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Original Article

PRDM16 inhibits atherosclerosis plaque forming through enhancing the function of periaortic brown adipose tissue

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Abstract: In this study, we aimed to explore the role of PRDM16 in atherosclerosis plaque and the related mechanism. Rats were randomly divided into 3 groups and injected with normal saline, pAdenoG empty vector and PRDM16-pAdenoG vector. HE staining on the abdominal aorta atherosclerosis plaque and adipose surrounding artery of rats was performed. The mRNA levels of Leptin, Adiponectin, MCP-1, IL-8, TNF-α, IL-6 and IL-10 in adipose rounding abdominal aorta were determined by qRT-PCR. Adipocytes 3T3-L1 was transfected with PRDM16- pAdenoG vector and the differentiation was studied. PRDM16-induced brown adipose cells were co-cultured with macrophages. The mRNA levels of LOX-1 and CD36 on the surface of macrophages were determined. The M1 and M2 macrophages were identified by iNOS and CD86, MR and Arg I. The expression values of MCP-1, TNF-α, IL-6 and IL-10 were determined by qRT-PCR and ELISA assay. Many atherosclerosis plaques were observed in pAdenoG and control group, while no similar plaque was found in PRDM16-pAdenoG group. White adipose occupied the main status in pAdenoG and the control group, while in PRDM16-pAdenoG group, brown adipose was found the most. mRNA levels of Leptin, Adiponectin and IL-8 in PRDM16-pAdenoG group increased markedly, while the values of MCP-1, TNF-α, IL-6 and IL-10 decreased significantly. PRDM16-induced Brown adipose cells downregulated the expression of LOX-1 and CD36. The expression of MCP-1 and TNF-α decreased, while the values of IL-6 and IL-10 increased. In conclusion, PRDM16 can inhibit the formation of atherosclerosis plaques by strengthening the function of periaortic brown adipose tissue.

Keywords: Atherosclerosis, PRDM16, brown adipose, macrophage

Introduction

Atherosclerosis is nowadays generally accepted as an inflammatory disease. It is known that local inflammation occurs in the formation the plaques, as macrophages and other immunocompetent cells are present in the lesions from an early stage [1]. Recent advances in basic science have established a fundamental role for inflammation in mediating all stages of this disease [2].

Inflamed adipose tissue is a culprit for the occurrence of metabolic syndrome and atherosclerosis. Perivascular adipose tissue surrounds (coronary) arteries and may be involved in local stimulation of atherosclerosis plaque formation. Adipose tissue in mammals can be divided into white and brown adipose tissue [3]. The transcriptional control of the adipocyte lineage has been studied extensively. Brown adipose tissue (BAT) is a specialized tissue that can dissipate energy and counteract obesity through a pattern of gene expression that greatly increases mitochondrial content and uncoupled respiration [4]. Stanford et al. also reported BAT can function in the dissipation of chemical energy in response to cold or excess feeding, and also has the capacity to modulate energy balance [5]. Its thermogenic capacity is such that it allows mammals to live below thermoneutral conditions without having to rely on shivering muscles [6]. In humans, BAT is present in newborns, but disappears rapidly during

postnatal periods and, in adults, is rather difficult to identify by conventional anatomical examinations [7]. Macrophage accumulation in obese adipose tissue has been observed to be localized around necrotic or apoptotic adipocytes [8]. Macrophages display remarkable plasticity and can change their physiology in response to environmental cues. They are widely distributed immune system cells that play an indispensable role in homeostasis and defense [9]. They can be phenotypically polarized by the microenvironment to mount specific functional programs. The recruitment of monocyte-macrophages into the artery wall is one of the earliest events in the pathogenesis of atherosclerosis and macrophage apoptosis occurs throughout all stages of atherosclerosis [10].

PPARγ, a member of the nuclear hormone receptor superfamily, plays a dominant role in the differentiation of both white and brown adipose cells. PRDM16 is the sixteenth member of PR family and is highly enriched in brown adipose cells compared to white adipose cells [11]. It controls brown adipose determination and differentiation by stimulating brown adipose-selective gene expression, while suppressing the expression of genes selective for white adipose cells [12]. It activates a robust brown adipose phenotype including induction of PGC-1α, UCP1, and type 2 deiodinase (Dio2) expression and a remarkable increase in uncoupled respiration. In rodents and newborn humans, brown adipose tissue helps regulate energy expenditure by thermogenesis mediated by the expression of uncoupling protein 1 (UCP1) [13]. However, the mechanism on how PRDM16 influence atherosclerosis remains unknown.

In this study, we aimed to explore the influence of PRDM16 on the differentiation of adipose cells and the role of BAT on macrophage.

Materials and methods

Experimental groups and cell culture

Apo E-/- rats (approximately 2 weeks old) with atherosclerosis weighing 120 to 170 g were used for the study and maintained on a 12-hour light/dark cycle with continuous access to food (regular feed combing 1% cholesterol and 20% adipose or high adipose feed) and water for 2 months. All animals were treated according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All animal procedures were conducted after gaining the approval of the animal care committee of Shanghai resident standardization training base.

