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MOLECULAR METABOLISM

Deletion of histone deacetylase 3 in adult beta cells improves glucose tolerance via increased insulin secretion

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ABSTRACT

Objective: Histone deacetylases are epigenetic regulators known to control gene transcription in various tissues. A member of this family, histone deacetylase 3 (HDAC3), has been shown to regulate metabolic genes. Cell culture studies with HDAC-specific inhibitors and siRNA suggest that HDAC3 plays a role in pancreatic β-cell function, but a recent genetic study in mice has been contradictory. Here we address the functional role of HDAC3 in β-cells of adult mice.

Methods: An HDAC3 β-cell specific knockout was generated in adult *MIP-Cre*ERT transgenic mice using the Cre-loxP system. Induction of HDAC3 deletion was initiated at 8 weeks of age with administration of tamoxifen in corn oil (2 mg/day for 5 days). Mice were assayed for glucose tolerance, glucose-stimulated insulin secretion, and islet function 2 weeks after induction of the knockout. Transcriptional functions of HDAC3 were assessed by ChIP-seq as well as RNA-seq comparing control and β-cell knockout islets.

Results: HDAC3 β-cell specific knockout (HDAC3βKO) did not increase total pancreatic insulin content or β-cell mass. However, HDAC3βKO mice demonstrated markedly improved glucose tolerance. This improved glucose metabolism coincided with increased basal and glucose-stimulated insulin secretion *in vivo* as well as in isolated islets. Cistromic and transcriptomic analyses of pancreatic islets revealed that HDAC3 regulates multiple genes that contribute to glucose-stimulated insulin secretion.

Conclusions: HDAC3 plays an important role in regulating insulin secretion *in vivo*, and therapeutic intervention may improve glucose homeostasis.

Keywords HDAC3; Glucose tolerance; Insulin secretion

1. INTRODUCTION

Glucose homeostasis is a tightly controlled process that is critical for normal physiology, which is maintained by pancreatic endocrine cells secreting peptide hormones and peripheral tissues responding accordingly. Specifically, the role of the β-cell in the pancreas is to secrete insulin in response to glucose, but dysfunction can lead to diabetes mellitus. Type 1 diabetes mellitus is of growing clinical significance in the population and is hallmarked by autoimmune recognition of pancreatic β-cells [1]. This proinflammatory environment created by the immune system leads to impaired function and death of the insulin producing β-cells of the pancreas, whereas type 2 diabetes mellitus is characterized by insulin resistance and failure of the insulin secreting β-cells, classically associated with obesity and low-grade inflammation [2,3]. Growing evidence suggests that changes in transcription, regulated by epigenetic changes may play a larger role in the pathogenesis of both these diseases than previously appreciated [4].

Histone deacetylase 3 (HDAC3) is a member of the class I HDACs. It functions as part of multi-protein complexes that deacetylate histone tails, thereby modifying chromatin structure and resulting in gene repression. HDAC3 has been shown to form stable complexes *in vivo* with, and be activated by, the nuclear receptor corepressor (NCoR1) and the silencing mediator for retinoic acid and thyroid hormone receptors (SMRT) [5,6]. Class I HDACs are ubiquitously expressed and have been implicated in regulation of metabolic gene signatures [7]. In the past several years, multiple studies of siRNA knockdown and pharmacological inhibition of HDAC3 have suggested a role for HDAC3 in β-cells, with loss of HDAC3 function protecting β-cells from cytokine-induced apoptosis and helping to maintain proper glucose-stimulated insulin secretion [8–12]. Furthermore, an HDAC3-specific inhibitor was reported to improve glucose homeostasis and insulin secretion in a diabetic rat model [11]. To determine the physiologic role of HDAC3 in β-cells, we applied mouse genetics to conditionally ablate HDAC3 *in vivo*. Here we demonstrate that the deletion of HDAC3 in β-

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Received October 31, 2016 • Revision received November 14, 2016 • Accepted November 17, 2016 • Available online xxx

http://dx.doi.org/10.1016/j.molmet.2016.11.007

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cells of adult male mice improves glucose tolerance by increasing insulin secretion.

2. METHODS

2.1. Animal studies

The *MIP-Cre*ERT and HDAC3$^{f/f}$ lines have previously been described [13,14]. Mice were maintained on a C57BL/6 background and normal chow unless otherwise noted. Analyses were restricted to male mice. Tamoxifen (Sigma T5648) was dissolved in corn oil at 20 mg/mL and administrated at 2 mg/day via gavage for five days. Animals were assayed 2 weeks after tamoxifen induction. Intraperitoneal glucose tolerance tests (IPGTTs) were performed as previously described [15]. Glucose-stimulated insulin secretion (GSIS) assays were performed by administering mice a bolus of glucose (3 g/kg) following a 16 h fast. Plasma was separated using heparinized tubes, and insulin and C-peptide were measured using ELISA kits (Crystal Chem #90080 and #90050, respectively). Total pancreatic insulin and glucagon content were determined by radioimmunoassay (RIA) in which acid–ethanol extractions were performed on whole pancreata (EMD Millipore). All the animal care and use procedures followed the guidelines of the Institutional Animal Care and Use Committee of the University of Pennsylvania in accordance with the guidelines of the US National Institutes of Health.

