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Cell death–inducing DFF45-like effector C is reduced by caloric restriction and regulates adipocyte lipid metabolism

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Abstract

Members of the cell death–inducing DFF45-like effector (CIDE) gene family have been shown to regulate lipid metabolism. In this article, we report that the third member of the human CIDE family, CIDEC, is down-regulated in response to a reduced caloric intake. The down-regulation was demonstrated by microarray and real-time polymerase chain reaction analysis of subcutaneous adipose tissue in 2 independent studies on obese patients undergoing treatment with a very low calorie diet. By analysis of CIDEC expression in 65 human tissues, we conclude that human CIDEC is predominantly expressed in subcutaneous adipocytes. Together, these observations led us to investigate the effect of decreased CIDEC expression in cultured 3T3-L1 adipocytes. Small interfering RNA–mediated knockdown of CIDEC resulted in an increased basal release of nonesterified fatty acids, decreased responsiveness to adrenergic stimulation of lipolysis, and increased oxidation of endogenous fatty acids. Thus, we suggest that CIDEC is a regulator of adipocyte lipid metabolism and may be important for the adipocyte to adapt to changes in energy availability.

1. Introduction

A core function of adipose tissue is to store and release energy in the form of lipids, and it responds promptly to the nutritional status of the body. Impairment in this function seems to be partly responsible for the development of obesity and obesity-related metabolic disturbances, such as insulin resistance and type 2 diabetes mellitus [1,2].

Recent reports indicate that the cell death–inducing DFF45-like effector (CIDE) gene family is involved in the regulation of lipid metabolism. The CIDE gene family consists of 3 members: CIDEA, CIDEB, and CIDEC (also referred to as *CIDE-3*). CIDEA knockdown increases the lipolytic capacity of mouse and human adipocytes, and CIDEA-deficient mice are lean and resistant to diet-induced obesity [3,4]. We have shown that levels of human CIDEA in subcutaneous adipose tissue are regulated by energy intake and associated with basal metabolic rate, body fat content, and serum insulin levels [5]. CIDEB-deficient mice are also less susceptible to diet-induced obesity and display a significant decrease in liver triglyceride content and secretion [6]. The mouse homologue of the CIDEC gene (fat-specific protein 27) is localized to intracellular lipid droplets and was recently shown to affect lipolysis [7]. However, no report on the function or expression of human CIDEC in relation to metabolism has been published.

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This study aimed to investigate the regulation of CIDEC during caloric restriction, its tissue distribution, and its role in adipocyte lipid metabolism.

2. Material and methods

All study subjects received written and oral information before giving written informed consent. These studies were approved by ethical review boards at Göteborg University and the University of Cape Town.

2.1. Subjects and samples

To determine the tissue distribution of CIDEC expression, microarray expression profiles of 65 human tissues were acquired from the GEO database (data set GSE3526). Each tissue in this data set is represented by Human Genome U133 plus 2.0 DNA microarray (Affymetrix, Santa Clara, CA) expression profiles from 3 to 9 different individuals.

For real-time polymerase chain reaction (rtPCR) analysis of CIDEC tissue distribution, RNA from adipose tissue and isolated adipocytes from 3 healthy women (body mass index [BMI] 23.0-27.6 kg/m^2) was used together with the Human Total RNA Master Panel II (Clontech, Mountain View, CA).

For the microarray study on adipose tissue depots, deep subcutaneous, superficial subcutaneous, and omental adipose tissue biopsies were obtained from 6 women with an average BMI of 27.4 ± 3.5 kg/m^2.

In the very low calorie diet (VLCD; 450 kcal/d) microarray study, 6 women and 18 men (starting BMI of 37.6 ± 4.9 kg/m^2; age, 25-61 years) were given a VLCD for 16 weeks. Subcutaneous adipose tissue biopsies were obtained at the start of VLCD treatment (week 0), twice during the VLCD phase (weeks 8 and 16), and 2 weeks after normal food was reintroduced (week 18). On average, patients lost 27.7 kg during the diet [5,8].