Rats were divided at random into the following 3 groups: (1) Sham group: rats of this group (n = 30) were injected with normal saline (NS) through abdominal aorta surrounding adipose tissues at 6 mg/kg per day for 7 days; (2) pAdenoG group: rats of this group (n = 30) were injected with pAdenoG empty vector through abdominal aorta surrounding adipose tissues at 6 mg/kg per day for 7 days; (3) PRDM16-pAdenoG group: rats of this group (n = 30) were injected with PRDM16-pAdenoG vector (multiplicity of infection = 100) through abdominal aorta surrounding adipose tissues at 6 mg/kg per day for 7 days.

Adipocyte 3T3-L1 and macrophage RAW264.7 were purchased from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

Biochemical indicator detection

To explore the effect of PRDM16 on atherosclerosis, the concentration of STG, TC and BG in rats after injection with NS, pAdenoG empty vector and PRDM16-pAdenoG vector. The concentration of TC and STG was determined with TC and STG test box (Nanjing institute of biological engineering research institute, China), respectively. Safe type Glucose meter (Changsha Sannuo biological sensor co., LTD, China) is designed to measure blood samples received on blood glucose test strips and to produce blood glucose values (mmol/L) from measurements of the blood samples.

Real-time PCR

To quantitatively determine the mRNA expression of PRDM16, Leptin, Adiponectin, MCP-1, IL-8, TNF-α, IL-6 and IL-10 mRNA in sham, pAdenoG and PRDM16-pAdenoG group, real-time quantitative PCR was used. For RNA isolation, total RNA was extracted and isolated from tissue samples using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. 10 μg RNA was reversely transcribed into cDNA using GoScript™ Reverse Transcrip-

Table 1. The detailed information of primers in RT-PCR

Name	Primer	Sequence
Leptin	Forward	5'-AGCTGCAAGGTGCAAGAAGA-3'
	Reverse	5'-TGAAGTCCAAGCCAGTGACC-3'
Adiponectin	Forward	5'-TCAGTGGATCTGACGACACC-3'
	Reverse	5'-TGCCATCCAACCTGCACAA-3'
MCP-1 (Ccl2)	Forward	5'-TTAAAAACCTGGATCGGAACCAA-3'
	Reverse	5'-GCATTAGCTTCAGATTTACGGGT-3'
IL-8 (CxCl15)	Forward	5'-TGGGTGAAGGCTACTGTTGG-3'
	Reverse	5'-GTCTCCCGAATTGGAAAGGGA-3'
PRDM16	Forward	5'-TGCGAAGGTGTCCAAACTGA-3'
	Reverse	5'-CCTTGGGAGTGAAGTCCTCG-3'
TNF-α	Forward	5'-ATGGCCTCCCTCTCATCAGT-3'
	Reverse	5'-TGGTTTGCTACGACGTGGG-3'
IL-6	Forward	5'-CGGCCTTCCCTACTTCACAA-3'
	Reverse	5'-TTCTGCAAGTGCATCATCGT-3'
TGF-β	Forward	5'-AGCTGCGCTTGCAGAGATTA-3'
	Reverse	5'-AGCCCTGTATTCCGTCTCCT-3'
IL-10	Forward	5'-AAGGGTTACTTGGGTTGCCA-3'
	Reverse	5'-TTCAGCTTCTCACCCAGGGA-3'

tion System (Promega, Southampton, UK). The cDNA was added to a 2 × Taq PCR MasterMix (Tiangen Biotech Co., LTD, Beijing, China) containing 10 pmol/L of each of the corresponding primer pairs. The details information of primers used in this analysis are listed in **Table 1**. PCR amplification was performed with corresponding cycles of 94°C for 3 min, 94°C for 30 s, annealing temperature for 30 s, 72°C for 1 min and 72°C for 5 min. The PCR products were separated on 1% agarose gels and were stained with ethidium bromide. The gels were scanned under a gel documentation system (Bio-Rad Co., Nanjing, China). β-actin expression was used as an internal reference to verify equal concentrations of cDNA in each sample.

Western blotting

To further determine the expression differences of PRDM16 after transfection with PRDM16-pAdenoG, western blotting was used. Abdominal aorta surrounding adipose tissues from sham, pAdenoG and PRDM16-pAdenoG group were washed thrice with PBS and transferred to buffer containing 25 mM HEPES; 2.5 mM EDTA; 0.1% Triton X-100, 1 mM PMSF, 5 μg/ml leupeptin. The mixture was centrifuged at 3000 r/min for 10 min and the supernatant was stored at 4°C. Total protein concentrations were determined with a UV spectrophotometer using a modified Bradford assay (Beckman Coulter, Fullerton, CA, USA). Equal amounts of protein from each sample (40 Ag) were mixed with 15-20 Al sample buffer and boiled for 5 min Samples were separated by electrophoresis on 7.5-12% polyacrylamide gels. Bands of proteins were then transferred to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad). The PVDF membrane was blocked TBS buffer (50 mM Tris-HCl, pH 7.5, containing 150 mM NaCl) containing 0.1% Tween-20 (TBST) for 1 h at room temperature and then incubated with antibodies against TJ claudin-5, VEGF, APP and Aβ for 3 h at room temperature. After an additional incubation for 1 h with horseradish peroxidase-conjugated secondary antibodies, the binding of antibodies to the PVDF membrane was and measured by KS 400 image analysis system (Carl Zeiss, Vision, Oberkochen, Germany).

