SIRT6 protects against palmitate-induced pancreatic β -cell dysfunction and apoptosis

Xiwen Xiong¹, Xupeng Sun¹, Qingzhi Wang¹, Xinlai Qian¹, Yang Zhang², Xiaoyan Pan^{2,3} and X Charlie Dong²

¹Department of Forensic Medicine, Xinxiang Medical University, Xinxiang, Henan, China

²Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana, USA

³Department of Endocrinology and Metabolism, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China

Correspondence should be addressed to X Xiong or X C Dong
Email
xwxiong@xxmu.edu.cn or xcdong@iu.edu

Abstract

Chronic exposure of pancreatic β -cells to abnormally elevated levels of free fatty acids can lead to β -cell dysfunction and even apoptosis, contributing to type 2 diabetes pathogenesis. In pancreatic β -cells, sirtuin 6 (SIRT6) has been shown to regulate insulin secretion in response to glucose stimulation. However, the roles played by SIRT6 in β -cells in response to lipotoxicity remain poorly understood. Our data indicated that SIRT6 protein and mRNA levels were reduced in islets from diabetic and aged mice. High concentrations of palmitate (PA) also led to a decrease in SIRT6 expression in MIN6 β -cells and resulted in cell dysfunction and apoptosis. Knockdown of *Sirt6* caused an increase in cell apoptosis and impairment in insulin secretion in response to glucose in MIN6 cells even in the absence of PA exposure. Furthermore, overexpression of SIRT6 alleviated the palmitate-induced lipotoxicity with improved cell viability and increased glucose-stimulated insulin secretion. In summary, our data suggest that SIRT6 can protect against palmitate-induced β -cell dysfunction and apoptosis.

Key Words

- ▶ sirtuin 6
- ▶ MIN6 cells
- ▶ insulin secretion
- ▶ apoptosis
- ▶ palmitate

Journal of Endocrinology
(2016) **231**, 159–165

Introduction

The prevalence of type 2 diabetes mellitus (T2DM), a chronic metabolic disorder, has been increasing steadily all over the world (Kahn *et al.* 2006, Doria *et al.* 2008). The pathogenesis of T2DM is multifactorial, but insulin secretory deficiency due to reduced pancreatic β -cell mass or function is a major pathogenic factor (Prentki & Nolan 2006, Muoio & Newgard 2008). In the prediabetic stage, because of insulin resistance, islets respond to increased insulin demand with enhanced insulin secretion and expanded β -cell mass in order to maintain euglycemia by compensatory hyperinsulinemia. However, as T2DM ensues, β -cells exhibit decompensation, a defect that has been attributed to both β -cell dysfunction and β -cell

death (Weir & Bonner-Weir 2004). A common feature of the prediabetic stage is an excess of circulating lipids, especially saturated fatty acids, which impairs not only peripheral insulin sensitivity but also islet β -cell function (Weir & Bonner-Weir 2004). Acute exposure to elevated free fatty acids (FFA) increases β -cell proliferation and insulin secretion, whereas prolonged exposure to FFA inhibits insulin secretion and induces β -cell apoptosis (Shimabukuro *et al.* 1998, Biden *et al.* 2004). However, the underlying mechanisms responsible for the β -cell lipotoxicity remain incompletely understood.

The sirtuin protein family has seven members in mammals (SIRT1–7) (Dong 2012). SIRT6 is a

chromatin-associated deacetylase that specifically deacetylates histone H3 at lysine 9 (H3K9), lysine 18 (H3K18) and lysine 56 (H3K56) residues (Michishita *et al.* 2008, 2009, Yang *et al.* 2009, Tasselli *et al.* 2016). In recent years, SIRT6 has been identified to modulate many important cellular processes, such as DNA repair, tumor suppression, anti-inflammation and metabolism. *Sirt6*-deficient mice display severe hypoglycemia and a multisystemic aging phenotype and died around 4 weeks after birth (Mostoslavsky *et al.* 2006, Xiao *et al.* 2010). Interestingly, SIRT6 transgenic mice treated with high-fat diet secrete more insulin in response to glucose compared with their wild-type littermates (Kanfi *et al.* 2010). In our recent study, we have shown that deletion of *Sirt6* in pancreatic β -cells in mice leads to impaired glucose-stimulated insulin secretion (GSIS). We have further identified that SIRT6 regulates insulin secretion by maintaining mitochondrial function and modulating intracellular Ca^{2+} dynamics (Xiong *et al.* 2016). However, whether SIRT6 is involved in β -cell lipotoxicity remains unclear. The aim of this study was to illustrate the role of SIRT6 in palmitate (PA)-induced β -cell dysfunction and apoptosis.

