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Research Paper

Adipocyte-derived Lysophosphatidylcholine Activates Adipocyte and Adipose Tissue Macrophage Nod-Like Receptor Protein 3 Inflammasomes Mediating Homocysteine-Induced Insulin Resistance

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ABSTRACT

The adipose Nod-like receptor protein 3 (NLRP3) inflammasome senses danger-associated molecular patterns (DAMPs) and initiates insulin resistance, but the mechanisms of adipose inflammasome activation remains elusive. In this study, Homocysteine (Hcy) is revealed to be a DAMP that activates adipocyte NLRP3 inflammasomes, participating in insulin resistance. Hcy-induced activation of NLRP3 inflammasomes were observed in both adipocytes and adipose tissue macrophages (ATMs) and mediated insulin resistance. Lysophosphatidylcholine (lyso-PC) acted as a second signal activator, mediating Hcy-induced adipocyte NLRP3 inflammasome activation. Hcy elevated adipocyte lyso-PC generation in a hypoxia-inducible factor 1 (HIF1)-phospholipase A2 group 16 (PLA2G16) axis-dependent manner. Lyso-PC derived from the Hcy-induced adipocyte also activated ATM NLRP3 inflammasomes in a paracrine manner. This study demonstrated that Hcy activates adipose NLRP3 inflammasomes in an adipocyte lyso-PC-dependent manner and highlights the importance of the adipocyte NLRP3 inflammasome in insulin resistance.

1. Introduction

Chronic inflammation, characterized by increased macrophage infiltration, is a common feature of adipose insulin resistance. The activated adipocytes and adipose tissue macrophages (ATMs) coordinately promote insulin resistance by increasing pro-inflammatory cytokines secretion [1,2], immune cells activation [3,4] and exosomes release [5,6]. Activation of adipocytes and ATMs relies on the pattern-recognition receptors (PRRs) to sense various pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs) [7,8]. Inflammasomes are a class of intracellular PRRs [9]. Once activated, inflammasome recruits Caspase1 (CASP1), and triggers its self-processing [10]. Processed CASP1 cleavages a number of cytokines, including interleukin (IL) 1β and IL18, participating in the onset of infectious diseases [11], autoimmune diseases [12] and cardiometabolic diseases [13,14]. Among the various types of inflammasomes, the nod-like receptor protein 3 (NLRP3) inflammasome has been extensively studied in adipose tissue. The *Nlrp3*, *Casp1* and *Il1b* knockout mice were protected from high-fat diet-induced adipose insulin resistance [15–18], but it is unclear if activation of the adipose NLRP3 inflammasome depends on adipocytes or ATMs. Some studies reported that the inflammasome components were highly expressed in the ATMs and co-localized only with ATM marker [17]. However, other studies proved the expression of inflammasome components in mouse and human primary adipocytes [19,20]. Activation of the adipocyte inflammasome was involved in adipogenesis and the depletion of macrophages did not affect the inflammasome activation in adipose tissue [15].

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Homocysteine (Hcy) is a sulfur-containing non-proteinogenic amino acid, involved in the methionine cycle. An increased plasma Hcy level (>15 μM), is an independent risk factor of cardiovascular disease and is clinically defined as hyperhomocysteinemia (HHcy) [21–23]. Apart from the cardiovascular effect of Hcy, a series of clinical studies have also revealed a closely association between HHcy and insulin resistance. Patients with insulin resistance and type II diabetes mellitus display increased plasma levels of Hcy [24–26]. A methylenetetrahydrofolate reductase C677T polymorphism that interferes with the methionine cycle and elevates plasma Hcy levels, is positively associated with insulin resistance [27,28]. Folic acid administration, in contrast, lowers the plasma level of Hcy and improves insulin sensitivity in obese children [29]. Our previous studies have established an HHcy mouse model by treating mice with Hcy in drinking water. The Hcy-treated mice mimic clinical HHcy patients well and exhibited an increased atherosclerosis [30]. The plasma levels of Hcy are positively associated with abdominal adiposity in humans [26]. In the mouse model of HHcy, Hcy was found to be enriched in adipose tissue, promoting insulin resistance and adipose inflammation [31,32], but the exact mechanisms is still elusive.

Our previous study has revealed that the activation of NLRP3 inflammasome aggravates Hcy-induced abdominal aortic aneurysm [33], but whether NLRP3 inflammasome is also involved in the Hcy-induced insulin resistance is unknown. In this study, Hcy-induced activation of the adipocyte and ATM NLRP3 inflammasomes was observed in adipose tissue and mediated insulin resistance. Hcy acted as a second signal activator of the adipocyte NLRP3 inflammasome, which was mediated by lysophosphatidylcholine (lyso-PC) through the adipocyte hypoxia-inducible factor 1 (HIF1)-phospholipase A2 group 16 (PLA2G16) axis. Finally, lyso-PC derived from the Hcy-treated adipocytes activated ATM NLRP3 inflammasomes in a paracrine manner.

