Histone acetylation of glucose-induced thioredoxin-interacting protein gene expression in pancreatic islets

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ARTICLE INFO

Article history:
Received 29 April 2016
Received in revised form 20 October 2016
Accepted 24 October 2016
Available online 29 October 2016

Keywords:
Glucotoxicity
Histone acetylation
Histone acetyltransferase p300
Histone deacetylase
Thioredoxin-interacting protein

ABSTRACT

Thioredoxin-interacting protein (TXNIP) has been shown to be associated with glucose-induced deterioration of pancreatic beta cell function in diabetes. However, whether epigenetic mechanisms contribute to the regulation of TXNIP gene expression by glucose is not clear. Here we studied how glucose exerts its effect on TXNIP gene expression via modulation of histone acetylation marks. To achieve this, we applied clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR/Cas9) to knock out histone acetyltransferase (HAT) p300 in a rat pancreatic beta cell line INS1 832/13. We also treated the cells and human islets with chemical inhibitors of HAT p300 and histone deacetylase (HDAC). In human islets, diabetes and high glucose resulted in elevated TXNIP and EP300 expression, and glucose-induced TXNIP expression could be reversed by p300 inhibitor C646. In INS1 832/13 cells, EP300 knock-out by CRISPR/Cas9 elevated glucose-induced insulin secretion and greatly reduced glucose-stimulated Tmp expression and cell apoptosis. This effect could be ascribed to decrease in histone marks H3K9ac and H4ac at the promoter and first coding region of the Txnip gene. Histone marks H3K9ac and H4ac in the Txnip gene in the wild-type cells was inhibited by HDAC inhibitor at high glucose, which most likely was due to enhanced acetylation levels of p300 after HDAC inhibition; and thereby reduced p300 binding to the Txnip gene promoter region. Such inhibition was absent in the Ep300 knock-out cells. Our study provides evidence that histone acetylation serves as a key regulator of glucose-induced increase in TXNIP gene expression and thereby glucotoxicity-induced apoptosis.

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1. Introduction

In diabetes, elevated glucose levels over time induce beta cell dysfunction, as well as loss of beta cell mass via accelerated apoptosis rate (Butler et al., 2003; Maechler et al., 1999; Robertson et al., 1992). Such hyperglycemia-induced deterioration of pancreatic beta cell is referred to as glucotoxicity, which may involve changes in gene profiles and its underlying mechanisms remained to be elucidated (Robertson et al., 2003).

Previous studies have shown that thioredoxin-interacting protein (TXNIP), an endogenous inhibitor of the antioxidant protein thioredoxin (Chen et al., 2008; Osłowski et al., 2012; Parikh et al., 2007), is among the most highly upregulated genes in glucose-treated human islets and diabetic mouse islets (Minn et al., 2005). Glucose-induced TXNIP gene expression resulted in oxidative damage and beta cell death, suggesting an important role for TXNIP in linking glucotoxicity and beta cell apoptosis (Chen et al., 2008; Minn et al., 2005). A recent study showed that changes in DNA methylation of the TXNIP gene in peripheral blood are strongly associated with type 2 diabetes (T2D) incidence (Chambers et al., 2015). It has also been shown that glucose-induced TXNIP gene expression involves interaction of the carbohydrate response element binding protein (ChREBP) with histone acetyltransferase p300 at the promoter of the gene (Cha-Molstad et al., 2009a). These observations support the view that glucose could induce TXNIP gene expression via epigenetic mechanisms, e.g. modification of histone acetylation.
Initiation of gene transcription involves relaxation of chromatin and unfolding of nucleosomes by various histone modifications including acetylation of the histones tails (Kouzarides, 2007; Narlikar et al., 2002). Histone acetylation is regulated by two groups of enzyme, HATs and histone deacetylases (HDACs). HATs add acetyl groups to the conserved lysine amino acids and this process can be reversed by removal of acetyl groups by HDACs. Gene transcription can be activated or repressed by lysine residue hyper-acetylation or hypoacetylation regulated by both HAT and HDAC (Kuo and Allis, 1998; Yuan et al., 2013).

