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Overexpression of TNF-α converting enzyme promotes adipose tissue inflammation and fibrosis induced by high fat diet

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ABSTRACT

Obesity is a state in which chronic low-grade inflammation persists in adipose tissues. Pro-inflammatory cytokines, including TNF-α, produced by adipose tissues have been implicated as active participants in the development of obesity-related diseases. Since TNF-α converting enzyme (TACE) is the major factor that induces soluble TNF-α, TACE has been noted as a pivotal regulator in this field. To reveal the role of TACE in adipose tissue inflammation, TACE-transgenic (TACE-Tg) and wild type (WT) mice were fed with high fat diet (HFD) or control diet for 16 weeks. At 13 weeks after the beginning of the diet, serum TNF-α and macrophage-related cytokine/chemokine levels were elevated in TACE-Tg mice fed with HFD (Tg-HFD mice), and the number of the so-called crown-like adipocyte was significantly increased in adipose tissues of Tg-HFD mice at the end of the experiment. Although macrophage infiltration was not detected in the adipose tissues at this time, fibrosis was observed around the crown-like adipocytes. These findings suggested that TACE overexpression induced macrophage infiltration and subsequent fibrosis in adipose tissues under HFD regimen. The collective evidence suggested that TACE could be a therapeutic target of HFD-induced obesity-related adipose tissue inflammation.

1. Introduction

Obesity is a growing problem that threatens the health of a large population of humans in the world. The representative life-threatening disorders in obese patients are cardiovascular diseases. Pro-inflammatory cytokines, including TNF-α, produced by adipose tissues have been implicated as active participants in the development of obesity-related cardiovascular diseases (Hajer et al., 2008). Currently, obesity is regarded as a state in which chronic low-grade inflammation persists in adipose tissues (Lee et al., 2013).

TNF-α converting enzyme (TACE), also known as a disintegrin and metalloproteinase (ADAM) 17, is the major factor that induces soluble TNF-α (Gooz, 2010). TACE can cleave transmembrane proteins to release their extracellular domains from the cell surface. It is initially produced as a 120 kDa inactive protein in the cytoplasm; thereafter, the N-terminus prodomain is removed by the furin proprotein-convertase at the trans-Golgi network. Consequently, the inactive TACE is converted to a 100 kDa active form. The active form of TACE is transported to the plasma membrane and binds to its substrates on the cell surface. The effects of TACE are dependent on its diverse array of substrates, including cytokines, growth factors, and their receptors. One of the most important substrates of TACE is the membrane-bound TNF-α. TACE can convert TNF-α from the membrane-bound form to the soluble form.

In terms of the critical role of TNF-α in obesity and adipose tissue inflammation, TACE has been noted as a pivotal regulator in this field (Menghini et al., 2013). It was shown that TACE activity was significantly higher in mice fed with high fat diet (HFD) compared with chow controls (Fiorentino et al., 2010). In addition, TACE heterozygously knockout mice were protected against HFD-induced obesity (Serino et al., 2007). Furthermore, mice that lack tissue inhibitor of metalloproteinase 3, an endogenous inhibitor of ADAM families, showed an accelerated development of complications of obesity under HFD regimen

Abbreviations: ADAM, a disintegrin and metalloproteinase; CD, control diet; ELISA, enzyme-linked immunosorbent assay; HE, hematoxylin and eosin; HFD, high fat diet; MCP-1, macrophage chemotactic protein-1; PKA, protein kinase A; PMA, phorbol myristate acetate; SD, standard deviation; SDS, sodium dodecyl sulfate; TACE, TNF-α converting enzyme; TACE-Tg, TACE transgenic; Tg, transgenic; Tg-CD, TACE-Tg mice fed with CD; Tg-HFD, TACE-Tg mice fed with HFD; WT, wild type; WT-CD, WT mice fed with CD; WT-HFD, WT mice fed with HFD.

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(Menghini et al., 2009; Fiorentino et al., 2010). The collective findings suggested that TACE could be a possible therapeutic target of obesity-related diseases (Menghini et al., 2013). In the present study, in order to determine how excessive expression and activation of TACE could contribute to adipose tissue inflammation, TACE-transgenic (TACE-Tg) mice and wild type (WT) mice were fed with HFD or control diet (CD).

2. Materials and methods

2.1. Mice

TACE-Tg mice established in C57BL/6 line (Fukaya et al., 2013) were used. The expression of transgene, which is a heterozygously inserted mouse TACE cDNA, is driven by the β-actin promoter; therefore, the transgene-derived TACE is expressed ubiquitously. In order to distinguish from the endogenous TACE, Flag tag was connected to the 3′ region of the transgene. For controls, WT C57BL/6 mice were used. Experiments using mice were permitted by the Animal Use and Care Committee (Permission No. 08-0367) and done in accordance with the Guidelines for the Care and Use of Laboratory Animals in Hokkaido University.

2.2. Diet regimen

Both TACE-Tg and WT mice (male, 12 weeks old) were fed with HFD (HFD-60, Oriental Yeast, Sapporo, Japan) or CD (AIN-93G, Oriental Yeast) for 16 weeks. The proportions of lipid-based calorie in HFD and CD were 60% and 10%, respectively. Each group, namely TACE-Tg mice fed with HFD (Tg-HFD mice), TACE-Tg mice fed with CD (Tg-CD mice), WT mice fed with HFD (WT-HFD mice), and WT mice fed with CD (WT-CD mice), included more than 10 mice.