HE staining

To study the influence of PRDM16 on the atherosclerosis plaque and adipose surrounding artery, HE staining on the abdominal aorta atherosclerosis plaque and adipose surrounding artery of rats was performed. Cells were collected by centrifuge after washed with phosphate-buffered saline (PBS) twice. Then, Hematoxylin-eosin was used to stain the cells for 5-10 min. Cells after dyeing were washed 3 times in PBS, shaken for 5 min each, dried, and mounted with 30% glycerol. Fluorescence microscope Q550CW (Leica, Wetzlar, Germany) with Cooke software (magnification, × 10) was used for apoptosis analysis.

Influence of PRDM16 on adipocytes differentiation

To explore the influence of PRDM16 on adipocytes differentiation, adipocytes 3T3-L1 was transfected with PRDM16-pAdenoG vector. The procedure was: First, 3T3-L1 cells were cultured with PRDM16-pAdenoG vector in 10% FBS DMEM for 2 hours. Then, the DMEM was changed and 3T3-L1 cells were incubated in incubator with 5% CO_2 for 48 hours. Following, RNA extraction and expression analysis were performed. To monitor the infection efficiency of PRDM16 on 3T3-L1 cells, GFP expressed from the adenoviral vectors was used and the value was over 50%. Next, adipocyte differenti-

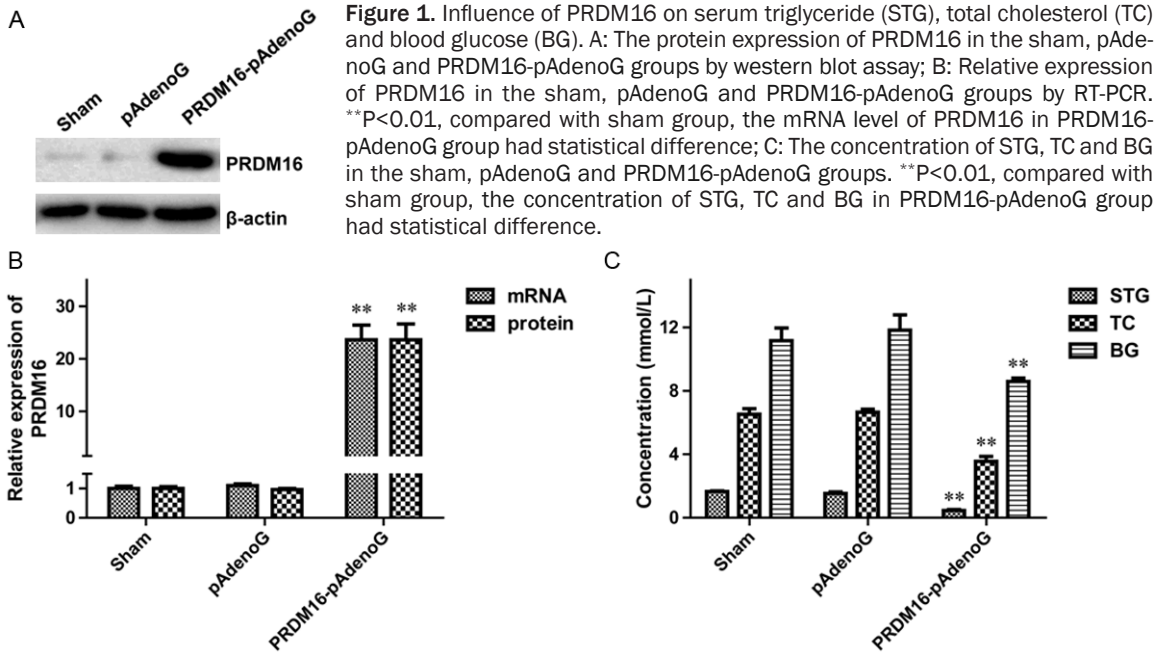

Figure 1. Influence of PRDM16 on serum triglyceride (STG), total cholesterol (TC) and blood glucose (BG). A: The protein expression of PRDM16 in the sham, pAdenoG and PRDM16-pAdenoG groups by western blot assay; B: Relative expression of PRDM16 in the sham, pAdenoG and PRDM16-pAdenoG groups by RT-PCR. $^{**}P<0.01$, compared with sham group, the mRNA level of PRDM16 in PRDM16-pAdenoG group had statistical difference; C: The concentration of STG, TC and BG in the sham, pAdenoG and PRDM16-pAdenoG groups. $^{**}P<0.01$, compared with sham group, the concentration of STG, TC and BG in PRDM16-pAdenoG group had statistical difference.

ation was performed and the procedure was: PRDM16-infected adipocytes 3T3-L1 were incubated in DMEM which contained 10% FBS, 0.5 mM isobutylmethylxanthine, 125 nM indomethacin, 1 μM dexamethasone, 850 nM insulin, 1 nM T3, and 1 μM rosiglitazone for 2 days. Then, cells were transferred to maintenance medium which contained 10% FBS, 850 nM insulin, 1 nM T3, and 1 μM rosiglitazone. The mRNA levels of BAT-selective genes (Ucp1, Cidea, Elovl3) and WAT-selective adipokines (resistin and angiotensinogen) before and after differentiation were determined by RT-PCR.

