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ChREBP regulates *Pdx-1* and other glucose-sensitive genes in pancreatic β-cells

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ABSTRACT

Carbohydrate responsive element-binding protein (ChREBP) is a transcription factor whose expression and activity are increased in pancreatic β-cells maintained at elevated glucose concentrations. We show here that ChREBP inactivation in clonal pancreatic MIN6 β-cells results in an increase in *Pdx-1* expression at low glucose and to a small, but significant, increase in *Ins2*, *GcK* and *MafA* gene expression at high glucose concentrations. Conversely, adenovirus-mediated over-expression of ChREBP in mouse pancreatic islets results in decreases in Pdx-1, MafA, Ins1, Ins2 and GcK mRNA levels. These data suggest that strategies to reduce ChREBP activity might protect against β-cell dysfunction in type 2 diabetes.

1. Introduction

Pancreatic β-cell glucolipotoxicity [1] is considered to play a significant role in the pathogenesis of type 2 diabetes. Carbohydrate responsive element-binding protein (ChREBP) is a member of the basic helix–loop–helix family of transcription factors and transactivates glucose-responsive genes by binding to DNA as a heterodimer with Max-like protein X1 at a well-defined carbohydrate-responsive element (ChoRE) [2–5]. In the liver, ChREBP is responsible for converting excess carbohydrate to fatty acids for long-term storage [6]. Mice deleted for both alleles of ChREBP display diminished rates of hepatic glycolysis and lipogenesis resulting in high liver glycogen content, low plasma free fatty acid levels and reduced adipose tissue mass [7]. Loss of ChREBP in leptin-null *ob/ob* mice protects against obesity [7,8].

We, and others, have previously shown that, in pancreatic β-cells, ChREBP is activated by high glucose and is responsible for the induction of the lipogenic genes fatty acid synthase (FAS) and L-type pyruvate kinase (L-PK) [9,10], and the proapoptotic gene *Txnip* [11,12]. ChREBP also represses aryl hydrocarbon receptor nuclear translocator/hypoxia inducible factor 1-β (ARNT/HIF1-β) [13] shown recently to be diminished in islets [14] and liver [15] of type 2 diabetic humans, and necessary for normal β-cell function and repression of hepatic gluconeogenesis. We sought here to investigate the effects of ChREBP silencing and over-expression on other key glucose-responsive genes in pancreatic islet β-cells, namely pancreatic and duodenal homeobox-1 (Pdx-1), MafA, glucokinase (GcK) and insulin, all critical for normal pancreatic β-cell function.

2. Materials and methods

2.1. Materials

Primers for siRNA construction and PCR were from MWG Biotech (Milton Keynes, UK). Antibodies were described in [9]. Other reagents were from Sigma or Invitrogen.

2.2. Plasmids, adenoviruses and siRNA

pChREBP and ChREBP siRNA have been described in [9]. Adenovirus encoding for ChREBP has been described in [13]. Plasmids and adenoviruses encoding GFP-null and constitutively active and dominant negative forms of SREBP-1c were described in [16]. pPdx1.Luc$_{FF}$, encoding the 5′ flanking region of the mouse pancreatic duodenum homeobox-1 (*Pdx-1*) gene (−2715 to 0 bp), was generated by PCR from MIN6 cell genomic DNA with the following primers: forward, 5′-ATAT GG TACC CTC CAG TAT CAG

Abbreviations: ARNT, aryl hydrocarbon receptor nuclear translocator; ChREBP, carbohydrate responsive element-binding protein; ChIP, chromatin immunoprecipitation; ChoRE, carbohydrate-responsive element; FAS, fatty acid synthase; GcK, glucokinase; GFP, green fluorescent protein; HIF, hypoxia inducible factor; L-PK, L-type pyruvate kinase; Pdx-1, pancreatic and duodenum homeobox-1; siRNA, small interfering RNA; SREBP-1c, sterol regulatory response-element-binding protein-1c; USF, upstream stimulatory factor.

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Table 1
Primers used for real-time RT-PCR.

mRNA	Forward primer 5′–3′	Reverse primer 5′–3′
MafA	CACCACGTGCGCTTGG	CAGAAAGAAGTCGGGTG
Pdx-1	TGGAGCTGGCAGTGATGTTGA	TCAGAGGCAGATCTGGCCAT
Ins1	GAAGCGTGGCATTGTGGAT	TGGGCCTTAGTTGCAGTAGTTCT
Ins2	AGCCCTAAGTGATCCGCTACAA	CATGTTGAAACAATAACCTGGAAGA
GcK	GCTTTTGAGACCCGTTTTGTG	GCCTTCGGTCCCCAGAGT
Cyclophilin	TATCTGCACTGCCAAGACTGA	CCACAATGCTCATGCCTTCTTTCA

Table 2
Effects of glucose and ChREBP silencing on mRNA levels in MIN6 cells.

