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RESEARCH PAPER

Class IIa HDACs do not influence beta-cell function under normal or high glucose conditions

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ABSTRACT

Inhibiting Class IIa Histone Deacetylase (HDAC) function is a promising approach to therapeutically enhance skeletal and cardiac muscle metabolic health in several chronic diseases including type 2 diabetes. However, the importance of Class IIa HDACs in the beta-cell remains unknown. As beta-cell function is vital to maintaining glycaemia it is essential that the importance of Class IIa HDACs in the beta-cell is determined. Here we used the INS-1E cell line cultured in normal glucose (11.1 mM) or hyperglycaemic (20 mM) conditions for 48 hrs to represent cells in a normal and diabetic environment respectively. Cells cultured in high glucose showed significantly reduced insulin secretory function and increased apoptotic signalling compared to cells cultured in normal glucose. Class IIa HDACS, HDAC-4 and −5, were not regulated at the transcript or protein level under normal or hyperglycaemic conditions suggesting that they may not play a role in beta-cell dysfunction. Furthermore, overexpression of wild-type HDAC-4 and −5 or dominant negative HDAC-4 and −5 did not alter insulin secretion, insulin mRNA expression or apoptotic signalling under normal or hyperglycaemic conditions. This suggests that Class IIa Histone Deacetylases do not play an important physiological role in the beta-cell under normal or diabetic conditions. Thus, Class IIa Histone Deacetylase inhibitors are not likely to have a detrimental effect on beta-cells supporting the use of these inhibitors to treat metabolic diseases such as type 2 diabetes.

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Introduction

Diabetes is characterised by two defects based around the blood glucose lowering hormone insulin: (1) insulin resistance, where the tissues such as muscle do not respond to insulin adequately and (2) beta-cell failure, where the insulin-producing beta-cells in the pancreas don't produce enough insulin. One area of research within type 2 diabetes, specifically insulin resistance, which has been particularly promising is the therapeutic potential of inhibiting the Class IIa Histone Deacetylases (HDACs) HDAC-4 and HDAC-5.

Class IIa HDACs lack intrinsic HDAC catalytic activity but repress gene expression by associating with the myocyte enhancer factor-2 (MEF2) transcription factors and recruiting a transcriptional corepressor complex to MEF2-dependent genes.[1] We have shown that disruption of the Class IIa HDAC corepressor complex (class IIa HDACs HDAC4 and HDAC5 interacting with HDAC3, nuclear receptor corepressor 2 (SMRT) and nuclear receptor corepressor 1 (Ncor)) is a promising therapeutic target to enhance metabolic health in chronic diseases including type 2 diabetes as well as obesity, cardiovascular disease and non-alcoholic fatty liver disease.[2,3] This corepressor complex is naturally disrupted in skeletal muscle during exercise due to phosphorylation-dependent nuclear export of HDAC4 and HDAC5.[4] Disruption of this complex in skeletal muscle by expressing active site mutants of HDAC4 and HDAC5 (that act in a dominant negative manner), results in increased MEF2-dependent transcription and increased expression of genes involved in several metabolic pathways as well as mitochondrial function.[2,4] Therefore, pharmacological targeting of the Class IIa HDAC corepressor complex is a potential therapeutic strategy to enhance metabolic health in chronic diseases. However, while the effect of disrupting this complex in skeletal muscle has been well validated, the effect on the beta-cell is currently unknown. Given that beta-cell function plays such a vital role in type 2

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diabetes, it is important that the effects of disrupting the Class IIa HDAC corepressor complex specifically on beta-cells is determined.

Some previous work has identified a role for Class IIa HDACs in the pancreas. HDAC4 and HDAC5 have been determined to be regulators of beta and delta cell lineage.[5] The absence of HDAC5 during embryonic development results in an increase in beta-cells, while the absence of HDAC4 results in an increase in delta-cells.[5] Conversely increased expression results in a decrease in these cell types.[5] It is unknown whether these results are due to the activity of HDAC4 and HDAC5 as a complex with HDAC3, SMRT and Ncor. Nonetheless, this research indicates that pharmacological disruption of Class IIa HDAC activity during development may be beneficial to enhancing endocrine mass and provide a more robust insulin secretory capacity in adulthood, which would be beneficial in maintaining glycaemia and preventing/delaying type 2 diabetes. However, the effect of disrupting HDAC4 and HDAC5 in adulthood is unknown. As this is time point at which Class IIa HDAC corepressor complex inhibitors would be administered, it is important to determine what, if any, effect the class IIa HDACs have on adult beta-cells.

