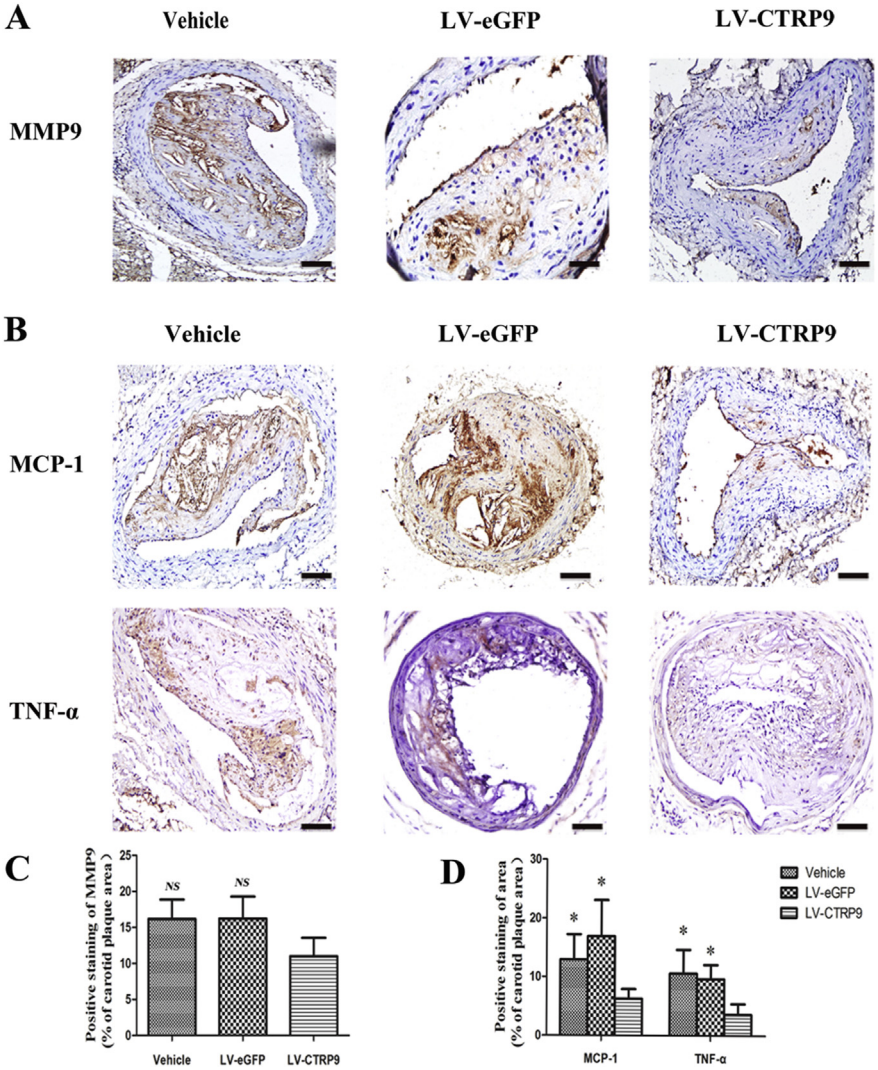

Fig. 3. CTRP9 reduces pro-inflammatory cytokines secretion in the carotid plaque. (A) Representative immunohistochemical staining of MMP9 in the carotid plaques in each group. Bar = 100 μm. (B) Representative immunohistochemical staining of MCP-1 and TNF-α in the carotid plaques in each group. Bar = 100 μm. (C) Quantitative analysis of MMP9 in each group as in (A). (*NS vs.* LV-CTRP9 group). (D) Quantitative analysis of MCP1 and TNF-α in each group as in (B) (*$p < 0.05$ *vs.* LV-CTRP9 group). Data are presented as means ± SEM, n = 5–6 in each group.

eGFP group or vehicle group (Fig. 4B). These data indicated that CTRP9 is a secreted protein, and we successfully constructed a macrophage cell line, which can stably express protein CTRP9.

3.6. Pro-inflammatory cytokines are decreased in RAW264.7 macrophages after LV-CTRP9 transfection

To determine whether CTRP9 has the potential function in suppression of pro-inflammatory cytokines, we first established the transfection cell lines as described above, and then stimulated these cells with oxLDL (50 μg/mL) for 24 h to induce foam cells. Two pro-inflammatory cytokines were detected by western blot. As shown in Fig. 4C and D, the expression of MCP-1 was significantly decreased in the LV-CTRP9 group compared with the LV-eGFP group. Similarly, TNF-α expression in the LV-CTRP9 group was significantly reduced compared with the LV-eGFP group (Fig. 4E and F). These results suggested that CTRP9 inhibit pro-inflammatory cytokines secretion from macrophage foam cells.