Although it is not clear if HAT gene expression and activity is affected by diabetes state in pancreatic islets, previous studies in other tissues have shown that HAT activity and histone acetylation is increased in kidneys of diabetic mice (Wang et al., 2015; Cai et al., 2016; De Marinis et al., 2016), as well as increased p300 gene expression in diabetic rat heart (Aziz et al., 2013). Inhibition of p300 by chemical inhibitor curcumin has been shown to prevent renal damage and dysfunction in streptozotocin (STZ)-induced diabetic mice (Wang et al., 2015). It could be reasonable to explore whether a similar mechanism also operates in pancreatic islets and, hence if p300 could be a potential therapeutic target to prevent beta cell damage in diabetes.

Here we examined HAT p300 (EP300) and TXNIP gene expression in diabetic human islets as well as rat insulinoma cell line INS1 832/13 exposed to high glucose. We have investigated whether inhibition of histone acetylation by either CRISPR/Cas9 silencing or a HAT p300 inhibitor could reverse glucose-induced TXNIP gene expression and rescue cells from glucose-induced apoptosis. Our study therefore shed light on a novel non-glucose lowering strategy for prevention of glucotoxicity in pancreatic islets by targeting at epigenetic mechanisms.

2. Materials and methods

2.1. Human Islets, cell culture and treatment

Human islets from cadaver donors were provided by the Nordic Islet Transplantation Program. RNA-seq on islet samples was performed using Illumina’s TruSeq RNA Sample Preparation Kit. All procedures were approved by the ethics committee at Lund University. Insulin secreting rat insulinoma cell line INS1 832/13 cells were cultured in complete RPMI 1640 medium (Invitrogen) containing 5 or 25 mM glucose. In the experiments testing the effect of p300 inhibitor, cells were treated with 25 μM C646 (Calbiochem, CA, USA) or 25 μM CI994 (Selleckchem, Houston, TX, USA) for 24 h in 5 or 25 mM glucose.

2.2. mRNA extraction and quantitative RT-PCR

Human islet and INS1 832/13 cell mRNA were extracted using RNeasy kit (Qiagen) according to the manufacturer’s instructions. mRNA was then reverse transcribed to cDNA using the first strand cDNA synthesis kit (Fermentas). mRNA expression was assessed by quantitative RT-PCR performed on a Prism 7900 Sequence Detection System by Taqman assay (Applied Biosystems). Ep300 expression in human islets was normalized to two housekeeping genes HPRT1 and PPIB. The assay numbers are: HPRT1 Hs02800695_m1; PPIB Hs00168719_m1; EP300 Hs00914223_m1; TXNIP Hs00197750. Txnip expression in rat pancreatic beta cell line INS1 832/13 was normalized to two housekeeping genes Ppib and Gapdh. The assay numbers are: Gapdh Rn01775763_g1; Ppib Rn03302274_m1; Txnip Rn01533885_g1.

2.3. Genome editing by CRISPR/Cas9

Ep300 −/− cells were generated applying CRISPR/Cas9-mediated genome editing (Wu et al., 2014). INS1 832/13 cells were edited using nuclease Cas9 together with two guide RNA pairs that specifically target at exon 1 of Ep300 (5’-AGATGAGGTCTAGGCCCT-3’ and 5’-GGTCCCACCGATGGCACT-3’). Guide sequence oligos were cloned into plasmid pX330-U6-Chimeric_BB-CBh-5SpCas9 (Addgene plasmid # 42230), a generous gift from Prof. Feng Zhang (Broad Institute of MIT and Harvard, Cambridge, MA, USA), and validated by Sanger sequencing. INS1 832/13 cells were then transfected with the constructed plasmids and single cell colonies were isolated by limiting dilution and expansion. Clones were then genotyped by Sanger sequencing in total 700 bp starting from 100 bp before the exon 1 of rat Ep300 gene.

