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Fibronectin Type III Domain Containing 4 attenuates hyperlipidemia-induced insulin resistance via suppression of inflammation and ER stress through HO-1 expression in adipocytes

Wonjae Lee [a], Subin Yun [b], Geum Hee Choi [c], Tae Woo Jung [d, *]

[a] Department of Biotechnology, Yonsei University, Seoul, South Korea
[b] Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY, USA
[c] Department of Surgery, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, South Korea
[d] Research Administration Team, Seoul National University Bundang Hospital, Seongnam, South Korea

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ABSTRACT

Although Fibronectin Type III Domain Containing 4 (FNDC4) has been reported to be involved in the modulation of inflammation in macrophages, its effects on inflammation and insulin resistance in adipose tissue are unknown. In the current study, we investigated the effects of FNDC4 on hyperlipidemia-mediated endoplasmic reticulum (ER) stress, inflammation, and insulin resistance in adipocytes via the AMP-activated protein kinase (AMPK)/heme oxygenase-1 (HO-1)-mediated pathway. Hyperlipidemia-induced nuclear factor κB (NFκB), inhibitory κBα (IκBα) phosphorylation, and pro-inflammatory cytokines such as TNFα and MCP-1 were markedly mitigated by FNDC4. Furthermore, FNDC4 treatment attenuated impaired insulin signaling in palmitate-treated differentiated 3T3-L1 cells and in subcutaneous adipose tissue of HFD-fed mice. FNDC4 administration ameliorated glucose intolerance and reduced HFD-induced body weight gain in mice. However, FNDC4 treatment did not affect calorie intake. Additionally, treatment with FNDC4 attenuated hyperlipidemia-induced phosphorylation or expression of ER stress markers such as IRE-1, eIF2α, and CHOP in 3T3-L1 adipocytes and in subcutaneous adipose tissue of mice. FNDC4 treatment stimulated AMPK phosphorylation and HO-1 expression in 3T3-L1 adipocytes and in subcutaneous adipose tissue of mice. siRNA-mediated suppression of AMPK and HO-1 abrogated the suppressive effects of FNDC4 on palmitate-induced ER stress, inflammation, and insulin resistance. In conclusion, our results show that FNDC4 ameliorates insulin resistance via AMPK/HO-1-mediated suppression of inflammation and ER stress, indicating that FNDC4 may be a novel therapeutic agent for treating insulin resistance and type 2 diabetes.

1. Introduction

Obesity-associated low-grade chronic inflammation is responsible for the impairment of insulin signaling, which makes obesity a major risk factor for insulin resistance, type 2 diabetes, and cardiovascular disease [1]. Elevated levels of pro-inflammatory cytokines that are regulated by NFκB, such as tumor necrosis factor-α (TNFα), monocyte chemoattractant protein 1 (MCP-1), interleukin 6 (IL-6), and IL-12, were observed in obese subjects with insulin resistance [2]. These cytokines work locally in adipose tissue, but they also affect systemic insulin sensitivity by increasing the levels of circulating insulin [3]. Several reports have suggested that inflammation is a causative factor for the development of insulin resistance. TNFα and IL-6 directly induce insulin resistance via an increase in serum free fatty acid levels through lipolysis in adipocytes [4]. MCP-1 secreted by adipose tissue causes macrophage infiltration into adipose tissue, leading to the production of various pro-inflammatory cytokines [5] and insulin resistance [6]. Conversely, suppression of TNFα-mediated signaling ameliorates metabolic syndromes in animal models [7]. Moreover, neutralization of TNFα improves insulin signaling in patients with chronic inflammation [8]. MCP-1 deficiency attenuates insulin resistance in

Abbreviations: FNDC4, Fibronectin Type III Domain Containing 4; AMPK, AMP-activated protein kinase; HO-1, Heme oxygenase-1; siRNA, Small interfering RNA.

* Corresponding author. Research Administration Team, Seoul National University Bundang Hospital, 166 Gumi-ro, Bundang-gu, Seongnam, 463-707, South Korea.
E-mail address: ohayo2030@hanmail.net (T.W. Jung).

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diabetic mice through the inhibition of ERK1/2 and p38-mediated pathways [9]. These findings suggest that inflammation promotes insulin resistance through up-regulation of pro-inflammatory cytokines.

The endoplasmic reticulum (ER) is responsible for the assembly of a wide range of proteins. The ER is an important organelle for protein synthesis, maturation, and the release of correctly folded proteins in conjunction with the Golgi apparatus. Since the ER plays a crucial role in various metabolic signaling pathways that ensure cellular homeostasis, maintaining appropriate ER function is important for cell survival [10]. Various environmental and genetic insults lead to the accumulation of unfolded proteins in the ER lumen, which leads to ER stress. Excessive ER stress induces apoptosis. Therefore, eukaryotic cells have evolved a system to ameliorate ER stress, known as the unfolded protein response (UPR) [11]. Several studies have demonstrated that there is a close relationship between ER stress and insulin resistance. Elevated ER stress has been detected in the adipose tissue of obese subjects that have insulin resistance [12]. Recently, Suzuki et al. had reported that adipose ER stress plays a crucial role in the development of insulin resistance and glucose intolerance [13]. In addition, it has been reported that obesity-induced ER stress causes chronic adipose tissue inflammation, leading to insulin signaling impairment in mice [14]. Therefore, modulation of ER stress in adipose tissue may be an effective therapeutic approach for treating insulin resistance.

Irisin, which was identified by Bostrom et al., is a cleaved form of Fibronectin Type III Domain Containing 5 (FNDC5). FNDC5 is a membrane-anchored protein in skeletal muscle, which is released during exercise and, in white adipose tissue, leads to browning and modulates energy expenditure [15]. It has been reported that FNDC5/Irisin has anti-inflammatory and anti-insulin resistance effects [16]. FNDC4, which had been characterized by Bosma et al. [17], is a member of the fibronectin type III domain family (FNDC1-5). FNDC4 is the closest homolog to FNDC5. FNDC4 shares 57% amino acid identity with FNDC5 in the functional and extracellular domains. In the Bosma et al. study, they had shown that FNDC4 has anti-inflammatory properties similar to FNDC5, and that observation led us to investigate its potential effects on hyperlipidemia-induced inflammation, ER stress, and insulin resistance in adipocytes.

In the present study, we showed that treatment of 3T3-L1 adipocytes and mouse adipose tissue with FNDC4 ameliorated hyperlipidemia-induced insulin resistance through attenuation of inflammation and ER stress via an AMP-activated protein kinase (AMPK)/heme oxygenase-1 (HO-1)-mediated pathway. Our results indicate that FNDC4 could play a role in a therapeutic strategy for the treatment of insulin resistance and type 2 diabetes.

2. Materials and methods

2.1. Cell culture, reagents, and antibodies

Mouse pre-adipocytes (3T3-L1; ATCC, Manassas, VA, USA) were cultured in Dulbecco's modified eagle medium (DMEM; Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Invitrogen), 100 units/mL penicillin, and 100 μg/mL streptomycin (Invitrogen). Cells were cultured in a humidified atmosphere containing 5% CO_2 at 37 °C. Differentiation was induced 48 h post-confluence (day 2) by incubating in culture medium supplemented with 1 μM insulin, 0.5 mM IBMX, and 0.5 μg/ml dexamethansone for 2 days. After an additional 4 days in medium containing 1 μM insulin, differentiated 3T3-L1 cells were treated for 24 h with 200 μM palmitate (Sigma, St Louis, MO, USA) and 0–500 nM mouse recombinant FNDC4 (Adipogen, San Diego, CA, USA). Sodium palmitate (Sigma, St Louis, MO, USA) was conjugated to 2% BSA (fatty acid free grade; Sigma) dissolved in DMEM. In all experiments, cells were treated with palmitate-BSA for 24 h, and 2% BSA was used as a control. Recombinant FNDC4 was conjugated with biotin using the Basic Biotinylation Sulfo-NHS Kit (Thermo Scientific Pierce, Rockford, IL, USA) at 4 °C. 3T3-L1 adipocytes were treated with various concentrations of biotin-conjugated FNDC4 (0–1 μM), or various concentrations of unconjugated FNDC4 (0–500 nM) in the presence of biotin-conjugated FNDC4 (500 nM) for 30 min. The cells were washed 2 times with PBS and treated with streptavidin-HRP. The results were normalized to protein content. Insulin (10 nM) was used to stimulate insulin signaling (insulin receptor substrate [IRS-1]) and Akt for 3 min.

2.2. Animals, feeding, and treatment

This study was approved by the institutional animal review board of the Institutional Animal Care and Use Committee of Bundang Seoul National University Hospital, Seongnam, Korea. Animal studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication, 8th edition, 2011). A control group and two experimental groups of 8-week-old male C57BL/6 J (B6) mice were given a normal diet (ND; Brogaarden, Gentofte, Denmark) or a high-fat diet (HFD; Research Diets, New Brunswick, NJ, USA) for 8 weeks. The HFD plus FNDC4 group was also given recombinant mouse FNDC4 intravenously (2.5 mg/kg/day); the ND and HFD groups were given the same volume of vehicle intravenously for 8 weeks. Mouse subcutaneous adipose tissue samples were isolated 15 min after treatment with intraperitoneal injected human insulin (Novo Nordisk, Princeton, NJ, USA; 10 U/kg body weight). To perform the intraperitoneal glucose tolerance test (IPGTT), mice were fasted for 12 h (overnight) and then intraperitoneally injected with glucose (2 g/kg body weight). Serum glucose levels were measured at glucose challenge and 30, 60, 90, and 120 min thereafter. To perform the insulin tolerance test (ITT), mice were fasted for 6 h and then given an intraperitoneal injection of human insulin (1 U/kg body weight). Serum glucose levels were measured at glucose challenge and 15, 30, 45, and 60 min thereafter. IPGTT was performed three days before sacrificing of the mice treated with HFD and FNDC4 for 8 weeks. One day after the end of IPGTT, ITT was performed. Serum glucose levels were measured using an Accu-Check III glucose analyzer. Upon completion of the study period, all experimental mice were sacrificed under anesthesia after an overnight (12-hr) fast.

2.3. Western blot analysis

Differentiated 3T3-L1 cells were harvested and proteins were extracted with lysis buffer (PRO-PREP; Intron Biotechnology, Seoul, Republic of Korea) for 60 min at 4 °C. Protein samples (30 μg) were subjected to 10% or 12% SDS-PAGE, transferred to a nitrocellulose membrane (Amersham Bioscience, Westborough, MA, USA), and probed with the indicated primary antibodies followed by secondary antibodies conjugated with horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Signals were detected using enhanced chemiluminescence (ECL) kits (Amersham Bioscience). Anti-phospho Akt (Ser473; 1:1000), anti-Akt (1:1000), anti-phospho AMPK (Thr172; 1:1000), anti-AMPK (1:2500), anti-HO-1 (1:1000), anti-NFκBp65 (1:2500), anti-phospho IκB (1:1000), anti-IL-6 (1:1000), anti-phospho IRE-1 (1:1000), anti-IRE-1 (1:2000), anti-phospho eIF2α (1:1000), anti-eIF2α (1:2000), and anti-CHOP (1:2000) antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-β-actin (1:5000) was obtained from Santa Cruz Biotechnology.

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