4. Discussion

Current evidence indicates that adiponectin is effective in attenuating atherosclerotic lesions and plays an anti-inflammatory role [8–10,15]. CTRP9 shares the highest degree of amino acid identity to adiponectin in its globular C1q domain [6]. In addition,

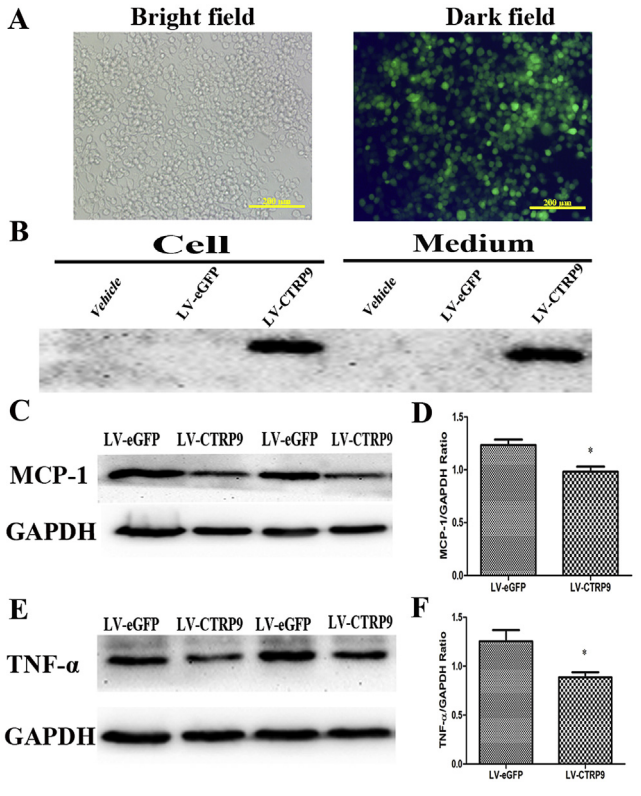

Fig. 4. CTRP9 overexpression in RAW264.7 cells successfully reduces pro-inflammatory cytokines expression in macrophage foam cells. (A) LV-CTRP9 transfection efficiency in RAW264.7 macrophages, which were observed both in the bright field and dark field by fluorescence microscope. The best MOI was found to be 60. Bar = 200 μm. (B) The expression of CTRP9 in cell lysates and cell culture medium were examined in three groups. (C) Representative blots for MCP-1 in LV-CTRP9 group and LV-eGFP group. (D) Quantitative analysis of MCP-1 in each group as in (C) (*$p < 0.05$ *vs.* LV-CTRP9 group). (E) Representative blots for TNF-α in LV-CTRP9 group and LV-eGFP group. (F) Quantitative analysis of TNF-α in each group as in (E) (*$p < 0.05$ *vs.* LV-CTRP9 group). Data are presented as means ± SEM from three independent experiments.

we hypothesized that CTRP9 could enhance carotid plaque stability for many other reasons: First, a recent study shows that the serum CTRP9 concentrations were associated with arterial stiffness, which was associated with the process of systemic atherosclerosis [14]. Secondly, CTRP9 induced vascular relaxation through the adiponectin receptor 1/AMPK/eNOS/nitric oxide signaling pathway [12]. Moreover, adiponectin receptor 1 is associated with carotid plaque stability [16]. Thirdly, the proliferation of vascular smooth muscle cells plays a crucial role in the process of atherosclerosis, and recent studies found that CTRP9 attenuated vascular smooth muscle cell proliferation after artery injury [11]. All of these data suggest that CTRP9 may, to some extent, associate with atherosclerosis.

To clarify the relationship between CTRP9 and atherosclerotic plaque, we constructed a mouse model for atherosclerosis. LV-CTRP9, LV-GFP and PBS were injected into the right jugular vein of mice, respectively. In the present study we found that: (1) CTRP9 enhances carotid plaque stability by altering the contents of the plaque, as shown by a significant decrease in lipids and macrophages whereas a substantial increase in collagen and VSMCs in carotid plaque; (2) CTRP9 decreases inflammatory cytokines in atherosclerotic plaque; (3) Macrophage foam cells expressing CTRP9 has less TNF-α and MCP-1 expression. To the best of our knowledge, our study has provided, for the first time, the experimental evidence of the effects of CTRP9 on plaque stabilization and the possible mechanisms.