In the beta-cell specifically, HDAC-1 and −3 are currently key targets for drug development.[6] The broad-spectrum HDAC inhibitors suberoylanilide hydroxamic acid (SAHA; also known as vorinostat) and trichostatin A (TSA) have been shown to protect from cytokine-induced apoptosis in INS-1 beta-cells.[7] Further, the pan HDAC inhibitor, Givinostat, in combination with CD3 antibody treatment, improved beta-cell survival in a mouse model of type 1 diabetes.[8] A general HDAC inhibitor (that induced hyperacetylation of the Txnip gene) also protected against glucotoxicity in INS-1 beta-cells in culture.[9] More specifically, a HDAC3 inhibitor preserved beta-cells by decreasing infiltration of mononuclear cells in a type 1 diabetes model[10] and improved beta-cell function and mass in the Zucker diabetic fatty rat model.[11] Further, this inhibitor was able to decrease cytokine- and glucose-induced apoptosis in INS-1E beta cells *in vitro*.[12] HDAC3 knock down in INS-1E cells with siRNA also protected against cytokine-induced apoptosis.[13] Therefore there is adequate evidence to conclude that HDAC3 plays a role in cytokine-induced apoptosis and some evidence that it may also play a role in apoptosis induced by high glucose. However, it remains unknown whether these effects are due to the inhibition of HDAC3's intrinsic activity or the inhibition of HDAC3 acting in the Class IIa HDAC corepressor complex along with HDAC-4 and −5. Further, the role of HDAC-4 and −5 in the adult beta-cell remains unknown.

Given that HDAC-4/5 play an important metabolic role in skeletal muscle, and that their partner HDAC-3 plays an important role in apoptosis in the beta-cell, it is possible that HDAC-4/5 may be important regulators of beta-cell metabolism, apoptosis and survival. As HDAC-4/5 inhibitors are currently under development for the treatment of several metabolic disorders, elucidating the role of HDAC-4/5 and the effect of their inhibition on the beta-cell is vital. In this study we investigated the regulation of HDAC-4 and −5 under normal and diabetic conditions, as well as the effect of increasing and inhibiting their activity to determine whether HDAC-4 and −5 inhibition is likely to be beneficial or detrimental to the beta-cell.

Results

To determine whether the expression of Class IIa HDACs, HDAC-4 and HDAC-5, are altered during beta-cell failure, INS-1E beta cells were used as a model. When INS-1E cells were cultured in normal conditions, insulin secretion was increased more than 2.5 fold in response to stimulatory glucose concentrations (Figure 1(a)). When INS-1E cells were maintained in high glucose culture conditions (20 mM) for 48 hrs they exhibited a reduced response to stimulatory conditions compared to those cultured in normal control conditions (Figure 1(a)), thereby mimicking a diabetes-like phenotype. Under these conditions HDAC-4 and HDAC-5 did not show any difference in expression at either the RNA or protein level (Figure 1(b, c)). As high fat conditions also contribute to the diabetic milieu we determined HDAC-4 and HDAC-5 mRNA and protein levels in response to a high palmitate environment as well as a combined high glucose and palmitate environment. In response to high palmitate HDAC-4 and HDAC-5 did not change at the level of mRNA ($p = .53$ and 0.40 respectively, $n = 4$) or protein ($p = .75$ and 0.76 respectively). In response to high glucose and palmitate HDAC-4 and HDAC-5 also did not change at the