Co-culture

For experiment group, macrophage RAW264.7 was inoculated into 24-well culture plates (1 mL suspension/well) which lied in the down room of transwell and adipocytes 3T3-L1 lied in the up room. For the control group, the same quantity of macrophage RAW264.7 was set in the up and down room of transwell. Then they were cultured at 37°C in a humidified atmosphere of 5% CO_2 in air. The mRNA levels of LOX-1 and CD36 on the surface of RAW264.7 cells in the two groups were determined by RT-PCR. To study the influence of adipocytes induced by PRDM16 on the polarization and secretion function of macrophage, first, iNOS and CD86 were used to identify M1 macrophage, MR and Arg I were used for the identification of M2 macrophage. Then, mRNA levels of MCP-1, TNF-α, IL-6, TGF-β and IL-10 in macrophage RAW264.7 were determined by RT-PCR. The protein levels of them were determined by ELISA assay.

ELISA assay

TNF-α was determined in the uterine secretions, serum and plasma using a commercially available ultra sensitive ELISA kit (Laboserv Diagnostica, Giessen, Germany). Following centrifugation the supernatants of the samples were analyzed. The ELISA was performed according to the instructions of the manufacturer. The serum and plasma concentration of TNF-α was measured to estimate the contamination of the samples with TNF-α from blood. The TNF-α concentration in the samples were expressed relative to the sample's protein content, determined according to Lowry et al. [14].

Statistical treatment and analysis

The data were represented as the means ± standard deviation (SD) and analyzed adopting PASW statistics 18 software. A value of $P<0.05$ is considered as statistically difference. Comparisons of two or more data sets were analyzed using one-way analysis of variance (ANOVA), and data with more than two variables were analyzed using two-way repeated-measures ANOVAs with post hoc Tukey's analysis.

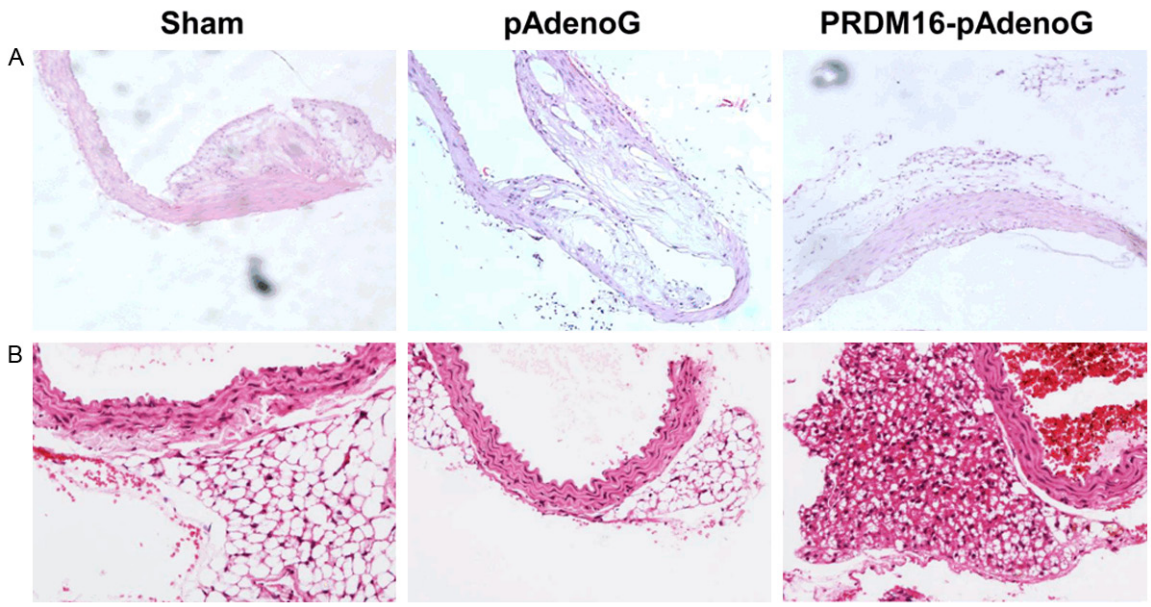

Figure 2. HE staining on the abdominal aorta atherosclerosis plaque and adipose surrounding artery of rats in the sham, pAdenoG and PRDM16-pAdenoG groups. A: PRDM16 inhibited the formation of atherosclerosis plaque. B: Adipose occupied the main status in the control and pAdenoG group, while in PRDM16-pAdenoG group, the main adipose was brown adipose.