2.2. Immunohistochemistry and immunofluorescence

Pancreata were dissected, weighed, fixed in 10% formalin for 16 h at 4°C, washed with PBS, and embedded in paraffin. Tissue sections were stained as previously described using HDAC3 (H-99 sc-11417), Insulin (ab7842), and Glucagon (N-17 sc-7780) antibodies [15]. Insulin immunohistochemistry (IHC) was performed as previously described [15]. Insulin signal was detected with Vectastain Elite ABC kit (standard; Vector, PK6100) and DAB Peroxidase Substrate Kit (Vector, SK4100). To quantify β-cell mass, sections were digitally scanned using an Aperio ScanScope CS2 and analyzed using ImageScope as previously described [16].

2.3. Islet isolation and static incubations

Islets were isolated using the standard collagenase (EC 3.4.24.3 Serva, 17449) digestion protocol as previously described [17]. For static incubations, islets were cultured for 3 days and then transferred to KREBS buffer. An equal number of islets were glucose starved for 30 min and then glucose-stimulated for 40 min. Supernatants were collected and insulin measurements performed by RIA.

2.4. RNA analysis

RNA was immediately extracted from isolated mouse islets two weeks after tamoxifen induction, and quantitative reverse transcription-PCR (RT-PCR) was performed as described [18], using primers as

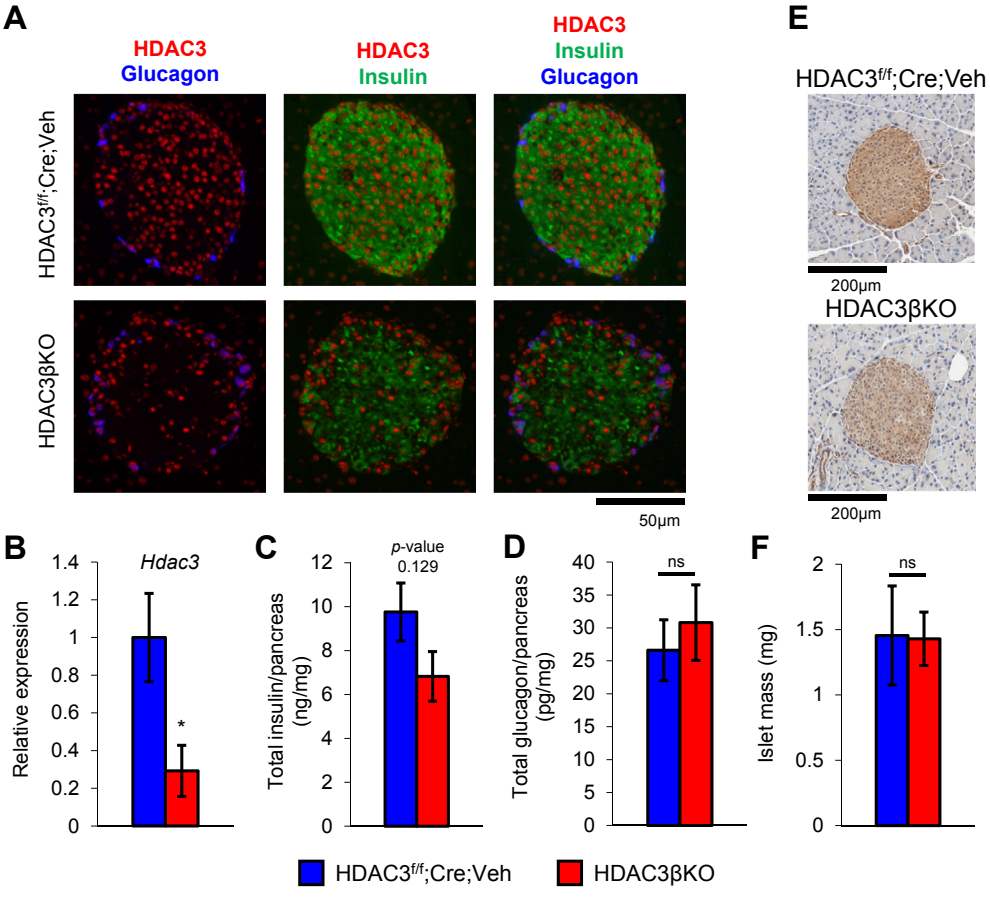

Figure 1: HDAC3 β-cell KO does not increase insulin content or β-cell mass. (**A**) Co-immunofluorescence for HDAC3, Insulin, and Glucagon (20×). (**B**) Quantitative RT-PCR of freshly isolated islets (n = 5). (**C, D**) Total pancreatic insulin and glucagon content normalized to pancreatic weight (n = 4–6). (**E**) Insulin immunohistochemistry (IHC) staining (20×). (**F**) β-Cell mass quantified from insulin IHC staining (n = 4). All error bars, s.e.m. (t-test, *$p < 0.001$).

