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Gene expression levels of Casein kinase 1 (CK1) isoforms are correlated to adiponectin levels in adipose tissue of morbid obese patients and site-specific phosphorylation mediated by CK1 influences multimerization of adiponectin

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ABSTRACT

White adipose tissue has now been recognized as an important endocrine organ secreting bioactive molecules termed adipocytokines. In obesity, anti-inflammatory adipocytokines like adiponectin are decreased while pro-inflammatory factors are over-produced. These changes contribute to the development of insulin resistance and obesity-associated diseases. Since members of the casein kinase 1 (CK1) family are involved in the regulation of various signaling pathways we ask here whether they are able to modulate the functions of adiponectin. We show that CK1δ and ε are expressed in adipose tissue and that the expression of CK1 isoforms correlates with that of adiponectin. Furthermore, adiponectin co-immunoprecipitates with CK1δ and CK1ε and is phosphorylated by CK1δ at serine 174 and threonine 235, thereby influencing the formation of adiponectin oligomeric complexes. Furthermore, inhibition of CK1δ in human adipocytes by IC261 leads to an increase in basal and insulin-stimulated glucose uptake. In summary, our data indicate that site-specific phosphorylation of adiponectin, especially at sites targeted by CK1δ *in vitro*, provides an additional regulatory mechanism for modulating adiponectin complex formation and function.

1. Introduction

Obesity is characterized by an increase in adipose tissue mass and is correlated to the development of obesity-related disorders including hypertension, heart disease, type 2 diabetes mellitus (T2D), and cancer (Kang, 2013; Kaur, 2014; Sundaram et al., 2013). In obesity, the level of circulating factors including insulin, adipocytokines, cytokines, vascular integrity factors, plasminogen activator inhibitor (PAI), and fatty acids as well as the deposition of lipids in liver and skeletal muscle are altered (Fan et al., 2013; Mlinar and Marc, 2011). For a long time, white adipose tissue was considered as a passive organ storing energy as triglycerides. To date, it is becoming increasingly clear that adipose tissue is a complex endocrine organ (Adamczak and Wiecek, 2013; Al-Suhaimi and Shehzad, 2013; Cao, 2014; Fischer-Posovszky et al., 2007; Leal Vde and Mafra, 2013; Wozniak et al., 2009). In addition to adipocytes, adipose tissue contains preadipocytes, nerve terminals, blood vessels, and macrophages. In particular, adipocytes and immune cells secrete a wide range of hormones and other factors, termed adipocytokines. At present, more than 20 adipocytokines have been identified as critical regulators of energy (lipid and glucose) homeostasis, appetite, insulin sensitivity, inflammation, and vascularization (Al-Suhaimi and Shehzad, 2013; Cao, 2014).

Adiponectin, an adipocytokine with anti-inflammatory, anti-diabetic, and anti-atherogenic features, is mainly secreted by

Abbreviations: aa, amino acid; Akt, protein kinase B; CLK2, CDC-like kinase 2; CK1δ, casein kinase 1 delta; CK1δKD, C-terminal truncated casein kinase 1 delta; CRC, colorectal cancer; FCS, fetal calf serum; FP, fusion protein; GST, glutathione-S-transferase; GST–ad, glutathione-S-transferase–adiponection; HMW, high molecular weight; LMW, low molecular weight; MCS, multiple cloning site; MO, morbid obese; MMW, middle molecular weight; mt, mutant; PKA, cAMP dependent protein kinase; PKCα, protein kinase Cα; PP1, protein phosphatase 1; wt, wild type; T2D, type 2 diabetes mellitus.

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adipocytes and its expression level is reduced in obesity (Fiaschi et al., 2014; Lim et al., 2014). Human adiponectin is a 244 amino acid (aa) polypeptide, containing a N-terminal secretory sequence, a collagen-like domain, and a C-terminal globular domain with large sequence and structural similarities to the complement factor C1q (Crouch et al., 1994; McCormack et al., 1997; Wong et al., 2004). Like other proteins of the C1q family adiponectin is able to build characteristic homomeres including low molecular weight (LMW) trimers, middle molecular weight (MMW) hexamers and high molecular weight (HMW) 12–18-mers (Liu and Liu, 2014; Pajvani et al., 2004). Posttranslational modifications, especially the formation of disulfide bridges, hydroxylation, and glycosylation, play an important role in regulating activity and complex formation of adiponectin (Peake et al., 2007; Richards et al., 2006; Wang et al., 2002, 2008). Phosphorylation is considered to be another most common and reversible covalent posttranslational modification that may alter the activity, life span, or cellular localization of proteins. However, the role of site-specific phosphorylation in regulating adiponectin complex formation and activity is still elusive. Since adiponectin contains several potential phosphorylation sites for tyrosine- and serine/threonine-specific kinases, we hypothesize that adiponectin is able to interact with cellular kinases and that its complex formation is modulated by site-specific phosphorylation. Among those potential adiponectin targeting kinases are also members of the serine/threonine-specific CK1 family, which are involved in the regulation of various processes, like immune response, differentiation, cell proliferation, transport processes, chromosome segregation, and lipid metabolism (Brookheart et al., 2014; Knippschild et al., 2014).

In the present study we show that CK1δ and ε are expressed in subcutaneous as well as in omental adipose tissue. Furthermore, the expression of CK1 isoforms on RNA level correlates with that of adiponectin. Adiponectin and CK1δ physically interact resulting in site-specific phosphorylation of adiponectin by CK1δ at serine 174 and threonine 235. In addition, site-specific phosphorylation of adiponectin influences HMW complex formation. Furthermore, inhibition of CK1δ in SGBS adipocytes by IC261 leads to an increase in basal and insulin-stimulated glucose uptake. Our data indicate that site-specific phosphorylation of adiponectin is an additional mechanism to regulate its biological activity.