CTRP9 is mainly expressed and secreted from adipocytes [6], which has not been previously detected in RAW264.7 macrophage cells. To investigate the anti-inflammatory effect of CTRP9 on macrophages, we expressed the gene coding for mouse CTRP9 in RAW264.7 macrophage foam cells by using a lentiviral vector. Furthermore, we demonstrated that the transfected macrophages could secret CTRP9 into the culture medium, which exerts similar alterations as exogenous (adipocyte-derived) CTRP9. The secreted CTRP9 could act on RAW264.7 macrophage cells in 'autocrine' manner. Recent report shows that RAW264.7 macrophages express adiponectin R1 receptor and adiponectin R2 receptor [17], in additionally, full-length adiponectin and globular form of adiponectin have the anti-inflammatory effect on RAW264.7 macrophages through adiponectin R1 receptor or adiponectin R2 receptor, respectively. In our study, we have shown that expression of CTRP9 in macrophage foam cells can significantly decrease the expression of anti-inflammatory cytokines, which suggest that CTRP9 may have the anti-inflammatory effect on RAW264.7 macrophages via adiponectin receptors. We demonstrated that CTRP9 produced by macrophages decreased the expression of TNF-α and MCP-1, which were important in the progress of atherosclerosis [18–22]. Recently, CTRP3, also a member of CTRPs, acts anti-inflammatory in mice [23]. Previous report also shows that adiponectin downregulate the expression of pro-inflammatory cytokines, TNF-α and MCP-1, in THP-1 Macrophage Foam cells [24], and transgenic mouse of adiponectin leads to a reduction of both the TNF-α and MCP-1 expressions [9]. These data suggest that CTRP9 may play an anti-inflammation role in atherosclerosis, which is similar to adiponectin. Therefore, our studies provide new insights for investigating the anti-inflammation role of CTRP9.

In addition, macrophage foam cells also express several matrix metalloproteinases (MMPs), such as MMP9 and extracellular matrix metalloproteinase [25], which, in turn, contribute to vulnerability of atherosclerotic plaques [26]. Higher MMP9 levels are associated with plaque vulnerability [27]. Recent studies show that anti-inflammatory cytokines like adiponectin could predict MMP2 plasma activity [28]. We further explored whether CTRP9 has an effect on extracellular matrix degradation [29,30]. In our study, though we observed that the MMP9 expression has the downward tendency in the LV-CTRP9 group compared with the other two

groups, there is no significant difference among the three groups. However, recent research shows that CTRP9 prevents LV-remodeling by reducing MMP9 activation [31]. Therefore, it remains unclear about the relationship between CTRP9 and MMPs in the progress of atherosclerosis, further experiments are needed to investigate the effects of CTRP9 on MMPs.

In conclusion, we demonstrated that CTRP9 alters the components of carotid plaque, and may act as an anti-inflammatory cytokine by reducing TNF-α and MCP-1 both *in vivo* and *in vitro*. All the results suggested that CTRP9 might enhance carotid plaque stability and play an anti-inflammation role against atherosclerosis.

Conflict of interest

None.

Acknowledgments

This work was supported by the grants of the National Natural Science Foundation of China (No 81350025).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.02.054.