In the VLCD rtPCR study, 8 men and 20 women (starting BMI of 36.3 ± 3.7 kg/m^2; age, 18-59 years) were given a VLCD for 12 weeks. Subcutaneous adipose tissue biopsies were obtained at the start of VLCD treatment (week 0) and 3 times during the VLCD phase (weeks 2, 6, and 12). On average, patients lost 19.6 kg during the diet.

2.2. RNA preparation

Adipose tissue biopsies were stored at −80°C until analysis. Adipocytes were isolated as previously described [9]. Total RNA from human tissues was prepared with the RNeasy Lipid Tissue kit, and RNA from 3T3-L1 cells was prepared with the RNeasy kit (both from Qiagen, Germantown, MD).

2.3. Real-time PCR

Human low-density lipoprotein receptor–related protein 10 was used as reference to normalize the expression levels between adipose tissue samples, and peptidyl-prolyl isomerase A was used as reference in the tissue distribution panel. These genes have consistently shown small interindividual and intertissue variation, respectively [10].

Assay-On-Demands were used for detection of mouse CIDEC, low-density lipoprotein receptor–related protein 10, and peptidyl-prolyl isomerase A. Human CIDEC was analyzed with an Assay-on Demand targeted to exon 3 (detects full-length isoform) and an Assay-by-Design detecting the full-length and alternatively spliced α-isoform (primer sequences GGCTGCCTGCCTGTGA and GGGACTTCATGGCGTATTCCA, probe sequence ACTGCGTTGGACCTCT). Assays and reagents for rtPCR analysis were from Applied Biosystems (Foster City, CA) and were used according to the manufacturer's protocol. All standards and samples were analyzed in triplicates.

2.4. Microarray

Preparation of complementary RNA and hybridization to microarrays were performed as previously described [9,11,12]. The RNA from the VLCD study was analyzed with the Human Genome U133A DNA microarray, and the RNA from the adipose tissue depot was analyzed with the Human Genome U133 plus 2.0 DNA microarray (both from Affymetrix).

2.5. Cell culture and CIDEC gene silencing

Cell culture reagents were from PAA Laboratories (Pasching, Austria), Novonordisk (Bagsvaerd, Denmark), and Sigma-Aldrich (St Louis, MO). The 3T3-L1 cells were cultured in Dulbecco modified Eagle medium (high glucose) supplemented with sodium pyruvate, penicillin, streptomycin, nonessential amino acids, L-glutamine, and 10% fetal calf serum. Differentiation was started 2 days postconfluence by addition of 500 μmol/L 3-isobutyl-1-methylxanthine, 250 nmol/L dexamethasone, and 860 nmol/L insulin to cell culture medium. After 4 days, 3-isobutyl-1-methylxanthine and dexamethasone were excluded; and cells were cultured for 2 more days in 860 nmol/L insulin. Finally, cells were cultured in original culture medium for 4 more days.

Differentiated 3T3-L1 cells were electroporated with stealth small interfering RNA (siRNA) (Invitrogen, Carlsbad, CA) using the Amaxa Nucleofector (Amaxa, Cologne, Germany) according to the instructions of the manufacturer. Experiments were performed 24 hours after electroporation. All siRNA experiments were performed twice, with similar results. In each experiment, 2 separate anti-CIDEC siRNA oligos were used to control for nonspecific siRNA effects.

2.6. Analysis of lipolysis and fatty acid oxidation

Cells were kept for 3 hours in Dulbecco modified Eagle medium without phenol red ± 10 μmol/L isoproterenol. After incubation, medium was collected and analyzed with a nonesterified fatty acid (NEFA)–C kit (Wako Chemicals, Richmond, VA). To determine oxidation of fatty acids, cells were labeled with 3H-palmitic acid (4 hours; GE Healthcare, Uppsala, Sweden) and chased for 4 hours. Pulse and chase