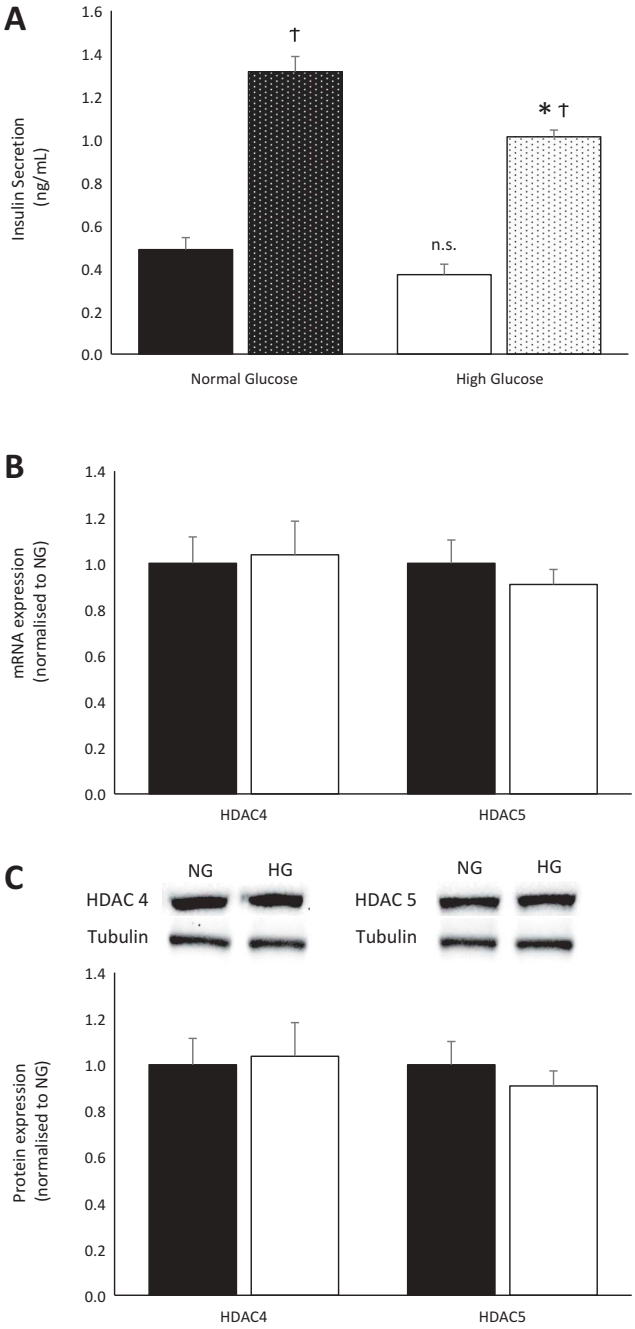

Figure 1. (a) Insulin secretion in response to basal (solid bars) and stimulatory (dotted bars) glucose conditions, (b) HDAC-4 and −5 mRNA expression and (c) representative images of HDAC-4, HDAC-5 and tubulin (loading control) western blots and HDAC-4 and −5 protein expression in INS-1E cells cultured for 48 hrs in normal (black bars) or high glucose (white bars) conditions. *$p < .05$ and n.s. not significant vs. normal glucose, †$p < .05$ vs. basal. N = 4 independent experiments with insulin secretion performed on 3 replicates for each condition.

mRNA (p = .40 and 0.41 respectively) or protein (p = .36 and 0.69 respectively) level.

While HDAC-4 and HDAC-5 did not change in response to high glucose and/or high palmitate, their activity may still impact beta-cell function and survival. In order to determine whether HDAC-4 and HDAC-5 can affect beta-cell function (insulin secretion) or survival (as measured by apoptotic signalling), INS-1E cells were transfected with WT HDAC-4 and HDAC5 and treated with normal and high glucose conditions. Transfected cells showed a high level of overexpression of both HDAC-4 and HDAC-5 (Figure 2(a)). High glucose significantly decreased insulin secretion (Figure 2(b)) while apoptotic signalling was significantly increased (Figure 2(c)). However, neither insulin secretion (Figure 2(b)) nor apoptotic signalling (Figure 2(c)) was altered by HDAC-4 and −5 overexpression compared with control cells. This indicates that increased HDAC-4 and −5 has no effect on beta cell function or survival under these conditions.

In order to determine whether disruption of the IIa HDAC corepressor complex could have a detrimental effect on beta-cells, INS-1E cells were transfected with DN HDAC-4 and HDAC-5 before beta-cell function and survival was examined under both control and high glucose conditions. Both DN HDAC-4 and −5 showed high levels of overexpression in the transfected cells (Figure 2(a)). The expression of DN HDAC-4 and −5 did not significantly alter insulin secretion (Figure 2(b)) or apoptosis (Figure 2(c)) under control or high glucose conditions. There was a visual trend for increased apoptotic signalling under both normal and high glucose concentrations with increased WT HDAC-4/5 however this was not statistically significant (p = .13 and 0.11 respectively). However when comparing DN HDAC-4/5 to WT HDAC-4/5 there was decreased apoptotic signalling under high glucose concentrations.