References

[1] A.S. Go, D. Mozaffarian, V.L. Roger, E.J. Benjamin, J.D. Berry, M.J. Blaha, S. Dai, E.S. Ford, C.S. Fox, S. Franco, H.J. Fullerton, C. Gillespie, S.M. Hailpern, J.A. Heit, V.J. Howard, M.D. Huffman, S.E. Judd, B.M. Kissela, S.J. Kittner, D.T. Lackland, J.H. Lichtman, L.D. Lisabeth, R.H. Mackey, D.J. Magid, G.M. Marcus, A. Marelli, D.B. Matchar, D.K. McGuire, E.R. Mohler 3rd, C.S. Moy, M.E. Mussolino, R.W. Neumar, G. Nichol, D.K. Pandey, N.P. Paynter, M.J. Reeves, P.D. Sorlie, J. Stein, A. Towfighi, T.N. Turan, S.S. Virani, N.D. Wong, D. Woo, M.B. Turner, Heart disease and stroke statistics–2014 update: a report from the American Heart Association, Circulation 129 (2014) e28–e292.
[2] J. Narula, P. Garg, S. Achenbach, S. Motoyama, R. Virmani, H.W. Strauss, Arithmetic of vulnerable plaques for noninvasive imaging, Nat. Clin. Pract. Cardiovasc. Med. 5 (Suppl. 2) (2008) S2–S10.
[3] P. Libby, P.M. Ridker, A. Maseri, Inflammation and atherosclerosis, Circulation 105 (2002) 1135–1143.
[4] P.K. Shah, Biomarkers of plaque instability, Curr. Cardiol. Rep. 16 (2014) 547.
[5] K.J. Moore, I. Tabas, Macrophages in the pathogenesis of atherosclerosis, Cell 145 (2011) 341–355.
[6] G.W. Wong, S.A. Krawczyk, C. Kitidis-Mitrokostas, G. Ge, E. Spooner, C. Hug, R. Gimeno, H.F. Lodish, Identification and characterization of CTRP9, a novel secreted glycoprotein, from adipose tissue that reduces serum glucose in mice and forms heterotrimers with adiponectin, FASEB J. 23 (2009) 241–258.
[7] E.J. Folco, V.Z. Rocha, M. Lopez-Ilasaca, P. Libby, Adiponectin inhibits pro-inflammatory signaling in human macrophages independent of interleukin-10, J. Biol. Chem. 284 (2009) 25569–25575.
[8] K. Ohashi, J.L. Parker, N. Ouchi, A. Higuchi, J.A. Vita, N. Gokce, A.A. Pedersen, C. Kalthoff, S. Tullin, A. Sams, R. Summer, K. Walsh, Adiponectin promotes macrophage polarization toward an anti-inflammatory phenotype, J. Biol. Chem. 285 (2010) 6153–6160.
[9] N. Luo, J. Liu, B.H. Chung, Q. Yang, R.L. Klein, W.T. Garvey, Y. Fu, Macrophage adiponectin expression improves insulin sensitivity and protects against inflammation and atherosclerosis, Diabetes 59 (2010) 791–799.
[10] N. Ouchi, S. Kihara, T. Funahashi, Y. Matsuzawa, K. Walsh, Obesity, adiponectin and vascular inflammatory disease, Curr. Opin. Lipidol. 14 (2003) 561–566.
[11] Y. Uemura, R. Shibata, K. Ohashi, T. Enomoto, T. Kambara, T. Yamamoto, Y. Ogura, D. Yuasa, Y. Joki, K. Matsuo, M. Miyabe, Y. Kataoka, T. Murohara, N. Ouchi, Adipose-derived factor CTRP9 attenuates vascular smooth muscle cell proliferation and neointimal formation, FASEB J. 27 (2013) 25–33.
[12] Q. Zheng, Y. Yuan, W. Yi, W.B. Lau, Y. Wang, X. Wang, Y. Sun, B.L. Lopez, T.A. Christopher, J.M. Peterson, G.W. Wong, S. Yu, D. Yi, X.L. Ma, C1q/TNF-related proteins, a family of novel adipokines, induce vascular relaxation through the adiponectin receptor-1/AMPK/eNOS/nitric oxide signaling pathway, Arterioscler. Thromb. Vasc. Biol. 31 (2011) 2616–2623.
[13] Y.C. Hwang, S. Woo Oh, S.W. Park, C.Y. Park, Association of serum C1q/TNF-Related Protein-9 (CTRP9) concentration with visceral adiposity and metabolic syndrome in humans, Int. J. Obes. (Lond.) (2013).
[14] C.H. Jung, M.J. Lee, Y.M. Kang, J.E. Jang, J. Leem, Y.L. Lee, S.M. Seol, H.K. Yoon, W.J. Lee, J.Y. Park, Association of serum C1q/TNF-related protein-9 concentration with arterial stiffness in subjects with type 2 diabetes, J. Clin. Endocrinol. Metab. 99 (2014) E2477–E2484.
[15] W.S. Kang, J.S. Kwon, H.B. Kim, H.Y. Jeong, H.J. Kang, M.H. Jeong, J.G. Cho, J.C. Park, Y.S. Kim, Y. Ahn, A macrophage-specific synthetic promoter for therapeutic application of adiponectin, Gene Ther. 21 (2014) 353–362.
[16] S. Takeuchi, K. Wada, Y. Uozumi, N. Otani, H. Osada, K. Nagatani, K. Mori, Adiponectin receptor 1 expression is associated with carotid plaque stability, Neurol. India 61 (2013) 249–253.