2.3. Primary culture of hepatocytes

Hepatocytes were isolated from TACE-Tg mice and cultured as previously described (Mei et al., 2011; Tomaru et al., 2012). In order to activate TACE, 0.5 mM palmitic acid was added to the medium for 30 min at 37 °C.

2.4. Immunoblotting

Samples were homogenized in lysis buffer [0.1% sodium dodecyl sulfate (SDS), 1% Nonidet-P40, 0.5% sodium deoxycholate, 100 μg/ml phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, protease inhibitor cocktail (Complete Mini, Roche, Basel, Switzerland)]. The lysates, which were adjusted to range from 10 to 40 μg/lane, were fractionated on 7.5% SDS polyacrylamide gel and then transferred onto PVDF membranes (GE Healthcare, Buckinghamshire, UK). After blocking by TBS-T (0.1% Tween-20 in Tris-buffered saline) containing 2% non-fat milk, the membranes were incubated overnight with the first antibody at 4 °C. After being washed 3 times by TBS-T, the membranes were next incubated overnight with 1:25000 dilution of peroxidase-labeled secondary antibodies (GE Healthcare) at 4 °C. Protein bands were detected using ECL Advance Western Blotting Detection kit (GE Healthcare).

2.5. Antibodies

Antibodies used in this study were anti-Flag (Sigma-Aldrich, St. Louis, MO), anti-F4/80 (Abcam, Tokyo, Japan), anti-protein kinase A (PKA) (Cell Signaling Technology, Tokyo, Japan), and anti-phosphorylated PKA (Cell Signaling Technology) antibodies.

2.6. Measurement of serum TNF-α and macrophage-related cytokines/chemokines

At 13 weeks after the beginning of the diet, peripheral blood samples were obtained from 3 mice and then the sera were mixed in each group. The serum concentration of TNF-α and macrophage-related cytokines/chemokines, including IFN-γ, IL-1β, and macrophage chemotactic protein-1 (MCP-1), was measured by enzyme-linked immunosorbent assay (ELISA) in Genetic Lab (Sapporo, Japan).

2.7. Measurement of serum leptin and adiponectin

At the end of the experiment, peripheral blood samples were obtained from all mice. The serum concentrations of leptin and adiponectin were determined using each corresponding ELISA kit (leptin: R&D Systems, Minneapolis, MN; adiponectin: Millipore, Billerica, MA) according to the manufacturer's protocol.

2.8. Statistics

Data were presented as mean ± standard deviation (SD). Student's *t*-test was applied for statistical analysis. The *p*-value of less than 0.05 was considered to be significant.

3. Results and discussion

3.1. Activation of transgene-derived TACE by palmitic acid

Since the transgene-derived TACE was expressed as the 120 kDa inactive form, no spontaneous phenotype appeared in TACE-Tg mice

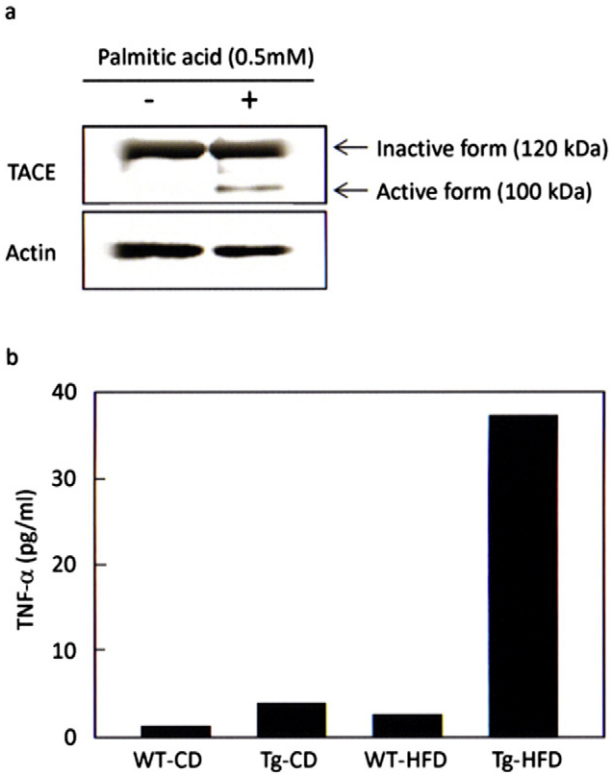

Fig. 1. (a) Activation of transgene-derived TACE. TACE-Tg hepatocytes were cultured with or without 0.5 mM palmitic acid for 30 min and then subjected to immunoblotting for the transgene-derived TACE, which could be detected by the anti-Flag antibody. The inactive and active forms of TACE were detected as the upper (120 kDa) and lower (100 kDa) bands, respectively. Actin was employed as an internal control. Experiments were carried out 2 times independently, and similar results were reproduced. The representative results are shown. (b) Serum TNF-α level. Peripheral blood was obtained from 3 mice in each group at 13 weeks after the beginning of the diet. Sera were separated by centrifugation and then combined in each group.