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Transcriptional regulation of mouse 6-phosphogluconate dehydrogenase by ADD1/SREBP1c

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Abstract

6-Phosphogluconate dehydrogenase (6PGDH) constitutes the pentose phosphate pathway and produces NADPH. 6PGDH is also considered as a lipogenic gene since NADPH is a pivotal cofactor for lipogenesis. Thus, it is important to elucidate how 6PGDH is regulated by various signals related to energy homeostasis. Here, we provide several evidences that ADD1/SREBP1c regulates the expression of mouse 6PGDH gene. DNase I footprinting assay and point mutation studies revealed that the E-box (CANNTG) motif in the promoter of mouse 6PGDH is an important *cis*-regulatory element for ADD1/SREBP1c. 6PGDH mRNA is highly expressed in white adipose tissue and tightly modulated by nutritional status. Furthermore, we found that ADD1/SREBP1c mediates insulin-dependent 6PGDH expression and that PI3-kinase is an important linker for its regulation. Taken together, these data suggest that ADD1/SREBP1c is a key transcription factor for 6PGDH gene expression and would coordinate glucose metabolism and lipogenesis for energy homeostasis.

Keywords: 6-Phosphogluconate dehydrogenase; NADPH; ADD1/SREBP1c; Insulin; Transcription

6-Phosphogluconate dehydrogenase (6PGDH: EC1.1.1.44) is an enzyme involved in the pentose phosphate pathway (PPP). 6PGDH and glucose 6-phosphate dehydrogenase (G6PDH), another enzyme of the PPP, are the main sources of NADPH in non-photosynthetic cells [1]. NADPH provides the reducing power for biosynthetic processes such as elongation of fatty acids, de novo synthesis of cholesterol [2,3]. Reducing power is also required to maintain the redox potential which is crucial for protection against oxidative stress and regulation of cellular proliferation and survival [4–6]. Since 6PGDH plays an essential role in maintaining cellular NADPH pool, any change of 6PGDH activity would affect energy homeostasis, growth rate, and cellular survival [7]. Both expression level and enzymatic activity of 6PGDH are regulated by diet and several hormones [8,9]. Furthermore, the fact that lipogenesis consumes large amounts of NADPH implies that 6PGDH is required to be activated when lipogenesis is stimulated [10].

ADD1/SREBP1c (adipocyte determination- and differentiation-dependent factor 1/sterol-regulatory element binding protein 1c) is a member of SREBP transcription factors. The SREBPs constitute a family of basic helix–loop–helix (bHLH) transcription factors and three isoforms have been identified [11–13]. A unique feature of SREBPs is dual DNA binding specificity to both classical palindromic E-box (CANNTG) and non-palindromic sterol-regulatory elements (SREs: ATCACCCCAC) [11,14,15]. Among three SREBPs, ADD1/SREBP1c plays a crucial role in fatty acid metabolism and insulin-dependent gene regulation especially in the regulation of lipogenic gene expression in fat and liver [16–19].

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Regulation of gene expression by insulin has been intensely studied for many years. It has been revealed that insulin regulates the expression of several key enzymes in fatty acid synthesis such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) [18,20,21]. Following insulin-induced insulin receptor (IR) autophosphorylation, insulin receptor substrate proteins are phosphorylated and mediate the transmission of insulin signaling pathway. Insulin signaling is also associated with several second messengers including phosphatidylinositol 3-kinase (PI3-kinase) and mitogen-activated protein kinase (MAPK) [22]. Phosphatidylinositol 3,4,5-phosphate regulates the activity or subcellular localization of phosphatidylinositol-dependent kinase and protein kinase B (also known as PKB/Akt) [23,24], and consequently modulates a number of target proteins including glycogen synthetase kinase-3 (GSK3), transcription factors, and coactivators. Recently accumulated evidences suggest that the effects of insulin on the expression of lipogenic genes are mediated by ADD1/SREBP1c [17,18,25]. Although further studies are required, it has been reported that ADD1/SREBP1c is stimulated by the PI3-kinase pathway as well as repressed by GSK3 to link insulin action to its target gene expression [26,27].

Although NADPH produced by 6PGDH is associated with lipogenesis in fat and liver, the transcription factor(s) responsible for 6PGDH expression has not been thoroughly investigated. In this study, we demonstrate that 6PGDH is highly expressed in fat tissue and its expression is enhanced by ADD1/SREBP1c via E-box motif in the proximal promoter region of mouse 6PGDH (m6PGDH) gene. Moreover, we reveal that ADD1/SREBP1c is also involved in insulin-dependent 6PGDH expression. These results suggest that ADD1/SREBP1c is a key transcription factor linking insulin signal and 6PGDH gene expression during lipogenesis.

Materials and methods

Animal treatment. Male C57BL/6J mice were housed (5 mice per cage), and water was given ad libitum, with 12 h light–dark cycle beginning at 07:00 a.m. In experiments, food was withdrawn during the daylight (12 h) before onset of the dark cycle.