Materials and methods

Cell culture and treatment

MIN6 cells between passages 15 and 20 were cultured in Dulbecco's modified Eagle's medium with 25 mM glucose supplemented with 15% fetal bovine serum, 100 U/mL penicillin, 100 $\mu\text{g}/\text{mL}$ streptomycin, 2 mM L-glutamine, and 50 mM β -mercaptoethanol. Palmitic acid (Sigma-Aldrich) was conjugated with fatty-acid-free BSA before addition to cell culture. PA was dissolved in 99% ethanol to a concentration of 100 mM, and then mixed with 10% BSA in serum-free high-glucose DMEM to make a 4 mM PA stock solution. The BSA-conjugated PA was added to MIN6 cells at a final concentration of 0.4 mM.

Adenovirus transduction

Adenoviruses carrying *SIRT6* or *GFP* were generated using pAdEasy system (Agilent) while adenoviral *Sirt6* (mouse) or *GFP* shRNAs were generated using BLOCK-iT system (Invitrogen). Adenoviruses were amplified in HEK293A cells and purified by CsCl gradient centrifugation. The viruses were titrated using

QuickTiter Adenovirus Titer Immunoassay Kit (Cell Biolabs) according to the manufacturer's protocol. Generally, we used 25–50 multiplicity of infection (MOI) for overexpression and 50–100 MOI for shRNA knockdown experiments.

Real-time RT PCR

Total islet RNA samples were prepared by using TRIzol reagent (Invitrogen) and converted into cDNA using a cDNA synthesis kit (Applied Biosystems). Real-time PCR analysis was performed using SYBR Green Master Mix (Promega) in an Eppendorf Realplex real-time PCR system.

Western blotting

Protein extracts from cells were made in lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM MgCl_2 , 1 mM EGTA) and an additional protease cocktail tablet from Roche at one tablet/10 mL final buffer volume. Protein extracts were resolved on an SDS-PAGE gel and transferred to nitrocellulose membrane (Santa Cruz Biotechnology). The membrane was incubated with the following antibodies: Sirt6 (Sigma-Aldrich), Actinin (Santa Cruz Biotechnology), Ac-H3K9, Cleaved Caspase 3 (Cell Signaling Technology). Detection of proteins was carried out by incubation with

Figure 1

SIRT6 is decreased in diabetic and aged islets. (A) Western blot analysis of SIRT6 and Ac-H3K9 in islet extracts of 5-month-old wild-type and *db/db* mice ($n=3$ per group, islets were pooled from 3 mice per genotype). (B) Western blot analysis of SIRT6 and Ac-H3K9 in islet extracts of young (2-month-old) and aged (18-month-old) mice ($n=2$ per group, islets were pooled from 3 mice per genotype).

HRP-conjugated secondary antibodies, followed by ECL detection reagents (Thermo Fisher Scientific).

Insulin secretion assay

On the day of experiment, MIN6 cells were preincubated with secretion assay buffer (SAB; 114 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4 , 1.16 mM MgSO_4 , 20 mM HEPES, 2.5 mM CaCl_2 , 25.5 mM NaHCO_3 , 0.2% BSA, pH 7.2) containing 2.5 mM glucose for 1 h. After removal of the incubation medium, cells were then incubated for 1 h in SAB containing the indicated glucose concentrations. Incubation medium was then collected and the amount of secreted insulin was analyzed using a mouse insulin ELISA kit (ALPCO). Protein concentration was determined by BCA protein assay kit (Thermo Fisher Scientific) and used to normalize insulin levels.

MTT assay

MIN6 cells were seeded in 96-well plates with 5×10^4 cells per well. The cells were exposed to 0.4 mM PA

treatment or transduced with indicated adenoviruses for the indicated periods of time. Cell viability was determined by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) kit (Sigma-Aldrich) according to the manufacturer's protocol.

Statistical analysis

All data are presented as mean $\pm$ S.E.M. Analysis was performed using 2-tailed unpaired Student's *t*-test for two-group comparisons and one-way ANOVA for multiple-group comparisons, and $P < 0.05$ was considered as significant.

Results

SIRT6 is decreased in both diabetic and aged pancreatic islets

Our recent study has shown that *Sirt6* deletion in β -cells results in β -cell dysfunction (Xiong *et al.* 2016), so we

Figure 2

Palmitate exposure decreases *Sirt6* expression in islets and MIN6 cells and leads to cell dysfunction and apoptosis. Mouse islets or MIN6 cells were treated with 1% BSA (BSA) or 0.4 mM palmitate (PA) complexed to 1% BSA. (A) Western blot analysis of SIRT6 and Ac-H3K9 in mouse islets exposed to PA for 72 h ($n=2$ per group, islets were pooled from six 2-month-old C57BL/6J male mice). (B) Real-time PCR analysis of *Sirt6* mRNA in MIN6 cells treated with PA for 48 h. (C and D) Western blot (C) and densitometric analysis (D) of SIRT6, Ac-H3K9 and cleaved caspase 3 in MIN6 cells exposed to PA at indicated time points. (E) MIN6 cell viability upon PA treatment for various time periods was analyzed by MTT assay. (F) MIN6 cells were incubated with PA for 48 h, and then GSIS was analyzed. $n=3$ per group for all the experiments. Data are presented as mean $\pm$ S.E.M. * $P < 0.05$ vs (B, D) BSA, vs (E) 0 h, vs (F) 16.7 mM BSA.

were curious about the SIRT6 status under diabetic and aging conditions. The leptin receptor mutant *db/db* mouse is a widely used type 2 diabetic mouse model. We analyzed SIRT6 protein levels in isolated islets from the *db/db* and control wild-type mice, and the data showed that SIRT6 protein levels were decreased in the *db/db* islets compared with controls (Fig. 1A). As a substrate of SIRT6, acetylated H3K9 was elevated in the *db/db* islets (Fig. 1A). Since SIRT6 has been recently identified as an important regulator of aging, we also checked whether SIRT6 is altered in the aged pancreatic islets. Indeed, SIRT6 protein levels were decreased in aged mouse islets compared with young controls (Fig. 1B).

Consistently, acetylated H3K9 was also elevated in the aged islets as expected (Fig. 1B).

Palmitate decreases *Sirt6* expression in mouse islets and MIN6 cells

As shown in Fig. 1, *Sirt6* expression is downregulated in islets from diabetic or aged mice. Since hyperlipidemia is one of the common features of type 2 diabetes, we also examined *Sirt6* expression in mouse islets exposed to PA (0.4 mM) for 72 h. SIRT6 protein levels in the mouse islets were decreased after 72 h of PA treatment relative to the controls (Fig. 2A). To further assess the effect of PA on *Sirt6* gene expression in pancreatic β -cells, MIN6 cells were treated with 0.4 mM PA. As shown in Fig. 2B, *Sirt6* mRNA levels were significantly reduced in MIN6 cells exposed to PA for 48 h. The Western blot data also confirmed that PA treatment decreased SIRT6 protein in a time-dependent manner (Fig. 2C and D). As expected, acetylation of the H3K9 residue was elevated after the PA treatment (Fig. 2C and D). It is known that lipotoxicity can ultimately lead to β -cell dysfunction and apoptosis (Sharma & Alonso 2014, Janikiewicz *et al.* 2015). To investigate the lipotoxic effect of PA on MIN6 cells, we further examined glucose-stimulated insulin secretion (GSIS) and cell viability in MIN6 cells in the presence or absence of PA. As shown in Fig. 2E, MIN6 cell viability was significantly decreased after exposure to PA for 48 h. As expected, a significant induction of cleaved caspase 3 by PA was observed. Consistent with the previous studies, although PA treatment did not reduce the basal insulin secretion in MIN6 cells, high-glucose-stimulated insulin secretion was remarkably attenuated. As shown in Fig. 2F, PA-treated MIN6 cells secreted ~30% less insulin compared with BSA-treated MIN6 cells when stimulated with 16.7 mM glucose.

Sirt6 knockdown in MIN6 cells leads to cell apoptosis and insulin secretory impairment

To verify whether *Sirt6* deficiency can lead to apoptosis in MIN6 cells, we performed *Sirt6* knockdown by transduction with *Sirt6* shRNA adenoviruses. *Sirt6* knockdown was confirmed by Western blot analysis (Fig. 3A and B). As expected, *Sirt6* knockdown in MIN6 cells increased the levels of Ac-H3K9 and cleaved caspase 3 (Fig. 3A and B). Moreover, PA treatment further exaggerated the elevation of Ac-H3K9 and cleaved

Figure 3

Knockdown of *Sirt6* increases cell apoptosis and impairs glucose stimulated insulin secretion in MIN6 cells. MIN6 cells were transduced with *shSirt6* or control *shGFP* adenoviruses for 48 h. (A and B) Western blot (A) and densitometric analysis (B) of SIRT6, Ac-H3K9 and cleaved caspase 3 in MIN6 cells. (C) MIN6 cell viability was measured by MTT assays. (D) GSIS was analyzed in control and *Sirt6* knockdown MIN6 cells. $n=3$ per group for all experiments. Data are presented as mean $\pm$ S.E.M. * $P<0.05$ vs palmitate + *shGFP*; § $P<0.05$ vs BSA + *shSirt6*.