Lastly, as beta-cell failure and high glucose culture of beta-cell is well known to reduce insulin mRNA levels we also determined whether overexpression of WT or DN HDAC4 and −5 was associated with a change in insulin mRNA levels. High glucose was associated with a decrease in insulin mRNA in control, WT HDAC4/5 and DN HDAC4/5 transfected cells (Figure 2(d)). However there was no difference with WT HDAC4/5 or DN HDAC4/5 overexpression under normal or high glucose conditions (Figure 2(d)).

Overall this suggests that HDAC-4 and −5 play no role in beta-cell function. Furthermore, disruption of

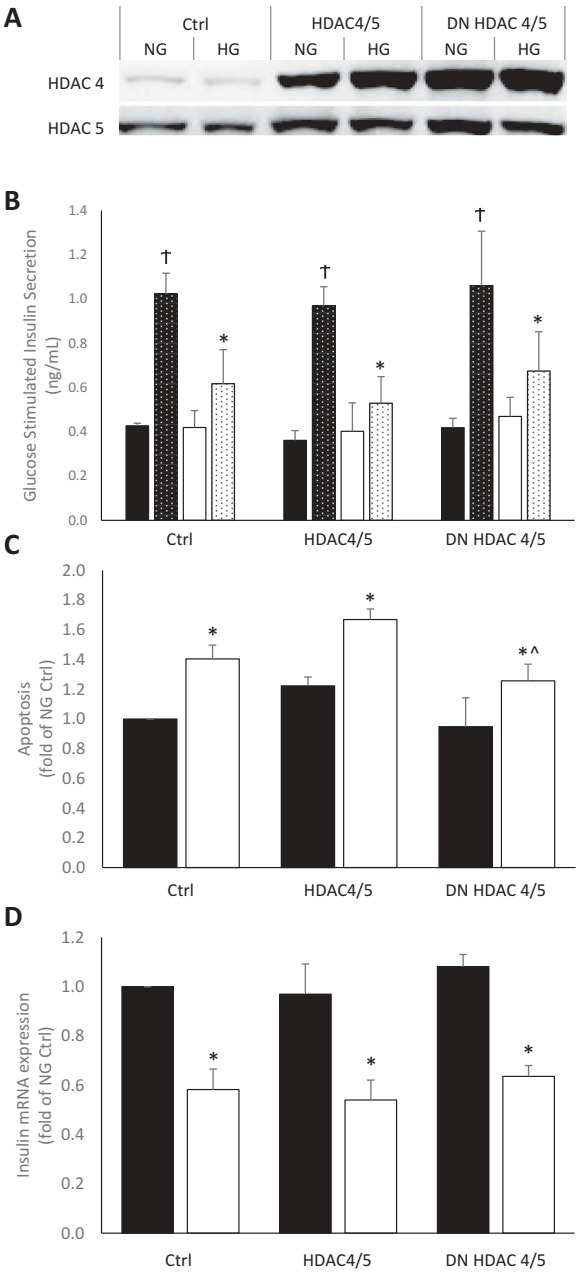

Figure 2. (a) Representative images of HDAC-4, HDAC-5 and tubulin (loading control) western blots, (b) insulin secretion in response to basal (solid bars) and stimulatory (dotted bars) glucose conditions, (c) apoptosis signalling and (d) insulin mRNA expression in INS-1E cells transfected with control plasmid (Ctrl), constitutively active HDAC 4 and HDAC-5 plasmids (HDAC4/5) or dominant negative HDAC-4 and HDAC-5 plasmids (DN HDAC4/5) and cultured for 48 hrs in normal (black bars) or high glucose (white bars) conditions. *$p < .05$ vs. control, †$p < .05$ vs. basal. N = 4–5 independent experiments with insulin secretion and apoptosis signalling performed on 3 replicates for each condition.

the IIa HDAC corepressor complex is likely to have no detrimental effect, and possibly a small positive effect to decrease apoptosis, on beta-cells.