Cell culture. 3T3-L1 and RatI-IR cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10%(v/v) bovine calf serum (BCS, Jeil Biotech, Daegu, South Korea) and 100 U of antibiotic–antimycotic at 10% CO_2 and 37 °C. Differentiation into adipocytes was achieved by allowing the cells to reach confluence before the addition of DMEM supplemented with 10% fetal bovine serum (FBS, JBI), 0.5 mM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone, and 5 μg/mL insulin at 5% CO_2 and 37 °C. After 2 days and every 2 days thereafter, fresh medium (DMEM plus 10% FBS and 5 μg/mL insulin) was changed. Human embryonic kidney 293 (HEK293) cells were maintained in DMEM supplemented with 10% FBS and 100 U of antibiotic–antimycotic and cultured at 37 °C in a 10% CO_2 incubator.

Cloning of mouse 6PGDH promoter and construction of luciferase reporters. Mouse genomic DNA was isolated from 3T3-L1 cells using lysis buffer (50 mM Tris, pH 7.5, 50 mM EDTA, 100 mM NaCl, and 2% SDS). The primers used for polymerase chain reaction (PCR) were as follows: −978 to +163 bp fragment—forward, 5′-CGT GGT ACC ACA TGC CTT-3′ and reverse, 5′-CCG GGC CCG ACT ACG CGT GTC GTC ACT CAC TGG GCC ATG-3′; −646 to +163 bp fragment—forward, 5′-TAC AAG CTT AAG GTA CCA CTC ACT TCC AGT CTT GCC-3′; −345 to +163 bp fragment—forward, 5′-GTA CAA GCT TAG GTA CCC ACA GAT AGG ACA GAC-3′. The primers included the sequences for the *Kpn*I and *Mlu*I restriction enzyme sites. The PCR products were digested with *Kpn*I and *Mlu*I, and cloned into the pGL3 basic vector (Promega).

Electrophoretic mobility shift assay. Electrophoretic mobility shift assays (EMSAs) were performed in 20 μL volume containing purified recombinant ADD1/SREBP1c protein (20 ng) in the reaction buffer (10 mM Tris, pH 7.6, 50 mM KCl, 2.5 mM $MgCl_2$, 0.05 mM EDTA, 0.1%(v/v) Triton X-100, 8.5%(v/v) glycerol, 1 μg of poly(dI–dC), 1 mM dithiothreitol, and 0.1 mg/mL non-fat dried milk). ^{32}P-labeled probe (0.1 pmole) was added into the reaction mixture and incubated at RT for 20 min. The samples were resolved in a 4% polyacrylamide gel with 0.25× Tris–borate–EDTA (TBE) buffer, and the gels were processed for autoradiography. For competition assays, unlabeled oligonucleotides (100-fold molar excess) were added into reaction mixture prior to the addition of radioisotope labeled probe. The DNA sequences of the double-stranded oligonucleotides were used as the following (only one strand is shown): ARE7, 5′-GAT CTG TGA ACT CTG ATC CAG TAA G-3′; SRE, 5′-GAT CCT GAT CAC CCC ACT GAG GAG-3′.

DNase I footprinting assay. DNA fragments of m6PGDH promoter were labeled in one strand and purified as described in below. m6PGDH promoter fragment was isolated by serial digestion with *Pst*I and *Nhe*I to obtain 5′- and 3′-overhanging ends. Subsequent DNA was labeled with Klenow fragment and [α-^{32}P]dCTP, and then purified by PAGE. DNA–protein binding reactions were performed with 50,000 c.p.m. of probe per reaction in the solution containing 10 mM Hepes, pH 7.9, 60 mM KCl, 1 mM EDTA, pH 8.0, 7%(v/v) glycerol, 1 mM dithiothreitol, 2 μg of poly(dI–dC), and indicated amount of recombinant ADD1/SREBP1c protein. After 30 min of incubation on ice, 5 U of DNase I, freshly diluted in a solution containing 10 mM Hepes, pH 7.6, 60 mM KCl, 25 mM $MgCl_2$, 5 mM $CaCl_2$, and 7%(v/v) glycerol, was added to the reaction and then kept at RT for 2 min. Digestion reactions were stopped by the addition of 80 μL of stop solution containing 20 mM Tris–HCl, pH 8.0, 20 mM EDTA, 250 mM NaCl, 0.5% SDS, 4 μg of yeast tRNA, and 10 μg of proteinase K. The samples were incubated for 1 h at 45 °C, extracted with phenol/chloroform, precipitated with ethanol, and resuspended in formamide dye. The samples were resolved in 6%(w/v) polyacrylamide/7 M urea sequencing gel. The protected regions were mapped with reference to the migration of Maxam-Gilbert A + G sequencing products.

Results

6PGDH is predominantly expressed in fat tissue and is induced during adipogenesis

In order to examine the tissue distribution of mouse 6PGDH mRNA expression, we performed Northern blot analysis. 6PGDH mRNA was highly expressed in white adipose tissue, and low amounts of 6PGDH were detected in several tissues including liver, spleen, kidney, and lung (Fig. 1A). Furthermore, we observed that the