2.4. ChIP assay

Briefly, cells were cross-linked by formaldehyde (final concentration 1%) and sonicated by Bioruptor sonicator (Diagenode) for 30 cycles of 30-s with a 30-s interval (medium intensity) period between cycles. Genomic DNA fragment lengths of 200–1000 bp were achieved after sonication. Lyesates were then centrifuged, and the supernatants (sonicated chromatin) were collected. 10% volume of each sample was removed as the input control. The sonicated chromatin was incubated overnight at 4 °C with 2.5 μg of antibody lysine 9-acetylated histone H3 (H3K9ac, ab4441, Abcam), H4ac (06-866, Millipore), p300 (sc-48343X, Santa Cruz Biotechnology) or a normal rabbit polyclonal IgG (12–370, Upstate/Millipore) as a negative control. Immune complexes were captured with 10 μl of 50% protein G beads, eluted by reverse cross-linking and protease K digestion. DNA fragments were purified using MinElute PCR Purification Kit (Qiagen) and quantified by SYBR Green PCR (Applied Biosystems) with primers designed for Txnip promoter region (forward primer AATGTTCACCCAGCTACAG and reverse TTCGTTTTCATGCTCTATGT); and the first coding region of the gene (forward primer CGAGTCAAAGCGTCAGGAT and reverse TTCATGCGCAGTCCAAAGT). The DNA quantitation value of each sample was analyzed by the 2^(-ΔΔCt) method and results were expressed as fold over control after normalizing with input samples. In all experiments, we verified that ChIP precipitation enrichment obtained was relative to IgG controls.

2.5. Western blotting

Cells were homogenized in ice-cold RIPA buffer containing complete protease inhibitor (Roche) by shaking on ice for 30 min. Supernatant was collected by centrifugation (10,000 × g, 10 min, 4 °C). Extracted total protein content was measured by Pierce BCA Protein Assay Kit (Thermo Scientific), and 8 μg of protein was electrophoresed by SDS-PAGE (BIO-RAD). The transferring and blocking were performed and the membrane was incubated overnight at 4 °C with anti-Acetyl-p300 antibody (Cell signalling, 1:1000) followed by incubation with anti-rabbit IgG (Cell Signaling, 1:2000) for 2 h at room temperature. Normalization was carried out by incubating membrane with anti-beta actin antibody (Sigma, 1:2000). Final signal was indicated by ChemiDoc MP System (BIO-RAD).

2.6. Insulin secretion assay

Insulin secretion was measured from cell culture medium by Merckodia High Range Rat Insulin ELISA kit (Merckodia AB), and nor-
Fig. 1. Elevated EP300 and TXNIP gene expression in human diabetic islets and inhibition of glucose-stimulated TXNIP expression by HAT p300 inhibitor. (A) EP300 gene expression in human islets from non-diabetic (ND, white bar, n = 7) and type 2 diabetic donor (T2D, black bar, n = 11) was measured by TaqMan assay quantitative PCR and normalized to housekeeping genes HPRT and PPIB. *p < 0.01 vs. ND. (B) Correlation between EP300 gene expression in human islets and HbA1c levels (between 4.6–8.1) in 23 donors. p < 0.05 was considered to be significant. (C) TXNIP gene expression was quantified by RNA sequencing in human islets from non-diabetic (ND, white box, n = 114) and diabetic (T2D, black box, n = 17) donors. *p < 0.05 vs. ND. (D) Human islets from individual donors (n = 4–5) were treated in 5 (5G, white bars) or 25 mM glucose (25G, black bars), in the presence or absence of p300 inhibitor C646 (25 µM) for 24 h. TXNIP gene expression was measured by TaqMan assay quantitative PCR and normalized to housekeeping genes HPRT and PPIB. *p < 0.05 vs. 5G control; and †p < 0.01 vs. 25G control.

2.7. Cell growth measurement and apoptosis assay

Cells were trypsinized and collected after respective incubation conditions. Total cell number was then determined by automated cell counter (Orfio). Cell apoptosis was measured using Cell Death Detection ELISA kit (Roche).

2.8. Data analysis

Statistical comparisons were performed using 2-tailed Student’s t-test if not stated otherwise. Data are expressed as the mean ± SEM. Correlations were analyzed by Pearson’s correlation coefficient test. Differences with p < 0.05 were considered significant.

