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Accumulation of adiponectin in inflamed adipose tissues of obese mice

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ABSTRACT

Objective. Adipose tissue inflammation plays an important role in the pathogenesis of obesity-associated complications, such as atherosclerosis. Adiponectin secreted from adipocytes has various beneficial effects including anti-inflammatory effect. Obesity often presents with hypoadiponectinemia. However, the mechanism and adiponectin movement in obesity remain uncharacterized. Here we investigated tissue distribution of adiponectin protein in lean and obese mice.

Methods. Adiponectin protein levels were evaluated by enzyme-linked immunosorbent assay and western blotting. Adipose tissues were fractionated into mature adipocyte fraction (MAF) and stromal vascular fraction (SVF).

Results. Adiponectin protein was detected not only in MAF but also in SVF, which lacks adiponectin mRNA expression, of adipose tissue remarkably. SVF adiponectin protein level was higher in obese mice than in lean mice. The mechanism of adiponectin accumulation was investigated in adiponectin-deficient (APN-KO) mice after injection of plasma from wild-type mice. These mice showed accumulation of exogenous adiponectin, which derived from wild type mice, in adipose tissues, and the adiponectin was more observed in SVF of diet induced obese APN-KO mice than lean APN-KO mice. Among the adiponectin binding proteins, T-cadherin mRNA and protein levels in SVF of obese mice were remarkably higher than in lean mice. Oxidative stress levels were also significantly higher in SVF of obese mice than lean mice. Mechanistically, H_2O_2 up-regulated T-cadherin mRNA level in murine macrophages.

Conclusions. The results demonstrated adiponectin targets to adipose SVF of obese mice. These findings should shed a new light on the pathology of adipose tissue inflammation and hypoadiponectinemia of obesity.

1. Introduction

Visceral obesity associated with multiple cardiometabolic risk factors is a common basis of atherosclerotic vascular diseases. Obesity correlates with chronic inflammation of the adipose tissues, which is characterized by progressive infiltration of macrophages [1] and overproduction of reactive oxygen species (ROS) [2,3]. The chronic inflammation state results in

Abbreviations: APN, adiponectin; CLS, crown-like-structure; HF/HSD, high fat/high sucrose diet; MAF, mature adipocyte fraction; NAC, N-acetylcysteine; ROS, reactive oxygen species; SVF, stromal vascular fraction; TBARS, thiobarbituric acid reactive substance; WAT_{mes}, mesenteric white adipose tissue; WAT_{sub}, subcutaneous white adipose tissue.

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adipocyte dysfunction and dysregulated production of adipocytokines, leading to systemic metabolic disorders and atherosclerosis [4,5]. The inflamed adipose tissue in obesity is also characterized histopathologically by the presence of "crown-like structures", representing macrophages around dead adipocytes [6,7].

The normal blood level of adiponectin [also known as adipocyte complement-related protein of 30 kDa (ACRP30), adipoQ and gelatin binding protein of 28 kDa (GBP28)], an adipocytokine discovered by our laboratory and three other groups, independently [8–12], ranges from 3 to 30 μg/mL, but decreases in obese individuals [13]. However, the mechanism of plasma adiponectin reduction in obesity has not been fully elucidated. Adiponectin is exclusively synthesized by adipocytes and exhibits anti-diabetic, anti-atherosclerotic, and anti-inflammatory properties [14]. These actions are supposed to be mediated through the interaction of adiponectin with cell surface binding receptors. The first identified adiponectin receptor, AdipoR1, was isolated from a human skeletal muscle cDNA library while screening for globular adiponectin binding [15]. AdipoR2 was found based on its homology with AdipoR1. Both AdipoR1 and AdipoR2 are surface membrane proteins with seven transmembrane domains, with similar molecular structures, and are expressed in the liver, muscle, and adipose tissue in humans. Adiponectin also binds to calreticulin on the macrophage cell surface, and opsonizes apoptotic cells [16]. T-cadherin, a GPI-anchored molecule that lacks a cytoplasmic region, also binds with adiponectin on vascular endothelial cells and smooth muscle [17,18].