2. Materials and methods

2.1. Patients and tissue collection

The cohort consists of 57 unrelated morbid obese (MO) patients (37 females, 20 males) who underwent bariatric surgery in the Department of General and Visceral Surgery of the University Hospital of Ulm. Approval of the study was granted by the ethics committee of the University of Ulm (Permission Number 73/09). Specimens from MO patients and blood samples used in our study were obtained from the tissue bank of the Department of General and Visceral Surgery of the University Hospital of Ulm. Tissue samples were collected during the operation. Samples were either snap frozen and stored in liquid nitrogen or fixed in formalin before being embedded in paraffin. The demographic characteristics, age, sex, and family history were recorded meanwhile. Relevant anthropometric and clinical parameters of the MO patients are provided in Table 1.

Table 1
Clinical parameters of MO patients.

	Female median (minimum–maximum)	Male median (minimum–maximum)
n	37	20
Age (years)	47 (23–66)	40 (17–59)
BMI (kg/m^2)	53.5 (40.8–78.0)	55.1 (41.2–72.0)
WC (cm)	130.5 (98.5–160.0)	153.0 (110.0–177.0)
WHR	0.88 (0.74–1.10)	0.98 (0.65–1.09)
Insulin (mU/l)	19.80 (4.80–171.40)	26.70 (9.20–69.40)
TG (mmol/l)	138 (71–366)	135 (75–223)
Leptin (ng/ml)	92.18 (11.06–245.86)	60.92 (21.00–121.50)
Adiponectin (ng/ml)	7100 (1625–27,975)	3113 (1800–4175)
CRP (mg/l)	14.4 (2.5–33.7)	7.3 (2.7–30.5)

Data are presented as median (minimum–maximum).
Abbreviations: BMI, body mass index; CRC, colorectal cancer; CRP, C-reactive protein; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; LDL, low-density lipoprotein; MO, morbid obese; TG, triglycerides; WC, waist circumference; WHR, waist-to-hip ratio.

2.2. Antibodies

The rabbit polyclonal anti-CK1ε (1:350, antiserum 712 (Brockschmidt et al., 2008)), the rabbit polyclonal anti-CK1ε (1:50, H-60, Santa Cruz, USA), the goat polyclonal anti-CK1δ (1:1000, R-19, Santa Cruz, USA), the rabbit polyclonal anti-CK1δ (1:350, antiserum 108 (Stoter et al., 2005)), the goat anti-human adiponectin antibody (1:1000, R&D Systems, Inc., Germany), the goat polyclonal caspase-3 p11 (1:50, K-19, Santa Cruz, USA), and a mouse monoclonal anti-β-actin antibody (1:10,000, Sigma-Aldrich, Germany) were used. Horseradish peroxidase either conjugated with anti-rabbit or anti-mouse IgG was purchased from GE Healthcare, USA (anti-mouse HRP, 1:10,000, NA931V and anti-rabbit HRP, 1:1000, NA934V). Horseradish peroxidase conjugated with anti-goat IgG was purchased from Rockland Immunochemicals, USA (1:5000, 605–4302).

2.3. Immunohistochemistry

Immunohistochemistry analyses were performed to detect CK1δ and CK1ε in paraffin embedded omental adipose tissue and subcutaneous adipose tissue from morbid obese patients. Staining procedures included deparaffinization in xylene, followed by rehydration via transfer through graded alcohols. To inhibit endogenous enzyme activity, Peroxidase Blocking Reagent (DAKO, Denmark) was used. The sections were treated with the antigen retrieval solution Citra Plus, pH 6.03 (BioGenex, USA) in a microwave oven, according to the manufacturer's instructions. Sections were then incubated with the polyclonal rabbit antisera specifically recognizing CK1ε (712, 1:350) or CK1δ (108, 1:350) at 4 °C overnight. After washing in Tris–HCl buffer a horseradish peroxidase containing polymer conjugated anti-rabbit IgG antibody (N-Histofine®, Nichirei Corporation, Japan) was applied at room temperature (RT) for 30 minutes. The enzymatic reaction was developed in a freshly prepared solution of 3′-diaminobenzidine using DAKO Liquid DAB Substrate–Chromogen solution as a chromogen for horseradish peroxidase. The sections were then counterstained with hematoxylin, and permanently mounted in Entellan (Merck, Germany). Positive and negative controls were included for each case. As a negative control the primary antiserum was omitted and substituted with Tris–HCl buffer.

2.4. Construction of plasmids

Total RNA was isolated from human omental adipose tissue (Omentum majus) using the RNeasy Lipid Tissue Kit from Qiagen (Qiagen, Germany). One microgram of total RNA was transcribed into cDNA using the RT2 First Strand Kit (SuperArray Bioscience Corp., USA). Human adiponectin was amplified by PCR using cDNA isolated from human omental adipose tissue as a template and the primers 5′-GAATTCGCATGCTGTTGCTGGG-3′ (5′ primer) and 5′-GAATTCTCAGTTGGTGTCATGG-3′ (3′ primer), cloned into the multiple cloning site (MCS) of pcDNA3.1/V5-His-TOPO (Invitrogen, Germany), and sequenced. In addition, various adiponectin fragments were amplified by PCR using human adiponectin as a template and the