Results

PRDM16 decreases the characteristics of atherosclerosis

In order to study the influence of PRDM16 on the occurrence of atherosclerosis, experimental rats were first injected with pAdenoG empty vector and PRDM16-pAdenoG vector, and the mRNA levels and protein expression values of PRDM16 in the three groups were determined by RT-PCR and western blot assay. As the results (**Figure 1A**) shown, after Apo E-/- rats were injected with PRDM16-pAdenoG for 7 days, the mRNA levels and protein expression values were significantly higher than rats injected with pAdenoG and the control ($P<0.01$). It indicated PRDM16 was markedly expressed in rats injected with PRDM16-pAdenoG. Serum triglycerides (STG), total cholesterol (TC) and blood glucose (BG) were risk factors for cardiovascular diseases and significantly high in atherosclerosis patients. Therefore, the concentration of STG, TC and BG in the three groups was determined to explore the relationship between PRDM16 and atherosclerosis. As **Figure 1B** shown, the concentration of STG, TC and BG in PRDM16-pAdenoG group was significantly lower than the other groups, which indicated high PRDM16 expression will cause the decrease of the levels of STG, TC and BG in Apo E-/- rats.

PRDM16 inhibit atherosclerosis plaque and induce BAT

To study the influence of PRDM16 on the occurrence of atherosclerosis plaque in abdominal aorta and the transition from white adipose tissue (WAT) to BAT, HE staining was performed on abdominal aorta and adipose around the artery collected from rats in pAdenoG, PRDM16-pAdenoG and the control group. As **Figure 2A** shown, atheromatous plaque was found in abdominal aorta in pAdenoG and the control group, while in PRDM16-pAdenoG group, almost no analogous plaques were found in abdominal aorta. Observing adipose around the artery, we would find in pAdenoG and the control group, WAT occupied the main position, while in PRDM16-pAdenoG group, BAT was the most important (**Figure 2B**). From those results, we concluded PRDM16 can inhibit the occurrence of atherosclerosis plaque in abdominal aorta and induce the formation of BAT.

PRDM16 changed the expression of inflammatory factors

Recent research has shown that inflammation plays a key role in coronary artery disease

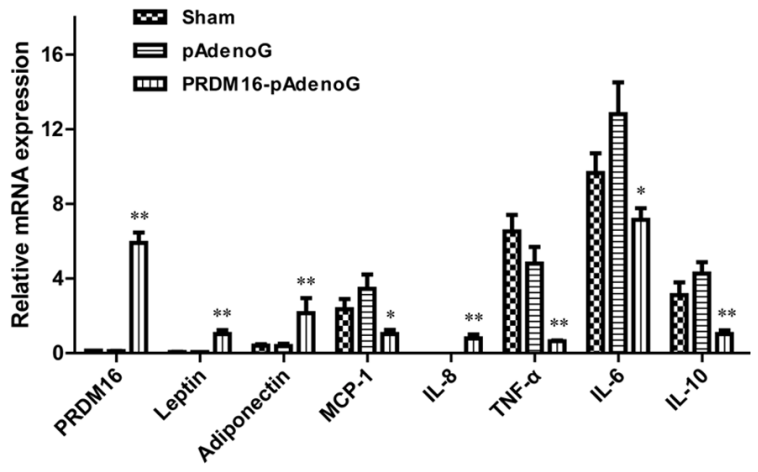

Figure 3. The mRNA levels of Leptin, Adiponectin, MCP-1, IL-8, TNF-α, IL-6 and IL-10 in the sham, pAdenoG and PRDM16-pAdenoG groups. *P<0.05, compared with the mRNA level of genes in the sham group, the values in PRDM16-pAdenoG group had statistical difference; **P<0.01, compared with the mRNA level of genes in the sham group, the values in PRDM16-pAdenoG group had significantly statistical difference.

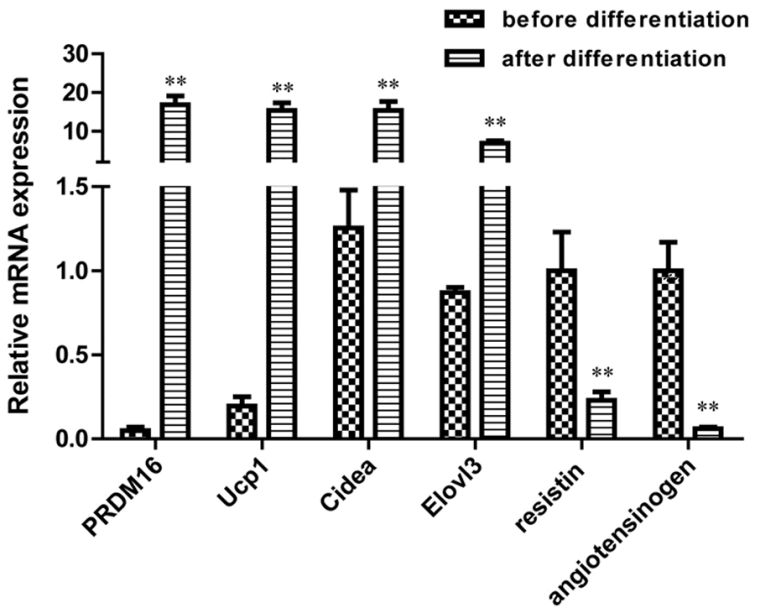

Figure 4. The mRNA levels of BAT-selective genes (Ucp1, Cidea and Elovl3) and WAT-selective adipokines (resistin and angiotensinogen) before and after adipocyte differentiation. **P<0.01, compared with the mRNA level of genes in before adipocyte differentiation group, the values in the after adipocyte differentiation group had significantly statistical difference.