Discussion

Class IIa HDACs have been shown to regulate islet cell lineage during development, resulting in a reduced beta-cell fraction.[5] However, their role in the beta-cell beyond the developmental stages is unclear. Here we have shown that HDAC-4 and HDAC-5 play little to no role in beta-cell function or survival in cultured INS-1E beta cells. This indicates that inhibiting HDAC-4 and HDAC-5 therapeutically is not likely to negatively impact the beta-cells. Therefore, HDAC-4/5 inhibitors under development could be viable treatments to improve metabolic health in other tissues with no deleterious effects on the beta-cell. However, it is important to note that these inhibitors will still need to have their specific effect on beta-cells carefully investigated.

In this study, HDAC-4 and HDAC-5 expression was unaltered under glucose-induced beta-cell dysfunction conditions indicating that there is no compensatory change in response to either a high-glucose environment or beta-cell dysfunction. Further, increasing their expression or inhibiting their activity had no effect on cell function or apoptosis. We believe that this indicates that while other HDACs may play important roles in the beta-cell,[6-13] HDAC-4 and HDAC-5 specifically do not on their own. Nevertheless, it must be noted that while the increased expression of HDAC-4 and HDAC-5 did not affect beta-cell function or apoptosis, they may still play as part of a complex under certain conditions. For example, it has been shown that altering the HDAC corepressor complex in skeletal muscle results in altered fatty acid oxidation but not glucose oxidation.[2] Therefore it is possible that the lack of effect on beta-cell function may be substrate specific and that fatty-acid modulated insulin secretion may be altered. However, given that inhibition of HDAC-4 and HDAC-5 activity (with DN HDAC4/5) also showed little effect, this would suggest that their activity as part of any complexes is also not physiologically important under normal or glucotoxic conditions. A minor effect may be indicated by the trend for increased apoptosis with increased HDAC4/5 and decrease apoptosis with DN HDAC4/5 however this trend is not significant. Either way, given that this possible minor effect is positive for HDAC4/5 inhibition it would only support the use of HDAC4/5 inhibitor's clinically.

Given that this study was carried out in INS-1E cells, it is possible that HDAC4/5 inhibition may have different effects *in vivo*. Cell lines, given that they are immortalised or have an increased proliferative activity, are different to native cells *in vivo*, and while the INS-1E cell line is an excellent model that shows comparable responsiveness to glucose across the physiological range,[14] it is susceptible to the inherent cell line limitations. In particular, INS-1E cells while being relatively differentiated, are still somewhat dedifferentiated compared to native beta-cells. As a major function of class IIa HDACs in skeletal muscle is to maintain dedifferentiation of myogenic precursors,[15] it is possible that the dedifferentiated state of INS-1E cells has masked the role of HDACs in beta-cells. This could be particularly relevant under hyperglycemic conditions where beta-cell dedifferentiation has been observed *in vivo*.-[16] However, other studies using INS-1E cells have shown effects of general HDAC inhibitors[9,11,12] indicating more broadly that other classes of HDACs still play important roles in this cell line suggesting this cell line is physiologically relevant. Further, the protective effects of HDAC 1, 2 and 3 inhibition against cytokine stress in INS-1E cells is transferrable to islets[11] suggesting that the roles of HDACs in INS-1E cells and islets may be equivalent. Nevertheless, further studies *in vivo* are warranted.

Overall, this study indicates that HDAC-4 and HDAC-5 may not play an important physiological role in the beta-cell and provides further evidence towards the suitability of HDAC-4/5 inhibitors for the treatment of metabolic disease.

Materials and methods

Cell Culture: INS-1E cells were maintained in RPMI 1640 media (Life Technologies) containing 11.1 mM glucose, 10% heat-inactivated foetal calf serum (In Vitro Technologies), 10 mM HEPES (Life Technologies), 1 mM Sodium Pyruvate (Life Technologies), 1% penicillin/streptomycin (Life Technologies) and 0.05 mM 2-mercaptoethanol (Sigma Aldrich) at 37 ^{0}C and 5% CO_2. For high glucose and high palmitate conditions this media was supplemented with glucose up to a final concentration of 20 mM and/or 0.1 mM palmitate (complexed to BSA at 1:3) and cells treated for 48 hrs.

