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

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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 *Txnip* 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; Oslowski 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.

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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 hyperacetylation 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-increased *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/Cas 9-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′-AGATGAGAGTTTAGGCCGCT-3′ and 5′-GCGTCCGCCAGCGATGGCAC-3′). Guide sequence oligos were cloned into plasmid pX330-U6-Chimeric_BB-CBh-hSpCas9 (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. Lysates 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 AATGTTCCCCAACCTCACAG and reverse CTTCGTTCCATGCCCTATGT); and the first coding region of the gene (forward primer CGAGTCAAAGCCGTCAGGAT and reverse TTCATAGCGCAAGTAGTCCAAGGT). The DNA quantitation value of each sample was analyzed by the $2^{-D\Delta Ct}$ 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 Mercodia High Range Rat Insulin ELISA kit (Mercodia AB), and nor-

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