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Mediation of glucolipotoxicity in INS-1 rat insulinoma cells by small heterodimer partner interacting leucine zipper protein (SMILE)

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ABSTRACT

Sustained elevations of glucose and free fatty acid concentration have deleterious effects on pancreatic beta cell function. One of the hallmarks of such glucolipotoxicity is a reduction in insulin gene expression, resulting from decreased insulin promoter activity. Sterol regulatory element binding protein-1c (SREBP-1c), a lipogenic transcription factor, is related to the development of beta cell dysfunction caused by elevated concentrations of glucose and free fatty acid. Small heterodimer partner (SHP) interacting leucine zipper protein (SMILE), also known as Zhangfei, is a novel protein which interacts with SHP that mediates glucotoxicity in INS-1 rat insulinoma cells. Treatment of INS-1 cells with high concentrations of glucose and palmitate increased SREBP-1c and SMILE expression, and decreased insulin gene expression. Adenovirus-mediated overexpression of SREBP-1c in INS-1 cells induced SMILE expression. Moreover, adenovirus-mediated overexpression of SMILE (Ad-SMILE) in INS-1 cells impaired glucose-stimulated insulin secretion as well as insulin gene expression. Ad-SMILE overexpression also inhibited the expression of beta-cell enriched transcription factors including pancreatic duodenal homeobox factor-1, beta cell E box transactivator 2 and RIPE3b1/MafA, in INS-1 cells. Finally, in COS-1 cells, expression of SMILE inhibited the insulin promoter activity induced by these same beta-cell enriched transcription factors. These results collectively suggest that SMILE plays an important role in the development of beta cell dysfunction induced by glucolipotoxicity.

1. Introduction

The main pathophysiological features of type 2 diabetes are beta cell dysfunction and insulin resistance [1]. The effects of high glucose and free fatty acid (FFA) concentrations on beta-cell dysfunction have been widely studied [2–4]. Chronic hyperglycemia (glucotoxicity [5,6]) or chronic dyslipidemia (lipotoxicity [7,8]) is known to have detrimental effects on beta-cell function in many experimental systems, and the combined effects of high glucose and high FFA concentrations are synergistically harmful, which has led to the concept of glucolipotoxicity [9,10]. Prolonged exposure to high FFA concentrations impairs insulin gene expression in the presence of high glucose concentrations [11–14]. The transcriptional mechanisms by which palmitate inhibits insulin gene expression do not involve changes in insulin mRNA stability but, rather, inhibition of glucose-induced insulin promoter activity [15]. This is associated with decreased binding activity of the transcription factors pancreatic duodenum homeobox 1 (PDX-1) and RIPE3b/MafA [16]. Accumulating evidence suggests that sterol regulatory element binding protein-1c (SREBP-1c), a key lipogenic transcription factor, plays a critical role in the development of beta-cell dysfunction caused by elevated glucose and FFA [17,18]. SREBP-1c is a membrane-bound transcription factor of the basic helix-loop-helix (bHLH) leucine zipper family of proteins, and has been described as a regulator of lipogenic enzymes in liver, adipocytes, myocytes and beta-cells [19]. Overexpression of SREBP-1c induced beta-cell dysfunction, as shown by the apoptosis of these cells, impaired glucose-stimulated insulin secretion (GSIS), and increased lipid accumulation [17,20]. However, it is still unclear how hyperlipidemia induces beta cell failure under the hyperglycemic condition of type 2 diabetes.

Small heterodimer partner interacting leucine zipper protein (SMILE) belongs to the basic region leucine zipper (bZIP) family

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of proteins [21,22]. SMILE gene expression produces two isoforms, SMILE-L (long isoform of SMILE, also known as CREBZF) and SMILE-S (short isoform of SMILE, previously known as Zhangfei) [22]. SMILE has been identified as an interacting partner of herpes simplex virus related host-cell factor (HCF) and inhibits the replication of the herpes simplex virus [21,23]. SMILE has been also reported as a coactivator of ATF4 and as a corepressor of CREB3, another cellular HCF-binding transcription factor [24,25]. Recent studies showed that SMILE acts as a transcriptional co-repressor of nuclear receptors such as glucocorticoid receptor, constitutive androstane receptor, hepatocyte nuclear factor 4α, and ERRγ. In another study, SMILE was shown to regulate ER stress-mediated gene transcription [26], and induced the expression of nerve growth factor receptor [27]. In adult tissues, the expression of SMILE is most abundant in heart, liver and skeletal muscle, and is moderately abundant in kidney and pancreas [21]. Little is known about the effect of SREBP-1c on SMILE expression related to glucolipotoxicityor the role of SMILE in insulin gene expression and/or secretion in pancreatic beta cells.

The present study reports the effects of glucolipotoxic conditions or adenovirus-mediated overexpression of SREBP-1c in INS-1 cells on SMILE expression and SMILE regulation of insulin gene expression and glucose-stimulated insulin secretion.