Transfections: performed using Lipofectamine LTX and Plus reagent (Thermo Fischer Scientific) according to the manufacturer's instructions. Two controls groups were employed, a non-transfected group and a control empty plasmid transfected group, no statistical difference was found between the two in either of the main outcomes (insulin secretion and apoptosis) therefore only the control plasmid is shown. INS-1E cells were transfected with plasmids expressing wild type (WT) HDAC4 (pFLAG CMV5 HDAC4) and HDAC5 (pDest26 HDAC5) or mutant HDAC4 (D840N) and HDAC5 (D870N) that disrupt interactions with Ncor1 and act in a dominant negative (DN) manner.[2,17] Following an overnight transfection, cells were treated for 48 hours with normal glucose (11.1 mM) or high glucose (20 mM).

Glucose-Stimulated Insulin Secretion: as previously described.[18] Briefly treated INS-1E cells were set to basal conditions with 2 mM glucose for 30 mins followed by 45 min incubation at 2 mM (basal) or 20 mM (stimulated) glucose. The amount of insulin secreted was determined using the mouse insulin ultra-sensitive ELISA kit (ALPCO) as per the manufacturer's instructions.

Real Time PCR: Total RNA was extracted from INS-1E cells using the RNeasy Mini Kit (Qiagen), and cDNA synthesised using the Maxima H minus First Strand cDNA synthesis kit (ThermoFisher Scientific). *HDAC-4* and *HDAC-5* expression levels were determined via real-time PCR using Luminaris SYBR (ThermoFisher Scientific) and calculated as $2^{\wedge -Ct}$ and normalised to total cDNA content as measured by Quant-IT OliGreen ssDNA Assay Kit (Molecular Probes, Life Technologies). Primers; HDAC4 F: GCA GAG GTT GAG CGT GAG, HDAC4 R: AAG TTC CCA TCG TAG CG; HDAC5 F: AGC AGC GGC AGG AAG, HDAC5 R: TGT GGG AGG GAA TGG TTG AGG.

Western Blotting: as previously described.[2] Briefly INS-1E cells were collected in protein lysis buffer (0.05 M Tris, pH7.5, 0.001 M EDTA. 10% EGTA, 1% Glycerol, 0.05 M Triton X-100, 0.005 M NaF, 0.001 M NaPyrophosphate, 0.001 M Na3PO4, DTT, PIC, MilliQ H2O). 20 µg of sample was separated via SDS-PAGE (8% Acrylamide/Bis-acrylamide gel), transferred to PVDF membrane (Thermo Scientific) and

blocked with 1% BSA (Bovogen Biologicals). Primary antibodies; HDAC4 (4A3) (Cell Signalling), HDAC5 (B-11) (Santa Cruz Biotechnologies) and α-tubulin (B-512) (Sigma Aldrich), and secondary antibodies: Anti mouse (7076s) and anti-rabbit (7074s) (Cell Signalling). For visualisation, ECL HRP Chemiluminescent Substrate Reagent Kit (Invitrogen) was used.

Apoptosis: measured using the Caspase-Glo 3/7 Assay kit (Promega) as per the manufacturer's instructions.

Statistical Analysis: All data was found to be normally distributed (Kruskal-Wallis test). Groups were compared by ANOVA with LSD post-hoc analysis using SPSS software. A $p < .05$ was considered significant. All data are presented as average + Standard Error of the Mean (SEM).

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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References