(CAD) and other manifestations of atherosclerosis. Moreover, our study showed PRDM16 can induce the formation of BAT. Therefore, we determined the influence of PRDM16 on the mRNA levels of Leptin, Adiponectin, MCP-1, IL-8, TNF-α, IL-6 and IL-10 in adipose around the artery by RT-PCR. Results showed after rats were injected with PRDM16-pAdenoG for 7 days, the mRNA levels of Leptin, Adiponectin, MCP-1, IL-8, TNF-α, IL-6 and IL-10 changed significantly. Compared with pAdenoG and the control group, mRNA levels of Leptin, Adiponectin and IL-8 in PRDM16-pAdenoG group increased markedly, while the values of MCP-1, TNF-α, IL-6 and IL-10 decreased significantly (**Figure 3**).

PRDM16 induce the differentiation into BAT

To study the role of PRDM16 on the differentiation of adipose tissue to WAT and BAT, PRDM16-pAdenoG vector was used to transfect the preadipocyte 3T3-L1 of rats for experiment and adipocyte differentiation was observed. The mRNA level changes of BAT-selective genes Ucp1, Cidea and Elovl3, and WAT-selective adipokines resistin and angiotensinogen were determined by RT-PCR. As **Figure 4** shown, the mRNA levels of Ucp1, Cidea and Elovl3 increased observably, while the expression of adipokines resistin and angiotensinogen was down-regulated significantly. All those indicated 3T3-L1 cells transfected with PRDM16 were more likely to differentiate into BAT.

BAC inhibits the secretion function of macrophages

Macrophages are widely distributed immune system cells that play an indispensable role in homeostasis and defense. Excessive lipid accumulation in macrophages plays an important role in the development of atherosclerosis. To study the role of brown adipocytes on macrophages, macrophage RAW264.7 was co-cultured with brown adipocyte 3T3-L1. Then the