Studies from our laboratories have demonstrated the severer tissue damage in the heart and kidney of adiponectin-deficient (APN-KO) mice compared to wild type mice, by various pathological overload. Administration of adenovirus-induced adiponectin ameliorates such tissue injury and also suppressed progression of fatty streak lesion in apoE KO mice [19–21]. The anti-proinflammatory property of adiponectin [14] might be mediated partly through the release of IL-10 by macrophages [22]. Adiponectin also promotes macrophage polarization toward the M2 phenotype and reduces ROS generation [23]. Here, in this study, we postulate adiponectin may stay or return to interstitial space of adipose tissue adipose tissue, and may play a role in the control of adipose tissue inflammation in obesity. The present study was designed to localize adiponectin in adipose tissues of obese and lean mice, and the mechanisms involved in such distribution pattern.

2. Materials and Methods

2.1. Materials

Rabbit polyclonal antibody to mouse adiponectin was generated at Otsuka Pharmaceutical (Tokyo, Japan). Monoclonal antibodies to mouse F4/80 (Abcam, Cambridge, MA) and T-cadherin (Santa Cruz Biotechnology, Santa Cruz, CA) were purchased. Goat anti-rabbit IgG horseradish peroxidase (HRP) conjugate and Goat anti-rat IgG HRP conjugate were purchased from GE Healthcare (Uppsala, Sweden).

2.2. Animal models

Male BKS.Cg-+LeprDB/+Leprdb/J (*db/db*) mice and their respective lean control male BKS.Cg-m+/m+/J (+*m*/+*m*) mice were purchased from CLEA Japan (Osaka, Japan). Male wild-type (WT) mice with a C57BL/6 J background were obtained from Clea Japan (Tokyo). Adiponectin-knockout (APN-KO) mice were generated and backcrossed as described previously [24]. Mice were acclimated to the new environment for at least one week before the experiments and kept in rooms set at 22 °C with a 12–12 h dark–light cycle (light cycle, 8 am to 8 pm). Mice were sacrificed, blood samples were collected, and the subcutaneous (WAT_{sub}) and mesenteric white adipose tissues (WAT_{mes}) as well as various organs were excised. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Osaka University School of Medicine. Each experiment was repeated at least three times.

2.3. Plasma infusion study

APN-KO mice were fed normal chow (NC) or high fat/high sucrose diet (HF/HS) (F2HFD2, Oriental Yeast, Osaka, Japan). In this study, 10 μL/body weight (BW) g of stocked mixed plasma from several WT mice (n = 10–15 plasma from 12 to 18-week-old mice were mixed just before injection) was injected intravenously into the cervical vein of APN-KO mice. Before and after plasma injection, venous blood samples (50 μL each) were collected from the tail vein at the indicated time periods for measurement of adiponectin levels.

2.4. Isolation of mouse mature adipocyte fractions and stromal vascular fractions

WAT_{sub} and WAT_{mes} from each mouse were fractionated, as described previously in detail by our group [25]. The adipose tissues were minced in Krebs–Ringer bicarbonate HEPES (KRBH) buffer [120 mmol/L NaCl, 4 mmol/L KH_2PO_4, 1 mmol/L $MgSO_4$, 1 mmol/L $CaCl_2$, 10 mmol/L $NaHCO_3$, 30 mmol/L HEPES, 20 μmol/L adenosine, and 4% (wt/vol) bovine serum albumin (Calbiochem, San Diego, CA)]. Tissue suspensions were centrifuged at 500 × g for 5 min to remove erythrocytes and free leukocytes. Collagenase was added to a final concentration of 2 mg/mL and incubated at 37 °C for 30 min under continuous shaking. The cell suspension was filtered through a 250 μm filter and then spun at 300 × g for 1 min to separate floating mature adipocytes fraction (MAF) from the stromal vascular cell fraction (SVF) pellet. The fractionation and washing procedures were repeated twice with KRBH buffer. Finally, both fractions were washed with phosphate buffered saline (PBS).

2.5. Western blot analysis

Specimens were frozen in liquid nitrogen immediately and lysed in a buffer containing 1% Triton X-100, 10% glycerol, 1% NP-40, 50 mmol/L HEPES, 10 mmol/L 140 sodium pyrophosphate, 100 mmol/L sodium fluoride, 10 mmol/L EDTA, 5 mmol/L sodium orthovanadate, 10 μg/mL aprotinin, 5 μg/mL leupeptin, 1.5 mg/mL benzamidine, and 1 mmol/L phenylmethylsulfonyl fluoride (PMSF). Lysates were incubated in