2. Materials and methods

2.1. Materials and plasmids

D-mannitol, D-glucose, and palmitate were purchased from Sigma–Aldrich (St. Louis, MO). Radiochemical[a-32P]dCTP was purchased from Perkin Elmer (Boston, MA). The full ORF (open reading frame) of human SMILE was amplified by PCR. The purified PCR products were digested and subcloned into the EcoRI and XhoI sites of pcDNA3. To generate −1131-bp-SMILE-Luc, the SMILE promoter region spanning −1131 to −15 bp was PCR-amplified from human genomic DNA and cloned into pGL3-basic vector (Promega) between the SacI and XhoI sites.

2.2. Cell culture

The INS-1 rat insulinoma cell line was cultured at 5% CO2–95% air at 37 °C in RPMI-1640 (Gibco BRL, Grand Island, NY) containing 11.2 mmol/l glucose and 2 mmol/l L-glutamine. The medium was supplemented with 10% FBS, 1 mmol/l pyruvate, 10 mmol/l HEPES, 50 μmol/l 2-mercaptoethanol, 100 U/ml penicillin, and 100 μg/ml streptomycin (INS-1 medium). All experiments were performed using INS-1 cells between passages 20 and 30.

2.3. Preparation of recombinant adenovirus

cDNAs encoding human SMILE or mouse SREBP-1c were inserted in the KpnI/XbaI sites of the pAdTrack-CMV shuttle vector. The resulting vector was then electroporated into BJ5138 cells containing the Adeasy adenoviral vector to produce the recombinant adenoviral plasmid. The recombinants were amplified in HEK-293 cells and isolated and purified by using CsCl(Sigma) gradient centrifugation. The preparations were collected, desalted, and the titers were determined by using Adeno-XTM Rapid titer (BD Bioscience, San Jose, CA) according to the manufacturer's instructions.

2.4. Northern blot analysis

Total RNA was isolated from cells using Trizol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. An amount of 20 μg total RNA was used from each sample. The probes for SMILE, insulin, PDX-1, MafA and BETA2 were labelled with [α-32P]dCTP by using a random-primer DNA-labelling system (Amersham Biosciences, Little Chalfont, UK).

2.5. Western blot analysis

Cell lysates were prepared using IPH lysis buffer [50 mmol/l Tris (pH 8.0), 150 mmol/l NaCl, 5 mmol/l EDTA, 0.1 mmol/l PMSF, 0.5% NP-40] containing proteinase inhibitors and DTT. The proteins were then electrotransfered to PVDF membranes (Millipore, Bedford, MA). The membranes were blocked by incubation in blocking buffer, incubated with anti-SREBP-1c antibody (BD bioscience), anti-SMILE antibody (Abcam, Cambridge, UK), anti-PDX-1 antibody (Santa Cruz, Santa Cruz, CA), anti-MafA antibody (Bethyl Laboratories Inc., Montgomery, TX) or anti-BETA2 antibody (Santa Cruz), washed, and incubated with horseradish peroxidase-conjugated secondary antibody. Signals were visualized by chemiluminescent detection according to the manufacturer's instructions (Amersham). The membrane was reblotted with anti-actin antibody to verify equal loading of protein in each lane. Densitometric measurements of the bands were made by using the digitalized scientific software program UN-SCAN-IT (Silk Scientific Corporation, Orem, UT).

2.6. In vitro transient transfection and gene reporter assays

INS-1 or COS-1 cells were plated at a density of 3×10^5 cell per well in a 12-well plate and subcultured for 2 days. SMILE promoter or insulin promoter constructs (300 ng/well) were transiently transfected by using LipofectamineTM 2000 Transfection Reagent (Invitrogen). β-Galactosidase plasmids were co-transfected as an internal control. Cells were transfected for 4 h, washed to remove plasmids, and then cultured in conditioned medium. Cells were harvested approximately 24 h after transfection for luciferase and β-galactosidase assays. An amount of 20 μl cell lysate was analyzed by using the Luciferase assay system according to the manufacturer's instructions (Promega, Madison, WI). Luciferase activity was detected using a SIRUS Luminometer (Berthold, Pforzheim, Germany). The luciferase activity was normalized by using the β-galactosidase activity.

2.7. Insulin secretion

To examine the effect of Ad-SMILE on GSIS in INS-1 cells, INS-1 cells were infected with indicated doses of SMILE or Null adenoviruses for 2 h. The culture medium was then changed to RPMI 1640 containing 10% FBS and the cells were cultured for 24 h. The cells was then starved in medium containing 3 mmol/l glucose and 2% FBS for 5 h, and subsequently incubated for 1 h at 37 °C in modified KRBB solution [114 mmol/l NaCl, 4.4 mmol/l KCl, 1.28 mmol/l $CaCl_2$, 1 mmol/l $MgSO_4$, 29.5 mmol/l NaHCO3, 10 mmol/l HEPES, 3 mmol/l glucose, 0.1% bovine serum albumin, and pH 7.4 (adjusted with NaOH)] with or without 16.7 mmol/l glucose. The supernatant (200 μl) was carefully collected and subjected to the rat insulin radioimmunoassay (Linco Research, St. Charles, MO).

2.8. Statistical analysis

All results are expressed as the mean ± SEM. Analysis of variance was used to determine significant differences in multiple comparisons and was performed by using the Duncan test. Values of $P < 0.05$ were considered to be statistically significant. All experiments were performed at least three times.