Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

High glucose, insulin and free fatty acid concentrations synergistically enhance perilipin 3 expression and lipid accumulation in macrophages☆

Bin Fan[a], Jian-Qiu Gu[b,*], Rong Yan[a], Hong Zhang[a], Juan Feng[a], Shoichiro Ikuyama[c]

[a] Department of Neurology, Shengjing Hospital, China Medical University, Shenyang 11004, China
[b] Department of Endocrinology and Metabolism, the First Affiliated Hospital, China Medical University, Shenyang 110001, China
[c] Division of Metabolism, Hematology and Rheumatic Diseases, Department of Internal Medicine, Kyushu University Beppu Hospital, Beppu 874-0838, Japan

ARTICLE INFO

Article history:
Received 9 November 2012
Accepted 28 February 2013

Keywords:
Perilipin
PLIN3
PLIN2
Metabolic syndrome
Foam cell

ABSTRACT

Objective. Perilipin (PLIN) 3, an intracellular lipid droplet (LD)-associated protein, is implicated in foam cell formation. Since metabolic derangements found in metabolic syndrome, such as high serum levels of glucose, insulin and free fatty acids (FFAs), are major risk factors promoting atherosclerosis, we investigated whether PLIN3 expression is affected by glucose, insulin and oleic acid (OA) using RAW264.7 cells.

Methods. Real-time PCR and Western blotting were performed to detect PLIN3 or PLIN2 expression. Oil-red O staining and Lipid Analysis were employed to measure cellular content of triacylglycerides (TAG) and cholesterol.

Results. PLIN3 mRNA was stimulated by high glucose or insulin concentrations individually, but not by OA. A combination of any two factors did not enhance PLIN3 expression any more than that evoked by glucose alone at 24 h. Interestingly, however, simultaneous addition of all three factors synergistically enhanced the PLIN3 expression. This synergistic effect was not apparent for PLIN2 mRNA expression. Inhibitors of Src family tyrosine kinase and/or phosphatidylinositol 3-kinase, both of which are activated by insulin and FFA signaling, partially suppressed PLIN3 expression induced by the combination of the three factors. While simultaneous addition of glucose, insulin and OA remarkably increased the cellular content of TAG and cholesterol, knocking-down of PLIN3 predominantly reduced TAG content.

Conclusions. These results indicate that PLIN3 expression is synergistically stimulated by high glucose, insulin and FFA concentrations, in parallel with TAG accumulation in macrophages. This finding raises new evidence of PLIN3 involvement in conversion of macrophages into foam cells

Abbreviations: PLIN, perilipin; LDs, lipid droplets; FFAs, free fatty acids; OA, oleic acid; PPAR, peroxisome proliferator-activated receptor; TAGs, triacylglycerides; ADRP, adipose differentiation-related protein; TIP47, tail interacting protein of 47 kDa; OXPAT, oxidative tissue-enriched PAT protein; GPR, G protein-coupled receptor; PI3K, phosphatidylinositol 3-kinase.

☆ There are no ethical/legal conflicts involved in the article.

* *Corresponding author.* Department of Endocrinology and Metabolism, The First Affiliated Hospital of China Medical University, Heping District, Shenyang 110001, China. Tel.: +86 24 83283135; fax: +86 24 83283073.
E-mail address: gu.jq@hotmail.com (J.-Q. Gu).

0026-0495/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.metabol.2013.02.013

1. Introduction

It is known that the perilipin (PLIN) family proteins, which comprise PLIN1 (perilipin), PLIN2 (adipose differentiation-related protein, ADRP), PLIN3 (tail interacting protein of 47 kDa, TIP47), PLIN4 (S3-12) and PLIN5 (oxidative tissue-enriched PAT protein, OXPAT), are dominant lipid droplet (LD)-associated proteins and are implicated in intracellular LD formation [1–4]. It has been reported that foam cells found in atherosclerotic lesions show enhanced expression of PLIN2 [5,6]. In addition, genetic abrogation of PLIN2 prevents atherosclerosis formation [7]. These findings strongly indicate that PLIN2, a representative and ubiquitously expressed LD protein, is closely related to atherosclerosis formation. We have also demonstrated that macrophages activated by inflammatory stimuli or pathogens enhance PLIN2 expression in macrophages [8–10].

On the other hand, PLIN3 was initially identified as a cytosolic protein that binds to the cytoplasmic domain of cation-dependent and cation-independent mannose 6-phosphate receptors [11]. PLIN3 is also LD-associated and ubiquitously expressed [12]. Recent reports have shown that PLIN3 acts as a carrier protein for free fatty acids (FFAs), promotes conversion of macrophages into foam cells and reduction of PLIN3 mRNA levels by antisense oligonucleotide treatment attenuated hepatic steatosis and serum triglyceride (TAG) levels, and improves glucose homeostasis in mice fed a high-fat diet [13,14]. We have recently demonstrated that PLIN3 expression is enhanced by stimulation of the Toll-like receptor 9 (TLR9)-mediated pathway, in part through inflammatory cytokine production in macrophages [15]. To date, however, little is known about the physiological or pathological regulatory mechanism of the PLIN3 gene in macrophages.