[17] P. Mandal, B.T. Pratt, M. Barnes, M.R. McMullen, L.E. Nagy, Molecular mechanism for adiponectin-dependent M2 macrophage polarization: link between the metabolic and innate immune activity of full-length adiponectin, J. Biol. Chem. 286 (2011) 13460–13469.
[18] L. Zhong, W.Q. Chen, X.P. Ji, M. Zhang, Y.X. Zhao, G.H. Yao, P.F. Zhang, C. Zhang, Y. Zhang, Dominant-negative mutation of monocyte chemoattractant protein-1 prevents vulnerable plaques from rupture in rabbits independent of serum lipid levels, J. Cell. Mol. Med. 12 (2008) 2362–2371.
[19] E. Mori, K. Komori, T. Yamaoka, M. Tanii, C. Kataoka, A. Takeshita, M. Usui, K. Egashira, K. Sugimachi, Essential role of monocyte chemoattractant protein-1 in development of restenotic changes (neointimal hyperplasia and constrictive remodeling) after balloon angioplasty in hypercholesterolemic rabbits, Circulation 105 (2002) 2905–2910.
[20] S. Yla-Herttuala, B.A. Lipton, M.E. Rosenfeld, T. Sarkioja, T. Yoshimura, E.J. Leonard, J.L. Witztum, D. Steinberg, Expression of monocyte chemoattractant protein 1 in macrophage-rich areas of human and rabbit atherosclerotic lesions, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 5252–5256.
[21] T.J. Qi, W.Q. Chen, C.L. Jiang, T.H. Yang, M.Q. Zhai, D.Q. Li, B.A. You, G.P. An, X.B. Hu, Y.G. Chen, Y. Zhang, J.F. Li, [Roles of monocyte chemoattractant protein-1, RANTES and Fractalkine on promoting vulnerability of atherosclerotic plaques], Zhonghua Xin Xue Guan Bing Za Zhi 39 (2011) 797–801.
[22] P.H. Park, H. Huang, M.R. McMullen, P. Mandal, L. Sun, L.E. Nagy, Suppression of lipopolysaccharide-stimulated tumor necrosis factor-alpha production by adiponectin is mediated by transcriptional and post-transcriptional mechanisms, J. Biol. Chem. 283 (2008) 26850–26858.
[23] A. Schmid, A. Kopp, F. Hanses, T. Karrasch, A. Schaffler, C1q/TNF-related protein-3 (CTRP-3) attenuates lipopolysaccharide (LPS)-induced systemic inflammation and adipose tissue Erk-1/-2 phosphorylation in mice in vivo, Biochem. Biophys. Res. Commun. 452 (2014) 8–13.
[24] L. Tian, N. Luo, R.L. Klein, B.H. Chung, W.T. Garvey, Y. Fu, Adiponectin reduces lipid accumulation in macrophage foam cells, Atherosclerosis 202 (2009) 152–161.
[25] Z.S. Galis, G.K. Sukhova, R. Kranzhofer, S. Clark, P. Libby, Macrophage foam cells from experimental atheroma constitutively produce matrix-degrading proteinases, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 402–406.
[26] P.J. Gough, I.G. Gomez, P.T. Wille, E.W. Raines, Macrophage expression of active MMP-9 induces acute plaque disruption in apoE-deficient mice, J. Clin. Investig. 116 (2006) 59–69.
[27] D. Silvello, L.B. Narvaes, L.C. Albuquerque, L.F. Forgiarini, L. Meurer, N.C. Martinelli, M.E. Andrades, N. Clausell, K.G. dos Santos, L.E. Rohde, Serum levels and polymorphisms of matrix metalloproteinases (MMPs) in carotid artery atherosclerosis: higher MMP-9 levels are associated with plaque vulnerability, Biomarkers 19 (2014) 49–55.
[28] V. Miksztowicz, N. Fernandez Machulsky, D. Lucero, E. Fassio, L. Schreier, G. Berg, Adiponectin predicts MMP-2 activity independently of obesity, Eur. J. Clin. Investig. 44 (2014) 951–957.
[29] H.L. Li, H.L. Chen, H. Li, K.L. Zhang, X.Y. Chen, X.W. Wang, Q.Y. Kong, J. Liu, Regulatory effects of emodin on NF-kappaB activation and inflammatory cytokine expression in RAW 264.7 macrophages, Int. J. Mol. Med. 16 (2005) 41–47.
[30] P. Libby, Y. Okamoto, V.Z. Rocha, E. Folco, Inflammation in atherosclerosis: transition from theory to practice, Circ. J. 74 (2010) 213–220.
[31] Y. Sun, W. Yi, Y. Yuan, W.B. Lau, D. Yi, X. Wang, Y. Wang, H. Su, X. Wang, E. Gao, W.J. Koch, X.L. Ma, C1q/tumor necrosis factor-related protein-9, a novel adipocyte-derived cytokine, attenuates adverse remodeling in the ischemic mouse heart via protein kinase A activation, Circulation 128 (2013) S113–S120.