1. McKinsey TA, Zhang CL, Olson EN. Control of muscle development by dueling HATs and HDACs. Curr Opin Genet Dev. 2001;11:497–504.
2. Gaur V, Connor T, Sanigorski A, Martin SD, Bruce CR, Henstridge DC, Bond ST, McEwen KA, Kerr-Bayles L, Ashton TD, et al. Disruption of the class IIa HDAC corepressor complex increases energy expenditure and lipid oxidation. Cell Rep. 2016;16:2802–2810. doi:10.1016/j.celrep.2016.08.005.
3. Gaur V, Connor T, Venardos K, Henstridge DC, Martin SD, Swinton C, Morrison S, Aston-Mourney K, Gehrig SM, van Ewijk R, et al. Scriptaid enhances skeletal muscle insulin action and cardiac function in obese mice. Diabetes Obes Metab. 2017;19:936–943. doi:10.1111/dom.12896.
4. McGee SL, Fairlie E, Garnham AP, Hargreaves M. Exercise-induced histone modifications in human skeletal muscle. J Physiol. 2009;587:5951–5958. doi:10.1113/jphysiol.2009.181065.
5. Lenoir O, Flosseau K, Ma FX, Blondeau B, Mai A, Bassel-Duby R, Ravassard P, Olson EN, Haumaitre C, Scharfmann R. Specific control of pancreatic endocrine β- and δ-cell mass by class IIa histone deacetylases HDAC4, HDAC5, and HDAC9. Diabetes. 2011;60:2861–2871. doi:10.2337/db11-0440.
6. Vetere A, Choudhary A, Burns SM, Wagner BK. Targeting the pancreatic beta-cell to treat diabetes. Nat Rev Drug Discov. 2014;13:278–289. doi:10.1038/nrd4231.
7. Larsen L, Tonnesen M, Ronn SG, Storling J, Jorgensen S, Mascagni P, Dinarello CA, Billestrup N, Mandrup-Poulsen T. Inhibition of histone deacetylases prevents cytokine-induced toxicity in beta cells. Diabetologia. 2007;50:779–789. doi:10.1007/s00125-006-0562-3.
8. Besancon A, Goncalves T, Valette F, Dahllof MS, Mandrup-Poulsen T, Chatenoud L, You S. Oral histone deacetylase inhibitor synergises with T cell targeted immunotherapy to preserve beta cell metabolic function and induce stable remission of new-onset autoimmune diabetes in NOD mice. Diabetologia. 2018;61:389–398. doi:10.1007/s00125-017-4459-0.
9. Bompada P, Atac D, Luan C, Andersson R, Omella JD, Laakso EO, Wright J, Groop L, De Marinis Y. Histone acetylation of glucose-induced thioredoxin-interacting protein gene expression in pancreatic islets. Int J Biochem Cell Biol. 2016;81:82–91. doi:10.1016/j.biocel.2016.10.022.
10. Dirice E, Ng RWS, Martinez R, Hu J, Wagner FF, Holson EB, Wagner BK, Kulkarni RN. Isoform-selective inhibitor of histone deacetylase 3 (HDAC3) limits pancreatic islet infiltration and protects female nonobese diabetic mice from diabetes. J Biol Chem. 2017;292:17598–17608. doi:10.1074/jbc.M117.804328.
11. Lundh M, Galbo T, Poulsen SS, Mandrup-Poulsen T. Histone deacetylase 3 inhibition improves glycaemia and insulin secretion in obese diabetic rats. Diabetes Obes Metab. 2015;17:703–707. doi:10.1111/dom.12470.
12. Wagner FF, Lundh M, Kaya T, McCarren P, Zhang YL, Chattopadhyay S, Gale JP, Galbo T, Fisher SL, Meier BC, et al. An isochemogenic set of inhibitors to define the therapeutic potential of histone deacetylases in beta-cell protection. ACS Chem Biol. 2016;11:363–374. doi:10.1021/acschembio.5b00640.
13. Chou DH, Holson EB, Wagner FF, Tang AJ, Maglathlin RL, Lewis TA, Schreiber SL, Wagner BK. Inhibition of histone deacetylase 3 protects beta cells from cytokine-induced apoptosis. Chem Biol. 2012;19:669–673. doi:10.1016/j.chembiol.2012.05.010.
14. Asfari M, Janjic D, Meda P, Li G, Halban PA, Wollheim CB. Establishment of 2-mercaptoethanol-

dependent differentiated insulin-secreting cell lines. Endocrinology. 1992;130:167–178. doi:10.1210/endo.130.1.1370150.

15. McKinsey TA, Zhang CL, Lu J, Olson EN. Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. Nature. 2000;408:106–111. doi:10.1038/35040593.
16. Talchai C, Xuan S, Lin HV, Sussel L, Accili D. Pancreatic beta cell dedifferentiation as a mechanism of diabetic beta cell failure. Cell. 2012;150:1223–1234. doi:10.1016/j.cell.2012.07.029.
17. Fischle W, Dequiedt F, Hendzel MJ, Guenther MG, Lazar MA, Voelter W, Verdin E. Enzymatic activity associated with class II HDACs is dependent on a multiprotein complex containing HDAC3 and SMRT/N-CoR. Mol Cell. 2002;9:45–57.
18. Aston-Mourney K, Hull RL, Zraika S, Udayasankar J, Subramanian SL, Kahn SE. Exendin-4 increases islet amyloid deposition but offsets the resultant beta cell toxicity in human islet amyloid polypeptide transgenic mouse islets. Diabetologia. 2011;54:1756–1765. doi:10.1007/s00125-011-2143-3.