3. Results

3.1. Elevation of EP300 and TXNIP gene expression in human diabetic islets and reduction of glucose-stimulated TXNIP gene expression by inhibition of p300

To study whether HAT p300 gene (EP300) expression is affected by the diabetic state, we examined EP300 expression in human islets from non-diabetic and T2D donors by quantitative PCR. EP300 gene expression was almost doubled in T2D islets (Fig. 1A) and correlated strongly with HbA1c levels (Fig. 1B), suggesting that increased EP300 gene expression could be a consequence of hyperglycemia.
Fig. 2. CRISPR/Cas9 silencing of Ep300 in INS1 832/13 cells. (A) Two CRISPR guide RNAs were designed to respectively bind 3 bps before the end of Ep300 exon 1 (CRISPR guide RNA 1) and 41 bps from the beginning of exon 1 (CRISPR guide RNA 2). PAM sequences are highlighted in black bold letters and CRISPR cutting sites are highlighted in red bold letters. (B) Illustration of Ep300 exon 1 deletion with 44 bps deletion on one allele and 45 bps deletion on the other allele. (C) Chromatogram confirmation of the deletion by Sanger sequencing. (D) Ep300 expression in wild-type and Ep300 knock-out cells was quantified by qPCR and normalized to housekeeping genes Gapdh and Ppib expression. The values represent the mean ± SEM of three independent experiments. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
In the transcriptome from 131 human donors, we detected significantly increased TXNIP gene expression in T2D islets compared to non-diabetic islets (Fig. 1C). Previous studies have shown that glucose stimulates recruitment of p300 to the promoter region of the TXNIP gene in human islet (Cha-Molstad et al., 2009a). To investigate if glucose-stimulated TXNIP gene expression can be reduced by inhibition of p300, we incubated human islets for 24 h in 5 or 25 mM glucose in the presence or absence of inhibitor C646. We observed significant reduction in high glucose-induced TXNIP gene expression by C646 (Fig. 1D), which suggests that glucose-induced increase in TXNIP expression can be prevented by inhibition of p300.

3.2. Reduced glucose-stimulated increase in Txnip gene expression in a rat pancreatic beta cell line after ep300 knockout

To further delineate the role of p300 in histone acetylation of the TXNIP gene and its consequences on gene transcription, we created a target deletion of Ep300 exon 1 by CRISPR/Cas9 in a rat pancreatic beta cell line INS1 832/13. We designed two CRISPR guide RNAs (gRNAs) that respectively bind 41 bps after the beginning of exon 1, and 3 bps before the end of exon 1 (Fig. 2A). As Cas9 nuclease introduces DNA breaks 3–4 bps (Fig. 2A, sequences highlighted in red) before a PAM sequence (Fig. 2A, sequences highlighted in black), we obtained a clone with deletion of exon 1 in the Ep300 gene with 45 bps deletion in one allele and 44 bps deletion in the other allele (Fig. 2B), which was confirmed by Sanger sequencing (Fig. 2C). Quantitative PCR (qPCR) using Ep300 TaqMan assays showed complete absence of Ep300 expression (Fig. 2D). Wild-type, homozygous and heterozygous clones used in the following experiments were identified and confirmed by Sanger sequencing.

We then tested wild-type, Ep300 +/- (heterozygous deletion) and Ep300 −/− (homozygous deletion) INS1 832/13 cells in 5 or 25 mM glucose for 24 h. Wild-type cells were also treated with p300 inhibitor C646 at both glucose concentrations to compare the effect of the inhibitor on Txnip expression to that of CRISPR/Cas9 knockout. To rule out the possibility of off-targets by CRISPR/Cas9, we tested the effect of two Ep300 −/− clones with exon 1 deletion introduced by CRISPR/Cas9 (as shown in Fig. 3, Ep300 −/− Clone 1 and 2). Cells were collected after treatment and relative Txnip mRNA levels were measured by qPCR (Fig. 3). Basal Txnip expression at 5 mM glucose was significantly reduced by C646, as well as in Ep300 −/− clones. High glucose (25 mM) increased Txnip expression, which was greatly reduced by C646. Heterozygous Ep300 +/- had similar Txnip expression reduction compared to C646-treated wild-type. In homozygous Ep300 −/− clones, Txnip expression was significantly lower than in C646-treated wild-type or Ep300 +/- cells.