2. Materials and Methods

2.1. Subject Sample Collection

The subject plasma samples were collected under a study approved by the Ethics Committee of Beijing Chao-Yang Hospital [34]. The blood samples from all subjects were placed in tubes containing EDTA and aprotinin (500 kIU/ml), and centrifuged immediately, then stored at −80 °C. The subject adipose tissue samples were collected under a study approved by the Ethics Committee of Peking University People's Hospital. The subject adipose tissue (3–5 g per subject) was excised from the omental adipose tissue of metabolically healthy subjects, undergoing abdominal surgery. The pre-adipocytes were isolated and differentiated to adipocytes, which was described in detail in the cell culture section. The studies complied with the Code of Ethics of the World Medical Association (Declaration of Helsinki). All subjects provided written informed consent prior to participation.

2.2. Animals and Housing

All mice were housed under specific pathogen free condition in a temperature-controlled room (22 °C) with a 12 h light and dark cycle and were given free access to a normal chow diet (Cat. 1025, HFK Biosciences, Beijing, China) and drinking water. Wild type (WT) mice were C57BL/6 J background and were obtained from Vital River Laboratories (Beijing, China). The Casp1 knockout (*Casp1*$^{-/-}$) and corresponding WT mice were obtained from Jackson Laboratory (Cat. 004947, Bar Harbor, ME, USA, RRID: MGI_3574069) [35] and were backcrossed to the C57BL/6 J background after 10 generations. The adipocyte-specific *Hif1a* knockout (*Hif1a*$^{\Delta Ad}$), adipocyte-specific *Hif1a* transgenic (*Hif1a*^{LSL}) and corresponding WT mice were on the C57BL/6 J background and were generated using the Cre-loxP system. The *Hif1a* flox (*Hif1a*$^{fl/fl}$) mice (RRID: MGI_3815313) were published previously [36]. *Hif1a*^{LSL} mice were described in an earlier study [37,38]. To exclude the confounding effect of aP2-Cre in macrophages, the adiponectin-Cre mice was used to generate *Hif1a*$^{\Delta Ad}$ and *Hif1a*^{LSL} mice, which was obtained from Jackson Laboratory (Cat. 028020, Bar Harbor, ME, USA, RRID: IMSR_JAX: 024671) [39]. All animal protocols were approved by the Animal Care and Use Committee of Peking University.

2.3. HHcy Mouse Models

The HHcy mouse model has been reported previously [31]. In brief, male 6- to 8-week-old mice were fed drinking water containing DL-Hcy (1.8 g/l) or not for the indicated periods. The drinking water was loaded in a bottle protected from light and changed every day. The DL-Hcy was purchased from Sigma-Aldrich Chemicals (Cat. H4628, St. Louis, MO, USA).

2.4. Glucose Tolerance Test and Insulin Tolerance Test

For the glucose tolerance test (GTT), mice were fasted for 12 h before the administration of glucose (1.8 g/kg, i. p.). Blood samples were drawn from a cut at the tip of the tail at 0, 30, 60, 90 and 120 min after glucose administration, and blood glucose concentrations were measured immediately. For insulin tolerance test (ITT), mice were fasted for 4 h before the administration of insulin (1 IU/kg, i. p.). Blood samples were drawn from a cut at the tip of the tail at 0, 30, 60, 90 and 120 min after insulin administration, and blood glucose concentrations were measured immediately.

2.5. Bone Marrow Transplantation

Prior to bone marrow transplantation (BMT), 8-week-old recipient mice were provided antibiotic drinking water containing neomycin (100 mg/l) and polymyxin B sulfate (10 mg/l) for 1 week and were lethally irradiated (9 Gy, Co^{60} source). Four hours later, the recipient mice were transplanted with bone marrow (5×10^6 mononuclear cells per mouse) *via* tail vein injection. The bone marrow was isolated from the femurs of 2- to 4-week old donor mice. The recipient mice were maintained on antibiotic water for another 2 weeks. Six weeks after the transplantation, recipient mice were used for experiments.

2.6. Cytometric Bead Array and FLICA-CASP1 Assay

The inflammatory cytokine levels in plasma were investigated using a cytometric bead array mouse inflammation kit (Cat. 552364, BD Biosciences, San Jose, CA, USA), according to the manufacturer's instructions.

For fluorescent labeled inhibitors of CASP assay (FLICA)-CASP1 assay, the flowcytometry (FCM) analysis of adipocytes and SVF cells were conducted according to the previous studies [40,41]. In brief, epididymal white adipose tissue (eWAT) was minced and digested by type I collagenase (0.8 mg/ml) for 20–30 min. Primary adipocytes and stromal vascular fraction (SVF) cells were separated and stained with FAM-YVAD-FMK (Cat. 98, ImmunoChemistry Technologies, Bloomington, MN, USA) for 2 h, according to the manufacturer's instructions. The stained cells were analyzed using a BD FACS Calibur with Cell QuestPro software (BD Biosciences, San Jose, CA, USA).

2.7. Cell Culture

The 3T3-L1 cell line (Cat. 3111C0001CCC000155) and 293T cell line (Cat. 3111C0002000000112) were purchased from the Cell Resource Center of China (Beijing, China). The 3T3-L1 and 293T cells were cultured in DMEM-high glucose plus 10% fetal bovine serum (FBS). For the differentiation of 3T3-L1 cells, the cells were cultured for another 2 days after achieving confluent and were treated with insulin

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