Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Adrenergic receptor-mediated activation of FGF-21-adiponectin axis exerts atheroprotective effects in brown adipose tissue-transplanted *apoE*$^{-/-}$ mice

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ARTICLE INFO

Article history:
Received 19 February 2018
Accepted 24 February 2018
Available online xxx

Keywords:
Brown adipose tissue
Atherosclerosis
Fibroblast growth factor-21
Adiponectin
Sympathetic nervous system

ABSTRACT

Brown adipose tissue (BAT) has been found as an endocrine organ that maintains metabolic homeostasis; however, the effects on atherosclerosis remain undefined. Here, we investigated the effect of experimental BAT transplantation on atherosclerosis. Interscapular BAT was dissected from wild-type mice and transplanted into the visceral cavity of 12-week-old *apoE*$^{-/-}$ mice. Oil-red O staining of whole aortas after 3 months of a high-cholesterol diet showed a significant decrease in atherosclerotic lesion area in BAT-transplanted mice by 32% compared with the sham control mice. Lipid profiles, except for serum triglyceride level, showed no difference between the 2 groups. BAT-transplanted mice showed higher concentrations of serum noradrenalin, fibroblast growth factor 21 (FGF-21), and adiponectin. Treatment with the β3-adrenergic receptor (AR) blocker completely abrogated the atheroprotective effects of BAT transplantation, with serum concentrations of FGF-21 and adiponectin being equivalent between the 2 groups. Homologous transplantation of BAT from *apoE*$^{-/-}$ mice also showed a significant decrease in atherosclerotic lesion area by 28% without affecting lipid profiles, while epididymal white adipose tissue transplantation did not affect atherosclerosis. Serum and endogenous BAT concentrations of FGF-21 were significantly higher in BAT-transplanted mice than sham control mice. Concomitantly, serum adiponectin levels were elevated in BAT-transplanted mice and showed a significant inverse correlation with atherosclerotic lesion area. Our findings show for the first time that atheroprotective effect of BAT transplantation is BAT-specific and independent of lipid-lowering effect, accompanied by AR-mediated activation of the FGF-21-adiponectin axis.

1. Introduction

Brown adipose tissue (BAT) plays a crucial role in adaptive thermogenesis in small mammals and newborn infants, and has long been considered to have no physiological relevance in adult humans [1]. However, recent study using positron emission tomography and computed tomography (PET/CT) have revealed that BAT is active in adulthood during cold acclimation [2]. BAT is inversely correlated with BMI in humans, in contrast to white adipose tissue (WAT) [3]. These findings have led to the notion that activation of BAT is a potential therapeutic strategy to prevent the development of obesity and metabolic disease [4–6].

The effects of BAT activation on atherosclerosis have recently been investigated; however, experimental findings remain controversial [7–9]. Although BAT activation by cold acclimation significantly decreased plasma triglyceride, it increased the plasma levels of small low-density lipoprotein (LDL) remnants, leading to atherosclerotic plaque growth in apolipoprotein E deficient (*apoE*$^{-/-}$) mice accompanied by marked reduction of plasma adiponectin levels [7]. In contrast, BAT activation by β3-adrenergic receptor (AR) stimulation inhibits the development of atherosclerosis in APOE*3-Leiden.CETP mice, but not in hyperlipidemic *apoE*$^{-/-}$ mice or LDL receptor deficient (*LDLR*$^{-/-}$) mice [8].

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https://doi.org/10.1016/j.bbrc.2018.02.185
0006-291X/

Previous studies have investigated the atheroprotective effects of activated BAT by focusing on augmented energy expenditure, with a consequent improvement in lipid homeostasis; however, BAT, as well as WAT, has recently been found as an endocrine organ that regulates metabolic homeostasis [10]. BAT produces and releases various kinds of adipokines, termed “batokines”, which have different actions from those in WAT [4,5]. Gunawardana et al. [4]. showed that adiponectin, leptin, and insulin growth factor-1 (IGF-1) was significantly increased in BAT-transplanted mice, which corrected glucose homeostasis in type 1 diabetic rats. A recent study by Stanford et al. [5]. showed that BAT transplantation stimulated the production and release of fibroblast growth factor 21 (FGF-21) in endogenous BAT, leading to improvement in glucose homeostasis and insulin sensitivity. However, it remains to be determined whether activated BAT could function as an endocrine organ to inhibit atherosclerosis development.

Here, we showed, for the first time, that BAT transplantation exerts atheroprotective effects, accompanied by enhanced expression of FGF-21 in endogenous BAT. Serum FGF-21 levels were also increased in BAT-transplanted mice along with a higher concentrations of serum adiponectin, which was inversely correlated with atherosclerotic lesions. Our findings suggest that BAT could function as an endocrine organ to inhibit atherosclerosis and its activation could be a potential therapeutic strategy to prevent cardiovascular diseases, as well as obesity and metabolic diseases, in adults.

2. Materials and methods

For detailed experimental procedures, see Supplementary material online.