Metabolic syndrome is often associated with elevated serum levels of glucose, insulin and FFAs, and the complex of these metabolic derangements promotes atherosclerosis or cardiovascular disease [16–18]. We presumed that the expression of LD proteins in macrophages could be influenced by derangements of these metabolic factors.

In the present study, we aimed to disclose whether metabolic derangements, such as high glucose, insulin and FFA levels, affect PLIN3 expression in macrophages. We show here that these metabolic factors synergistically enhance PLIN3 expression and intracellular lipid accumulation in macrophages.

2. Methods

2.1. Materials

Glucose, insulin, oleic acid (OA) and GW501516 were purchased from Sigma-Aldrich (St. Louis, MO), OA was dissolved in water containing 5 mmol/L bovine serum albumin (BSA, Sigma-Aldrich), and then diluted in the medium. Fenofibrate and troglitazone were generous donations from Kaken Pharmaceuticals (Tokyo, Japan) and Sankyo (Tokyo, Japan), respectively. Antibodies against PLIN3 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). PP2 and LY-20094 (LY) were purchased from Calbiochem (LA Jolla, CA). Small interference RNA (siRNA) was purchased from Qiagen (HP-guaranteed siRNA; Hilden, Germany). A sequence-specific siRNA for PLIN 3 (sense: 5′-AACAGCACAGAGAAUGAGGAG-3′) was selected by its potency.

2.2. Cell culture

Murine macrophage cell lines, RAW264.7 and J774, were obtained from the American Type Culture Collection (Manassas, VA). 1×10^6 cells were routinely cultured in 10 cm tissue culture dishes (Falcon 3003; Becton Dickinson Labware, Franklin Lakes, NJ) in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, St. Louis, MO) supplemented with 10% charcoal-treated fetal calf serum (FCS), 1% non-essential amino acids and appropriate antibiotics. The FCS we used was shown to be the same in terms of the concentrations of FFAs, TAG and total cholesterol with charcoal treatment [8,10,13]. We preliminarily confirmed by trypan blue staining that concentrations of reagents we used in this study were appropriate and those that did not affect cell viability.

2.3. Western Blot analysis

The procedures were basically according to those we reported previously [8,15,19]. The cells were harvested using RIPA lysis buffer containing 50 mmol/L Tris–HCl (Ph 7.4), 150 mmol/L NaCl, 1% sodium deoxycholate, 1% NP-40, 1 mmol/L phenylmethylsulfonyl fluoride (PMSF) and 1 mmol/L EDTA. The proteins were heated at 95 °C for 5 min, applied to 10% SDS-PAGE and electro-blotted onto a polyvinylidene difluoride membrane (MSI, Westborough, MA) for 1 h at 100 V with a western blotting apparatus (Bio-Rad, Hercules, CA) in Tris-glycine transfer buffer (25 mmol/L Tris, 192 mmol/L glycine, 20% methanol and 0.1% SDS). The membranes were blocked for 1 h at room temperature with 5% non-fat milk in phosphate-buffered saline containing 0.1% Tween20 (PBS-Tween20). Next, the membranes were incubated with rabbit PLIN3 antibodies (0.5 μg/mL in PBS-Tween20) for 1 h. After washing four times (5 min each) with PBS-Tween20, the membranes were incubated with an appropriate secondary IgG-horseradish peroxidase conjugate (Santa Cruz Biotechnology, Santa Cruz, CA) diluted in PBS-Tween20 (0.08 μg/mL) for 1 h and then washed as above. The immunoreactivity was detected by the enhanced chemiluminescence technique. Three independent experiments were carried out and a representative result is shown.

2.4. Real-time PCR

Total RNA was extracted using TriReagent (Sigma-Aldrich, St. Louis, MO), according to the manufacturer's instructions. Single strand cDNA was synthesized with ReverTra Ace-α kit (Toyobo, Osaka, Japan) using 0.5 μg of total RNA. Real-time PCR was performed with the SYBR Green method using the ABI prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA) in 25 μL reactions [12.5 μL of 2 × iQ SYBR Green supermix (Bio-Rad, Hercules, CA), 320 nmol/L each primer, 5 μL of 1:20 diluted cDNA]. The primer sequences were as follows: PLIN3 (sense: CTGAGAAAGGCGTCAAGACC, antisense: CCATTTCTTGAGCCCCAGAC), PLIN2 (sense: CTGTCTACCAAGCTCTGCTC, antisense: