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Impaired insulin signaling upon loss of ovarian function is associated with a reduction of tristetraprolin and an increased stabilization of chemokine in adipose tissue

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ABSTRACT

Loss of ovarian function can activate inflammation and lead to insulin resistance (IR). IR is also a core feature of obesity and obesity-associated metabolic dysfunction. Tristetraprolin/zinc finger protein 36 (TTP) interferes with TNF-α production by destabilizing TNF-α mRNA, and mice deficient in TTP develop a complex syndrome of inflammatory disease (Carballo et al., 1998; Taylor et al., 1999). We hypothesized that ovariectomy (OVX) might also prime inflammation by reducing tristetraprolin/zinc finger protein 36 (TTP) levels. We used a mouse OVX model to study impaired insulin signaling due to loss of ovarian function by evaluating Akt activity upon insulin stimulus. Impaired insulin signaling was initially detected in adipose tissue (AT) at 4 weeks after OVX, and then spread to liver and muscle, finally resulting in systemic IR at 12 weeks after OVX. OVX decreased TTP protein levels and increased adipocyte size, oxidative stress, chemokine expression and fat mass in AT by 4 weeks after surgery. TTP deficiency due to TTP gene deletion induced aberrant insulin signaling and increased chemokine expression and macrophage numbers in AT but did not increase adipocyte size, oxidative stress, or fat mass, suggesting that it promotes insulin signaling by decreasing AT inflammation independent of oxidative stress and adiposity. OVX, like TTP deficiency, increased the stability of chemokine transcripts as assessed from their half-lives. Our data indicate that the impaired insulin signaling resulting from OVX is due to an OVX-induced reduction of TTP and the resulting stabilization of inflammatory chemokines.

1. Introduction

Postmenopausal women are subject to a constellation of metabolic diseases, such as insulin resistance (IR) (Stefanska et al., 2015). Loss of ovarian function is associated with increased visceral fat and weak chronic inflammation (Rogers et al., 2009; Kim et al., 2013), which is quite similar to the outcome of diet-induced obesity (DIO). DIO is considered to be caused by fat accumulation in adipose tissue (AT) and leads to cellular stress and inflammation and finally IR (Shoelson et al., 2007). The increased fat results in the accumulation of adipose tissue macrophages (ATMs), which play a critical role in chronic inflammation and the development of IR (Lumeng et al., 2007).

Tristetraprolin/zinc finger protein 36 (TTP) is an anti-inflammatory protein that binds and destabilizes certain mRNAs containing adenylate/uridylate-rich elements (AREs) and encode a variety of inflammation-related gene products (Lai et al., 2006). TTP-knockout (KO) mice develop a systemic inflammatory response with excessive secretion of pro-inflammatory cytokines resulting in erosive arthritis, autoimmunity, and myeloid hyperplasia (Carballo et al., 1998; Taylor et al., 1999). Several authors have

Abbreviations: ARE, adenylate uridylate-rich element; AT, adipose tissue; ATM, adipose tissue macrophage; BMM, bone marrow-derived macrophage; DIO, diet-induced obesity; GRO-α, growth-related oncogene; H&E, hematoxylin-eosin; HFD, high-fat diet; IR, insulin resistance; KC, keratinocyte-derived chemokine; KO, knockout; MIP-1α, macrophage inflammatory protein 1α; miR, microRNA; MCP-1, monocyte chemoattractant protein-1; OVX, ovariectomy; PI3K, phosphoinositol 3-kinase; qPCR, real-time quantitative PCR; ROS, reactive oxygen species; RPS, ribosomal protein of the small subunit; TTP, tristetraprolin/zinc finger protein 36; WT, wild type.

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suggested that lack of TTP causes obesity-associated metabolic disease. TTP transcripts and TTP protein itself are induced during adipocyte differentiation (Lin et al., 2007). Obese individuals with metabolic syndrome have much reduced levels of TTP transcripts (Bouchard et al., 2007a; Bouchard et al., 2007b). Moreover levels of TTP mRNA in AT are inversely related to the values of the IR index (Bouchard et al., 2007b), implying that a reduction of TTP has a critical role in insulin resistance.

One physiological target of TTP is TNF-α, and TTP interacts directly with AREs in TNF-α mRNA (Carballo et al., 1998). Many chemokines including keratinocyte-derived chemokine (KC), monocyte chemoattractant protein-1 (MCP-1; CCL2), and macrophage inflammatory protein 1α (MIP-1α; CCL3) also contain AREs in their 3′-UTRs (Bakheet et al., 2003), suggesting that they could also be targets for TTP. KC is homologous to human growth-related oncogene (GRO-α), but its expression pattern and function seem to be closer to those of IL-8 (Bozic et al., 1995). Binding of KC to its receptor contributes to macrophage infiltration and AT accumulation in high-fat diet (HFD)-induced obesity (Neels et al., 2009) and in atherosclerotic lesions (Boisvert et al., 2006). KC expression is elevated in both the blood and AT of genetically obese (ob/ob) and HFD-fed obese mice. Microarray profiling has revealed that MCP-1 and MIP-1α were strongly up-regulated in mature adipocytes (Lee et al., 2005) and cultured preadipocytes of obese individuals (Nair et al., 2005), suggesting that both chemokines cause obesity-induced metabolic complications. In a clinical study, serum MCP-1 levels were found to be correlated with levels of markers of obesity-related metabolic dysfunction, as well as IR and type-2 diabetes (Simeoni et al., 2004). MCP-1 plays a major role in recruiting macrophages and causing inflammation. Its circulating levels increased significantly in women undergoing the menopausal transition, suggesting that MCP-1 might serve as an indicator of hormonal change (Tani et al., 2013).

Ovariectomy (OVX) in mice is generally accepted as an animal model of the human menopause (Komori, 2015). We and others have demonstrated that OVX induces systemic IR (Rogers et al., 2009; Vieira Potter et al., 2012; Kim et al., 2013; Choi et al., 2014, 2015; Park et al., 2016), but the mechanism involved has not been elucidated. In the present study we investigated a key molecule (TTP) potentially involved in the initiation of impaired insulin signaling upon OVX, We present evidence that the defective insulin signaling is the result of a reduction in the level of TTP that slows the normal breakdown of transcripts of the inflammatory chemokines KC, MCP-1, and MIP-1α in AT.

2. Materials and methods

2.1. Animals and study design

Ten-week-old female C57BL/6J mice were subjected to sham operation or OVX under anesthesia using 2′,2′,2′-tribromoethanol (250 mg/kg, Sigma Chemical). $TTP^{-/-}$ (TTP knockout, KO) mice were kindly provided by Dr. Perry J. Blackshear (National Institute of Environmental Health Sciences, Research Triangle Park, NC). $MCP\text{-}1^{-/-}$ (MCP-1-KO) mice on a C57BL/6J genetic background were purchased from the Jackson Laboratory. The mice were bred in the animal facility of the University of Ulsan and were maintained under specific pathogen-free conditions with free access to water and standard chow diet. Sex- and age-matched littermates were used as controls In all experiments. All animal care and procedures were conducted according to the protocols and guidelines approved by the University of Ulsan Animal Care and Use Committee (UOUACUC) (HSC-14-060). Food intake and body weight were monitored daily and weekly, respectively.

Mice were fasted for 6 h and killed by CO_2 asphyxiation. Blood was collected by cardiac puncture, and tissues were harvested immediately. Serum MCP-1 and KC levels were measured by sandwich ELISAs using an anti-MCP-1 and anti-KC for Ab coating, and biotinylated anti-MCP-1 and anti-KC as recommended by the supplier (R & D Systems, Minneapolis, MN). Serum H_2O_2 levels were determined with an Amplex Red hydrogen peroxide/peroxidase assay kit (Invitrogen, Carlsbad, CA). To determine adipocyte size, adipose tissues were fixed in 10% formalin solution for 24 h, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin-eosin (H&E). Images were obtained with an Axio-Star Plus microscope (Carl Zeiss, Gottingen, Germany) and further analyzed with Image J software.

2.2. In vivo phospho-Akt signaling study

Sham or OVX mice were fasted for 16 h before i.p. injection of 4 unit/kg insulin (Lilly). After 5 min, the mice were anesthetized, and liver, skeletal muscle and white adipose tissue (WAT, gonadal fat) were collected and stored in liquid nitrogen. To isolate protein, WAT was homogenized in lysis buffer (50 mM Tris-Cl, pH 7.5; 150 mM NaCl; 1% Nonidet P-40; 0.5% sodium deoxycholate; 0.1% SDS; 0.01% protease inhibitor mixture, 0.5 mM phosphatase inhibitor), fractionated by 10% SDS-PAGE, and electroblotted to visualize phosphorylated (Thr308) Akt (diluted at 1:1000, Cell Signaling, Beverly, MA, USA) and total Akt (diluted at 1:1000, Cell Signaling). Tubulin (1:5000, Sigma Aldrich) was used as a loading control. Insulin-induced phospho-Akt was normalized to total Akt with Image J software.

2.3. Real-time quantitative PCR (qPCR)

Total RNA was reverse transcribed with random primers and M-MLV reverse transcriptase (Promega, Madison, USA). qPCR was carried out using SYBR Green 1 Taq polymerase (Qiagen, Hilden, Germany) and appropriate primers on a StepOnePlus™ Real-Time System (Applied Biosystems, Foster City, CA, USA). The specificity of each primer pair was confirmed by melting curve analysis and agarose-gel electrophoresis. The housekeeping ribosomal protein product of the small subunit (RPS) gene was amplified in parallel with the genes of interest. Copy numbers relative to RPS were calculated from the expression $2^{-\Delta\Delta Ct}$. Primer sequences used are shown in Table 1.

2.4. Detection of protein carbonyls in white adipose tissue

Protein carbonyls in WAT were assayed with an OxyBlot protein oxidation detection kit (Chemicon International, Temecula, CA,

Table 1
Sequence of oligonucleotides used for PCR.

Transcript		Sequences (5′ to 3′)
TTP	Up	ctctgccatctacgagagcc
	Down	gatggagtccgagtttatgttcc
MCP-1	Up	gaaggaatgggtccagacat
	Down	acgggtcaacttcacattca
KC	Up	ccgaagtcatagccacactcaa
	Down	gcagtctgtcttctttctccgttac
MIP-1α	Up	ttctctgtaccatgacactctgc
	Down	cgtggaatcttccggctgtag
TNF-α	Up	aagcctgtagcccacgtcgta
	Down	ggcaccactagttggttgtctttg
F4/80	Up	ctttggctatgggcttccagtc
	Down	gcaaggaggacagagtttatcgtg
RPS	Up	atcagagagttgaccgcagttg
	Down	aatgaaccgaagcacaccatag