[Glucose]	Scrambled siRNA		ChREBP siRNA	
	3 mM	30 mM	3 mM	30 mM
MafA	0.0294 ± 0.0002	0.0332 ± 0.0002***	0.0297 ± 0.0002	0.0395 ± 0.0002*,¶
GcK	0.397 ± 0.0002	0.927 ± 0.0006***	0.386 ± 0.0002	1.14 ± 0.0001***,¶
Ins2	3.52 ± 0.004	9.79 ± 0.004***	3.48 ± 0.0007¶	10.2 ± 0.004***,¶

Culture conditions, total RNA preparation and real-time RT-PCR conditions were as described in Fig. 3. Data are means ± SEM from three independent experiments performed in triplicates, and normalized to cyclophilin mRNA levels. *, *** indicate $p < 0.05$, 0.0001 for the effect of glucose and ¶ indicates $p < 0.05$, for the effect of ChREBP siRNA.

GGA GGA-3′ (KpnI site underlined); reverse, 5′-TTT GAGCTC CCA CCC CAG ATC GCT TTG A-3′ (SacI site underlined) and subcloned into pGL3 basic (Promega). Two point mutations [−106C > A; −102T > G] in the *Pdx-1* promoter were introduced using Quick-change™ (Stratagene) with the following sense primer: 5′-ATG GCT CCA GGG TAA ACA ACG GGG GGT GCC CCA GAG CCT ATG-3′.

2.3. MIN6 cell culture and islet of Langerhans isolation

MIN6 cells were cultured as in [9]. Mouse islets of Langerhans were isolated and cultured as in [13].

2.4. Single cell reporter gene assay

Intranuclear microinjection of plasmids, antibodies and siRNAs in MIN6 cells were performed at plasmid concentrations of 0.1 (pPdx1.Luc_{FF}), and 0.05 (pChREBP, pSREBP-1c, pCMV-RL) mg ml^{-1}, and antibody against ChREBP and SREBP at 1 mg ml^{-1}, before imaging as described in [9].

2.5. Real-time RT-PCR

Total mRNA isolation, cDNA generation and real-time quantitative PCR were performed with primers listed in Table 1, as in [13] and according to the manufacturer's instructions. Levels of mRNA encoding the indicated genes were normalized compared with cyclophilin mRNA and expressed as the fold change over control (null, 3 mM glucose) and presented as the means ± SEM.

2.6. Chromatin immunoprecipitation assay

Chromatin immunoprecipitation was performed essentially as described in [9,13].

2.7. Statistical analysis

Data are given as means ± SEM. Comparisons between means were performed by unpaired two-tailed Student's *t*-test with Bonferroni correction as appropriate, using Microsoft Excel.

3. Results

3.1. ChREBP silencing enhances glucose-responsive gene expression in MIN6 pancreatic β-cells

We have previously shown that ChREBP silencing in pancreatic murine insulinoma MIN6 β-cells improves glucose-stimulated insulin secretion, possibly through a decrease in total triglyceride content [9]. Here, we examined the impact of ChREBP silencing by RNA interference on other glucose-responsive genes in MIN6 β-cells. ChREBP knockdown increased the levels of mRNA encoding *MafA*, *GcK* and *Ins2* at high (30 mM) glucose concentrations, whereas ChREBP silencing increased the expression of the *Pdx-1* gene at low (3 mM) glucose concentrations (Table 2 and Fig. 1A). Correspondingly, we observed a similar increase in *Pdx-1* promoter activity at low glucose after ChREBP inhibition by microinjection of a specific anti-ChREBP antibody (Fig. 1C), while introduction of a ChREBP expression vector by microinjection suppressed the activity of *Pdx-1* promoter at high glucose (Fig. 1E). By contrast, SREBP1-c inactivation or over-expression was without effect on *Pdx-1* promoter activity or mRNA levels (Fig. 1B, D and F).

3.2. ChREBP modulation of Pdx-1 gene expression might be indirect

We next sought to identify the region on the *Pdx-1* promoter responsive to ChREBP repression. No consensus ChoRE exists on the *Pdx-1* promoter, but a proximal E-box, located at −105 bp (Fig. 2A) is highly conserved between species, is protected in DNAse footprints, and has been proposed to confer β-cell specificity to the *Pdx-1* promoter [17]. Up to now, it has been thought that this site predominantly binds USF, since mutations abolishing the binding of the latter factor impair the activity of the *Pdx-1* promoter, whereas over-expression of a dominant-negative USF2 reduces both *Pdx-1* promoter activity as well as Pdx-1 mRNA and protein levels [17,18]. Indeed, mutation of this site abolished both the glucose response and the repressive effect of ChREBP of the *Pdx-1* reporter construct (Fig. 2B). However, neither ChREBP, USF2 nor SREBP-1c binding could be detected to the proximal (−260 to +1) region of the promoter by chromatin immunoprecipitation (Fig. 2C). By contrast, and as previously reported [9], ChREBP binding was readily detectable on the proximal L-PK promoter at elevated glucose concentrations (Fig. 2C, bottom panel). We also used this approach to screen a further 11 E-boxes lying in the Pdx-1 promoter region between −2.7 and −0.26 kb (Fig. 3) but could not reveal evidence for ChREBP (not shown).

3.3. Adenovirus-mediated over-expression of ChREBP inhibits glucose-responsive gene expression in isolated mouse islets of Langerhans

We next examined the effects of ChREBP over-expression on the levels of mRNAs encoding the above glucose-responsive