3.3. Effect of Ep300 knock-out and HDAC inhibition on histone acetylation at the promoter and the first coding region of the Txnip gene and gene expression

To study in detail the effect of Ep300 knock-out on histone acetylation in the Txnip gene, we performed chromatin immunoprecipitation (ChIP) on wild-type and Ep300 −/− cells using antibodies binding to histone 3 lysine 9 acetylation (H3K9ac) and histone 4 acetylation (H4ac) which are frequently acetylated sites in active gene transcription. Since histone acetylation is regulated by both HATs and histone deacetylases (HDACs) in a counteracting and balanced manner, to study if the effect of Ep300 knock-out on histone acetylation can be reversed by HDAC inhibition, we also treated the cells with a class I HDAC inhibitor CI994. Wild-type and Ep300 −/− cells were incubated 24 h in 5 and 25 mM glucose, in the presence and absence of CI994. ChIP-quantitative PCR (ChIP-qPCR) for H3K9ac and H4ac enrichment was performed on sheared and precipitated DNA from the cells using primers flanking the promoter region and the first coding region of the Txnip gene (Fig. 4 A–F). We observed that at 5 mM glucose, CI994 increased H3K9ac and H4ac at promoter in both wild-type and Ep300 −/− cells to similar extend (Fig. 4B and C). In the first coding region, CI994 increased H3K9ac and H4ac in the wild-type, but only H3K9ac in the Ep300 −/− cells (Fig. 4E and F). High glucose significantly increased H3K9ac and H4ac in the Txnip gene in the wild-type cells, and this increase was greatly reduced in the Ep300 −/− cells (Fig. 4B–F). In both wild-type and Ep300 −/− cells, high glucose increased H4ac at the promoter (Fig. 4C), H3K9ac and H4ac at the first coding region (Fig. 4E and F). While high glucose-increased H3K9ac at the promoter was only observed in the wild-type but not in Ep300 −/− cells. Surprisingly, in wild-type, CI994 decreased H4ac at the promoter (Fig. 4C), H3K9ac and H4ac at the first coding region (Fig. 4E and F); but had no effect on H3K9ac at the promoter (Fig. 4B). In the Ep300 −/− cells, CI994 increased H3K9ac at the promoter (Fig. 4B), and had no effect on any other acetylation events examined (Fig. 4C–F).
To study if the observed changes in acetylation had any impact on Txnip gene expression, we performed qPCR on cells collected from the same experiments as in ChIP. C1994, 25 mM glucose, or in combination, significantly increased Txnip gene expression in both wild-type and Ep300 −/−, but to a much reduced extend in the Ep300 −/− cells (Fig. 4G).

3.4. An HDAC inhibitor decreased p300 binding to the Txnip promoter by increasing p300 autoacetylation

Previous studies have shown that there are multiple regions of p300 susceptible to autoacetylation by p300 (Stiehl et al., 2007), and p300 undergoes a dynamic cycle of autoacetylation by itself and deacetylation by HDACs (Black et al., 2008). Conformational changes caused by autoacetylation will change binding affinity of p300 to gene promoter. We therefore speculate that the reduction of H3K9ac and H4K9 at high glucose in the presence of HDAC inhibitor C1994 (Fig. 4C, E and F) is due to increased acetylated p300 and thereby decreased binding to the Txnip promoter. To test this hypothesis, we first performed western blotting using antibody binding to acetylated p300 (ac-p300) on INS1 832/13 cells treated with 5 and 25 mM glucose, in the presence and absence of C1994. Ac-p300 was significantly lower in high glucose compared to low glucose, and C1994 increase ac-p300 at both glucose concentrations (Fig. 5A). Next, we studied p300 binding at the Txnip promoter and first coding region by ChIP. High glucose had a tendency (p = 0.08) to increase p300 binding at the Txnip promoter, which was significantly reduced by C1994, while p300 binding at the first coding region of Txnip was relatively low and not affected by either glucose concentration or C1994 (Fig. 5B).

3.5. Reduced Txnip gene expression by Ep300 knock-out increased insulin secretion and prevented glucotoxicity by reducing apoptosis and promoting cell growth

TXNIP has been shown to act as a negative regulator of cell growth and metabolism (Dolgort et al., 2010; Parikh et al., 2007; Patwari et al., 2005) and induces beta cell apoptosis (Chen et al., 2010). To study if reduced Txnip gene expression in Ep300 −/− cells promotes insulin secretion, we treated wild-type and Ep300 −/− cells in 5 and 25 mM glucose for 24h. Insulin secretion was significantly increased in the knock-out cells at both glucose concentrations (Fig. 6) compared to the wild-type. To investigate if Ep300 knock out protects beta cells from glucotoxicity-induced apoptosis, cell apoptosis was measured in wild-type and Ep300 −/− cells treated in 5 and 25 mM glucose for 48 and 72 h. We observed that high glucose-induced cell death was greatly reduced; and cell growth was increased in Ep300 −/− cells compared to wild-type already after 48h and persisted for 72h (Fig. 7A). We performed similar cell apoptosis assay in INS1 832/13 cells treated with 5 or 25 mM glucose, with or without C646 and C1994. Both inhibitors increased cell death with less fold increase at high glucose, during