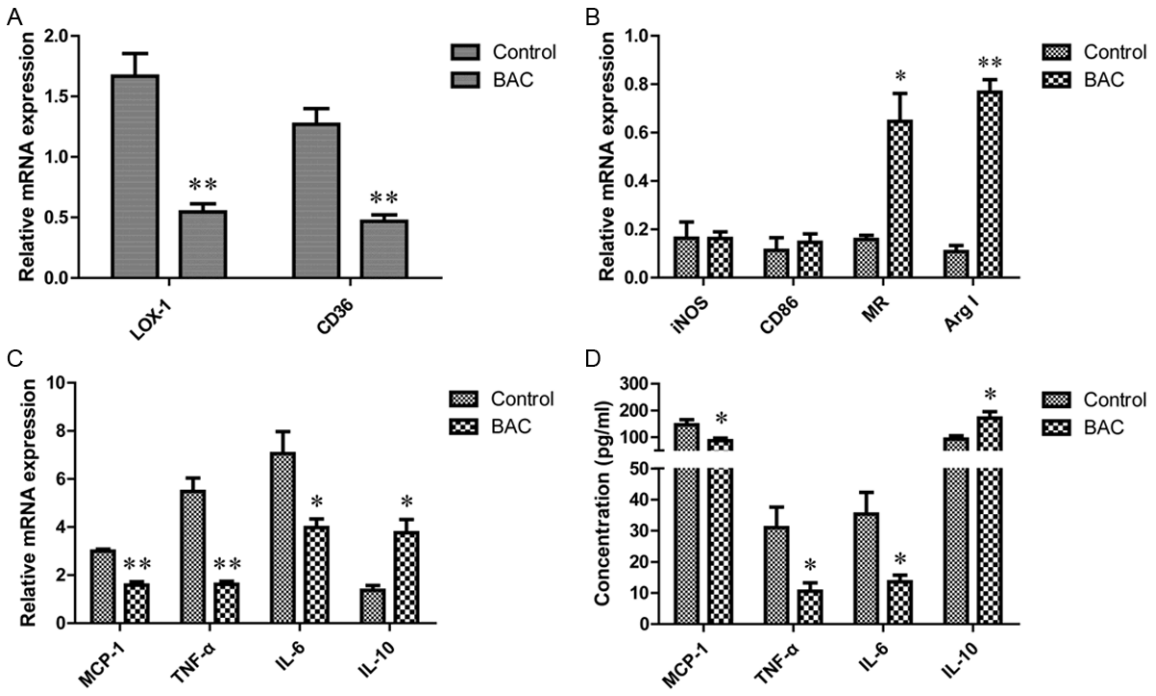

Figure 5. Influence of PRDM16-induced brown adipose cells on the polarization and secretion function of macrophage. A: Relative mRNA levels of LOX-1 and CD36 on the surface of macrophages in the co-culture group and the control. BAC: the co-culture group. *P<0.01, compared with relative mRNA level of LOX-1 or CD36 in the control group, the value in BAC group had significantly statistical difference; B: The relative mRNA levels of iNOS, CD86, MR and Arg I in the control and BAC group. BAC: the co-culture group. *P<0.01, compared with relative mRNA level of iNOS, CD86, MR and Arg I in the control group, the value in BAC group had significantly statistical difference; C: The relative mRNA levels of MCP-1, TNF-α, IL-6 and IL-10 in the control and BAC group by RT-PCR. *P<0.01, compared with relative mRNA level of MCP-1, TNF-α, IL-6 and IL-10 in the control group, the value in BAC group had significantly statistical difference; D: The concentrations of MCP-1, TNF-α, IL-6 and IL-10 in the control and BAC group detected by RT-PCR. *P<0.01, compared with control group.

mRNA levels of LOX-1 and CD36 on the surface of RAW264.7 cells by RT-PCR, results showed the expression of LOX-1 and CD36 was much lower than that in the control, respectively (**Figure 5A**). iNOS, CD86, MR and Arg I were used to identify the phenotype of macrophage and the mRNA levels of them were determined by RT-PCR (**Figure 5B**). As shown, the expression of MR and Arg I in BAC group was much higher than the control group, while the values of iNOS and CD86 kept almost the same. The mRNA and protein levels of MCP-1, TNF-α, IL-6, TGF-β and IL-10 were also determined by RT-PCR and ELISA assay, respectively. The results showed the mRNA levels for all of them in BAC group were much lower than the control (**Figure 5C**).

Discussion

Atherosclerosis, formerly considered a bland lipid storage disease, actually involves an ongoing inflammatory response. Atherosclerosis plaques have been shown to occur predominantly in epicardial coronary arteries. The mechanisms that cause atherosclerosis plaque to become symptomatic remain unclear. Evidence suggests that mediators of inflammation are instrumental in the formation of plaque [15]. Perivascular adipose tissues were known as a cause of atherosclerosis [16]. While adipocytes expressed PRDM16 is a known as a marker for brown adipose [17]. Therefore, we aimed to study the influence of PRDM16 on atherosclerosis.

First, rats were randomly divided into 3 groups, and injected with normal saline, pAdenoG group and PRDM16-pAdenoG. Results of RT-PCR and western blot assay showed the expression value of PRDM16 in PRDM16-pAdenoG group was significantly up-regulated. The determination on the concentration of STG, TC and BG showed the concentration of STG