2.1. Experimental animals

Donor wild-type mice (C57BL/6) were obtained from Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan). Recipient *apoE*$^{-/-}$ mice obtained from Jackson Laboratory (Bar Harbor, ME, USA) were B6.129P2-*Apoe*^{tm1Unc}/J background. BAT transplantation was performed as described previously [5], and studied in the following 3 protocols: 1) transplantation of interscapular BAT from wild-type mice into apoE$^{-/-}$ mice; 2) transplantation of interscapular BAT from wild-type mice into apoE$^{-/-}$ mice with β3-AR blocker treatment; 3) homologous transplantation of BAT or epididymal WAT (eWAT) into apoE$^{-/-}$ mice.

Recipient *apoE*$^{-/-}$ mice were fed a high-cholesterol diet (13.6% fat, 1.25% cholesterol; Oriental Yeast Co. Tokyo, Japan) until 24 weeks of age. In the experiment of protocol 2, β3-AR blocker (1 mg/kg/day, SR59230A, Sigma) was administered using an osmotic minipump. At 24 weeks, mice were sacrificed by *trans*-cardiac perfusion of physiological saline solution under anesthesia induced by inhalation of isoflurane, and atherosclerotic lesions of the aorta were evaluated.

2.2. Statistical analysis

All data are expressed as mean ± SE. Mean values were compared using one-way or two-way ANOVA with Tukey-Kramer's post hoc test to analyze differences between the groups. For correlation studies, *P* value and Spearman regression coefficient (r) were shown as the best-fit line generated by STATCEL2 (OMS, Saitama, Japan). $P < 0.05$ was considered significant.

3. Results

3.1. Atherosclerotic lesion development is reduced in BAT-transplanted *apoE*$^{-/-}$ mice accompanied by augmented activation of endogenous BAT

We first transplanted interscapular BAT from C57BL/6-Tg (CAG-EGFP) mice into the visceral cavity of age-matched *apoE*$^{-/-}$ mice. Histochemical analysis of the grafted BAT after 12 weeks of transplantation revealed the successful engraftment of transplanted BAT (Supplementary Fig. S1). We then examined the impact of BAT transplantation on atherosclerosis in *apoE*$^{-/-}$ mice. Atherosclerotic lesions in whole aortas was markedly reduced in BAT-transplanted mice compared with control mice (Fig. 1A and B). Mean body weight and BAT-to-body ratio were modestly, but significantly, smaller than control mice, while cumulative caloric intake was equivalent (Supplementary Fig. S2). Lipid profiles, except for serum triglyceride level, were comparable between the 2 groups, while blood pressure was significantly increased in BAT-transplanted mice (Supplementary Fig. S3). Consistent with earlier reports, the intraperitoneal glucose tolerance test showed significant improvement in BAT-transplanted *apoE*$^{-/-}$ mice, and oxygen consumption and energy expenditure were significantly increased in BAT-transplanted *apoE*$^{-/-}$ mice (Supplementary Fig. S4). We next examined the characteristics of grafted and endogenous BAT in *apoE*$^{-/-}$ recipient mice. Bilateral eWAT was dissected and then grafted BAT was extracted by carefully scraping off the surrounding eWAT (Fig. 1C). The histomorphological examination of the grafted BAT showed unilocular, but not multilocular, droplets like WAT (Fig. 1D). Consistently, the expressions of brown adipocyte-related genes were significantly lower than those in endogenous BAT (Supplementary Fig. S5A). In contrast, brown adipocyte-related genes expression in endogenous BAT were markedly increased in BAT-transplanted mice compared with those in sham control mice. We examined the mRNA expression of anti-inflammatory cytokines indicative of atherosclerosis development. Endogenous BAT in BAT-transplanted mice showed a 2.9-fold increase in *FGF-21* mRNA expression compared with that of sham control mice (Fig. 1E), while the mRNA expression profiles of pro-inflammatory cytokines did not differ (Supplementary Fig. S5B). We therefore focused on the augmented expression of FGF-21 as a candidate for atheroprotective action in BAT-transplanted mice.

3.2. Blockade of β3-AR abrogates the atheroprotective effects of BAT transplantation along with the reduction of serum FGF-21 and adiponectin levels

Serum FGF-21 levels in BAT-transplanted mice were significantly higher than those in sham control mice (Fig. 2A). However, there was no difference in the serum levels of IGF-1, TGF-β, TNF-α, and IL-6 between groups (Supplementary Fig. S6). FGF-21 has been reported to promote the differentiation and lipolysis of WAT, thereby contributing to the augmented production of adiponectin [11,12], which could inhibit atherosclerosis development [13]. As expected, inguinal white adipose tissue (iWAT) of BAT-transplanted mice showed a significant increase in *adiponectin* mRNA (Fig. 2B), accompanied by the increased serum adiponectin levels (Fig. 2C). We next examined the mechanism whereby the FGF-21-adiponectin axis is activated in BAT-transplanted mice. Because the mRNA expression levels of *UCP-1* and *Ppargc1a* in endogenous BAT were significantly elevated in BAT-transplanted mice (Supplementary Fig. S5A), we guessed that brown adipocyte is stimulated via the sympathetic nervous system (SNS). Consistently, serum noradrenalin concentration was significantly elevated in BAT-transplanted mice compared with sham control

Please cite this article in press as: M. Kikai, et al., Adrenergic receptor-mediated activation of FGF-21-adiponectin axis exerts atheroprotective effects in brown adipose tissue-transplanted *apoE*$^{-/-}$ mice, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.02.185