\[ p < 0.05, **p < 0.01, ***p < 0.001 \text{ vs. respective 5G (wild-type or knock out) or as indicated; } p < 0.05, **p < 0.01, ***p < 0.001 \text{ vs. respective 5G (wild-type or knock out) or as indicated; } p < 0.01, \|\|p < 0.001 \text{ vs. 25G.} \]

The values represent the mean ± SEM of four to five independent experiments.

Fig. 4. Effect of Ep300 knock-out on histone acetylation at the promoter and the first coding region of the Txnip gene and reduction in Txnip gene expression. (A) Schematic of the ChIP-qPCR primer location is indicated by large arrow. Primer 1 locates at ~600bp from the +1 transcription start site of Txnip gene. Exons are presented as filled rectangles. (B, C) Wild-type cells (white bars) and Ep300 −/− cells (black bars) were incubated in 5 (5G) or 25 mM glucose (25G), in the presence or absence of HDAC inhibitor C1994 (25 μM) for 24 h. Cells were then fixed and sonicated. DNA was precipitated using antibodies binding to H3K9ac (B) or H4ac (C). Quantitative PCR was run using primers flanking the promoter region as indicated in (A), and results are presented as fold change over 5G. The values represent the mean ± SEM of four to five independent experiments.
which condition p300 activity increases and is more susceptible to inhibition. Therefore the lower fold change suggests a protective effect of the inhibitors at high glucose (Fig. 8).

4. Discussion

Increased TXNIP gene expression in pancreatic islets is considered a hallmark of glucotoxicity and a mediator of the negative effects of glucose on beta-cells. The novel finding of this work was the demonstration of involvement of HAT p300 and HDACs on TXNIP gene expression via histone acetylation in pancreatic beta cells. We investigated in detail by applying pharmacological inhibitors of p300 and HDACs, as well as silencing of Ep300 gene using CRISPR/Cas9.

Previous studies have shown that high glucose increases acetylation at H4ac in the promoter region of the TXNIP gene (Chamolstad et al., 2009a). However, it has not been demonstrated if glucose-induced TXNIP gene expression can be reversed by targeting at HAT. Here we provide evidence that glucose-stimulated Txnip gene expression is dependent on p300-facilitated histone acetylation. Beside pharmacological inhibitor, we also applied p300 silencing by...

Fig. 5. Increased acetylated p300 and decreased p300 binding at Txnip promoter by CI994. (A) INS1 832/13 cells were incubated 24 h in 5 (5G) or 25 mM glucose (25G) with (n = 5, grey bars) or without CI994 (n = 5, white bars). Cells were then collected for western blotting using antibody binding to acetylated-p300 (A); or ChIP using antibodies binding to p300 (B). ChIP PCR was run using primers flanking the promoter and 1 st coding region, and results are presented as p300 binding % of input. The values represent the mean ± SEM. **p < 0.001 vs. 5G or as indicated; 'p < 0.05, †††p < 0.001 vs. 25G.

Fig. 6. Increased insulin secretion in Ep300 knock-out cells. Wild-type cells (n = 12, white bars) and Ep300 −/− cells (n = 12, black bars) were incubated in 5 (5G) or 25 mM glucose (25G) for 24 h. Insulin secretion was then determined by Mercodia High Range Rat Insulin ELISA kit (Mercodia AB). The values represent the mean ± SEM. *p < 0.05, **p < 0.01 vs. wild-type 5G or as indicated.