and TC in PRDM16-pAdenoG group was markedly lower than the other groups, while the value of BG was opposite. Therefore, we speculated PRDM16 play an important role on the happen of atherosclerosis. To verify the role of PRDM16 on atherosclerosis, HE staining on the abdominal aorta atherosclerosis plaque and adipose surrounding artery of rats was performed. Results showed atherosclerosis plaques were all found in the control and pAdenoG group, while in PRDM16-pAdenoG group, none was found. For observing adipose surrounding artery, white adipose occupied the main status in the control and pAdenoG group, while in PRDM16-pAdenoG group, the main adipose was brown adipose. Therefore, we concluded PRDM16 can inhibit the formation of atherosclerosis plaque and induce the differentiation of adipose to brown adipose. It indicated PRDM16 may inhibit the formation of atherosclerosis plaque by increasing the content of brown adipose. To explore whether the relationship between PRDM16 and brown adipose exists, we determined the expression values of Leptin, Adiponectin, MCP-1, IL-8, TNF-α, IL-6, IL-10 in adipose surrounding artery in the 3 groups. Results showed the expression of Leptin and Adiponectin were significantly up-regulated after transfection with PRDM16, while the values of MCP-1, IL-8, TNF-α, IL-6 and IL-10 were markedly down-regulated. Leptin, adipocyte hormone and the gene product of the obese gene, may play an important role in regulating body weight by signaling the size of the adipose tissue mass [18]. It is a crucial molecule for a number of diverse physiological processes, such as inflammation, immune function and atherosclerosis [19]. Previous data indicate that hyperleptinaemia is involved in the pathogenesis of atherosclerosis [18]. It can cause the incrassation of tunica media in artery and instability of plaque, and strengthen the expression of AS factor [20]. Adiponectin is an adipocyte-derived peptide, which has antiinflammatory and insulin-sensitising properties [21]. It possesses anti-atherogenic properties, such as the suppression of adhesion molecule expression in vascular endothelial cells and cytokine production from macrophages [22]. It has been identified recently as one of the adipocytokines with important metabolic effects and can suppress atherogenesis by inhibiting the adherence of monocytes, reducing their phagocytic activity, and suppressing the accumulation of modified lipoproteins in the vascular wall [23]. MCP-1, also known as CC chemokine ligand 2), is a potent chemoattractant and activator of mononuclear phagocytes. Monocyte chemoattractant protein-1 (MCP-1) is a potent chemokine synthesized by several cell types, eg, inflammatory cells, such as monocytes, and resident renal cells, such as human tubular epithelial cells (TECs) [24]. It play a key role in the migration of monocytes and T-cells into the vessel wall and in the evolution of atherosclerosis [25]. Expression of this proinflammatory chemokine is increased in atherosclerotic lesions. Moreover, MCP-1 appears to be a key molecule regulating atherosclerosis plaque instability by recruiting monocytes/macrophages into the plaques and thus eliciting proinflammatory responses [26]. IL-8, a member of the chemokine family, has been shown to play an important role in tumor growth, angiogenesis, and metastasis [27]. Aberrant production of IL-8 has been shown in various human inflammatory diseases and it is a novel target to alleviate acute inflammation [28]. It plays a causative role in acute inflammation mainly by recruiting and activating neutrophils [29]. TNF-α is a potent pro-inflammatory and immunomodulatory cytokine implicated in inflammatory conditions such as rheumatoid arthritis, Crohn's disease, multiple sclerosis and the cachexia associated with cancer or human immunodeficiency virus [30]. It induces the occurrence of atherosclerosis and the inhibition of it is therapeutic regarding inhibition of atherosclerosis [31]. IL 6 is typical example of multifunctional cytokines involved in the regulation of the immune response, hematopoiesis, and inflammation. Overproduction of it is associated with a spectrum of age-related conditions including cardiovascular disease, osteoporosis, arthritis, type 2 diabetes, certain cancers, periodontal disease, frailty, and functional decline [32]. IL-10, a cytokine with anti-inflammatory properties, has a central role in infection by limiting the immune response to pathogens and thereby preventing damage to the host [33]. It is expressed in advanced human atherosclerosis and is associated with low inducible nitric oxide synthase expression and low levels of apoptosis [34]. TNF-α has also been implicated in a range of inflammatory, infectious, and malignant disorders. At the cellular level, TNF-α modulates a broad spectrum of responses including inflammation, immuno-

regulation, proliferation and apoptosis [35]. It has been shown to be an important marker of vascular inflammation and a predictor of atherosclerosis [36]. From the study above, we concluded PRDM16 can induce the formation of brown adipose cells by influencing inflammatory factors.

To study the influence of PRDM16 on the differentiation to brown adipocyte, adipocyte 3T3-L1 was transfected with PRDM16-pAdenoG vector. Results showed after transfection with PRDM16, the expression of BAT-selective genes Ucp1, Cidea and Elovl3 increased, while the expression of WAT-selective adipokines resistin and angiotensinogen decreased. This indicated overexpression of PRDM16 can induce adipocyte differentiate more easily into brown adipocyte. Then, brown adipocyte induced by PRDM16 was co-cultured with macrophage to explore the effect of brown adipocyte on the function of macrophage. Macrophages play an essential role in the resolution of tissue damage through removal of necrotic cells, thus paving the way for tissue regeneration. Macrophages also directly support the formation of new tissue to replace the injury [37]. Results showed the expression levels of LOX-1 and CD36 on the surface of macrophage were much lower than the control. LOX-1, a lectin-like 52-kD receptor for oxidized low-density lipoproteins (ox-LDL), is present primarily on endothelial cells [38]. It plays a critical role in endothelial dysfunction and atherosclerosis and its activation also plays an important role in monocyte adhesion to endothelial cells [39]. CD36 has been identified in isolated cell studies as a putative transporter of long chain fatty acids [40]. It plays an important role in the uptake of oxidized forms of low density lipoprotein (LDL) and contributes to lesion development in murine models of atherosclerosis [41]. Identification on the phenotype of macrophage showed the expression of M2 macrophage increased significantly with co-culture with PRDM16-induced brown adipocyte. Polarization of macrophages to M1 or M2 cells is important for mounting responses against bacterial and helminth infections. Then the expression levels of MCP-1, TNF-α, IL-6 and IL-10 in macrophage were also determined. Results showed the expression of MCP-1 and TNF-α decreased significantly, while the values of IL-6 and IL-10 increased. MCP-1 is key agonists that attract macrophages to tumors. In obesity, MCP-1 can contribute to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis [42]. IL-6 is a cytokine produced by several cell types including antigen presenting cells (APC) such as macrophages, dendritic cells, and B cells [43]. It can also induce the differentiation of macrophages. IL-10 regulates inflammation by reducing cytokine and chemokine production from activated macrophages [44]. The increase of IL-6 and IL-10, and decrease of MCP-1 and TNF-α indicated PRDM16 weaken the secretion function of macrophages.

In conclusion, PRDM16 can inhibit the formation of atherosclerosis plaques, induce the differentiation of adipose cells to brown adipose cells, reduce inflammatory reaction and weaken the secretion function of macrophages.

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Disclosure of conflict of interest

None.

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