Fig. 7. Reduced cell apoptosis and promotion of cell growth in Ep300 knock-out cells. Wild-type cells (n = 6, white bars) and Ep300 −/− cells (n = 6, black bars) were incubated in 5 (5G) or 25 mM glucose (25G) for 48 or 72 h as indicated. (A) Cell apoptosis was then determined by Cell Death Detection ELISA kit (Roche). The values represent the mean ± SEM. ***p < 0.001 vs. 5G (wild-type or knock-out, respectively); ▼▼▼p < 0.001 vs. wild-type of the same condition. (B) Cells number was counted by automated cell counter (Origo). The values represent the mean ± SEM. ***p < 0.001 vs. 5C (wild-type or knock-out, respectively); ▼▼▼p < 0.001 vs. respective wild-type.
glucose (25 mM) increased histone acetylation in both wild-type and *Ep300−/−*, the latter had markedly reduced acetylation levels (Fig. 4b–f), indicating high glucose-increased histone acetylation in *Txnip* gene is largely dependent on p300. This was further supported by the finding that in *Ep300−/−* cells, glucose-increased histone acetylation was not affected by addition of CI994. A surprising observation was that in the wild-type, CI994 significantly decreased high glucose-increased histone acetylation (Fig. 4B–F). It has been shown that p300 is susceptible to autoacetylation by itself (Stiehl et al., 2007), and deacetylation by HDACs (Black et al., 2008). Conformational changes caused by autoacetylation and deacetylation will therefore change binding affinity of p300 to various complexes. Silencing of HDAC SIRT2 has been shown to increase p300 autoacetylation and thereby decrease p300 recruitment to the luciferase promoter and decrease downstream gene expression (Black et al., 2008). Here in our study, reduction of glucose-increased histone acetylation by CI994 was absent in *Ep300−/−*, suggesting that the inhibitory effect of HDAC inhibitor CI994 on histone acetylation at high glucose is p300 dependent. Furthermore, we provided evidence that p300 acetylation is decreased by high glucose and increased by HDAC inhibitor (Fig. 5A). The latter resulted in decreased p300 binding to the *Txnip* promoter (Fig. 5B); and as a consequence, reduced histone acetylation (Figs. 4 and 9). Although *Txnip* gene expression was also increased by CI994 at high glucose in the wild-type (Fig. 4G), the fold change (2.9-fold) was less than that seen in *Ep300−/−* (4.2-fold).

In this study, we investigated changes in histone acetylation marks H3K9ac and H4ac. However, there are many more histone marks that are susceptible to acetylation, such as H2AK5, H2BK8, H3K14, H3K18, H3K23 etc. At 5 mM glucose, *Ep300−/−* decreased; and HDAC inhibitor CI994 increased *Txnip* gene expression, but had no significant effect on H3K9ac and H4ac. This may be a consequence of modification of other histone acetylation marks. Previous studies have shown that acetyl-CoA concentrations alter the histone acetylation pattern by altering p300 specificity (Henry et al., 2015). Free energy analysis has shown that H3K9 has the least p300 affinity among histone acetylation marks at low acetyl-CoA levels, while having the highest affinity at high acetyl-CoA concentrations generated by metabolism of glucose. Therefore we speculate that the changes in H3K9ac and H4ac in association with gene expression at low/high glucose may be due to changes in p300 specificity and affinity to the modification sites.

Taken together, glucose-induced *Txnip* gene expression is greatly reduced by p300 silencing, and *Ep300−/−* cells are protected from high glucose-induced cell death and have elevated insulin secretion. Since expression of both *Ep300* and *Txnip* is elevated in human diabetic islets, prevention of glucose-induced histone acetylation could present a new therapeutic strategy to protect pancreatic beta cells from glucotoxicity.

**Duality of interest**

The authors declare that there is no duality of interest associated with this manuscript.

**Author contributions**

P.B. performed in vitro experiments and analysis. D.A. performed in vitro experiments and analysis. C.I. performed in vitro experiments and analysis. R.A. performed in vitro experiments and analysis. J.D. performed in vitro experiments and analysis. E.O.L. performed in vitro experiments and analysis. J.W. performed in vitro experiments. L.G. designed and supervised all parts of the study and drafted the report. Y.D.M. designed the study, performed in vitro experiments and analysis, supervised all parts of the study.


and drafted the report. All researchers took part in the revision of the report and approved the final version.

Acknowledgements

The work was supported by grants to Leif Groop from the Swedish Research Council: a project grant (Nr 2010-3490) and a Centre of Excellence Grant to the Lund University Diabetes Centre (Nr 2008-6589), and by the European Union’s Seventh Framework. This work was also supported by grants from the Hjelt Foundation.

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