Figure 4

Overexpression of SIRT6 protects against PA-induced cell apoptosis and GSIS impairment. MIN6 cells were transduced with SIRT6 or control GFP adenoviruses. After 4 h, the medium was changed and cells were treated with 1% BSA or 0.4 mM PA for 48 h. (A) Western blot of SIRT6, Ac-H3K9 and cleaved caspase 3. (B) Quantification of relative levels of Ac-H3K9 and cleaved caspase 3. (C) MIN6 cell viability was measured by MTT assays. (D) GSIS was analyzed in the absence or presence of 0.4 mM PA. $n=3$ per group for all the experiments. Data are presented as mean $\pm$ S.E.M. * $P<0.05$ vs palmitate + GFP.

caspase 3 induced by *Sirt6* knockdown (Fig. 3A and B). Consistently, *Sirt6*-deficient MIN6 cells exhibited decreased cell viability and impaired GSIS even without PA treatment (Fig. 3C and D).

SIRT6 protects MIN6 cells against PA-induced cell apoptosis and insulin secretory defects

To examine whether SIRT6 can protect MIN6 cells from lipotoxicity, we overexpressed SIRT6 in MIN6 cells by adenoviral transduction. Overexpression of SIRT6 in MIN6 cells under regular culture conditions exhibited no effect on cell survival and insulin secretory capacity (Fig. 4A, B, C, D). However, after 48-h PA treatment, SIRT6 overexpression resulted in reduced caspase 3 activation and enhanced cell viability (Fig. 4A, B, C). Moreover, overexpression of SIRT6 improved insulin secretory

capacity in response to glucose stimulation in PA-treated MIN6 cells compared with control GFP (Fig. 4D).

Discussion

Hyperlipidemia is known to cause β -cell dysfunction, which is characterized by impaired GSIS and increased apoptosis (Sharma & Alonso 2014, Janikiewicz *et al.* 2015). Individuals with T2DM have elevated levels of PA, which is one of the most prevalent saturated fatty acids in the circulation and has been linked to functional impairment of pancreatic β -cells (Kharroubi *et al.* 2004). Several molecular processes associated with lipotoxicity in β -cells have been reported, including endoplasmic reticulum (ER) stress, mitochondrial dysfunction, increased reactive oxygen species (ROS), elevated ceramide and impaired autophagy (Sharma & Alonso 2014, Janikiewicz *et al.* 2015).

SIRT6 is primarily characterized as a nuclear NAD⁺-dependent deacetylase of histone H3K9, H3K18 and H3K56 (Michishita *et al.* 2008, 2009, Yang *et al.* 2009, Tasselli *et al.* 2016). SIRT6 has been implicated in diverse cellular functions including anti-inflammation, metabolic homeostasis, stress resistance and tumor suppression (Kugel & Mostoslavsky 2014). In this study, we have examined the role of SIRT6 in protection against PA-induced β -cell lipotoxicity. Our data reveal that SIRT6 expression is significantly decreased not only in PA-treated MIN6 cells but also in diabetic and aged mouse islets. Knockdown of *Sirt6* in MIN6 cells increases cell apoptosis and impairs GSIS, whereas overexpression of SIRT6 protects β -cells from apoptosis and improves insulin secretory capacity in response to glucose.

We have demonstrated in our previous work that *Sirt6* knockout in pancreatic β -cells reduces ATP production and increases mitochondrial damage (Xiong *et al.* 2016). Mitochondrial oxygen consumption rates (OCR) are significantly decreased in *Sirt6*-knockdown MIN6 cells as well (Xiong *et al.* 2016). Consistent with our findings, mitochondrial defects have also been observed in *Sirt6*-knockout mouse ES cells and *Sirt6*-knockout breast cancer cells (Zhong *et al.* 2010, Choe *et al.* 2015). Chronic PA exposure induced β -cell dysfunction, and apoptosis is partly mediated by mitochondrial dysfunction (Janikiewicz *et al.* 2015). Thus, it is likely that SIRT6 may exert its protective effect against the PA-induced β -cell dysfunction through regulation of mitochondrial function.

Oxidative stress generated by fatty acid oxidation has been considered to be involved in the pathogenesis of PA-induced β -cell dysfunction and apoptosis (Janikiewicz *et al.* 2015). A recent report has shown that SIRT6 can protect human mesenchymal stem cells from oxidative stress through regulation of nuclear factor erythroid 2-related factor 2 (NRF2) (Pan *et al.* 2016). *SIRT6*^{-/-} hMSCs exhibit elevated ROS, dysregulated redox metabolism and increased sensitivity to oxidative stress. Mechanistically, SIRT6 acts as a transactivator of transcription factor NRF2 to modulate the expression of genes involved in antioxidant pathway (Pan *et al.* 2016). However, whether SIRT6 also plays an anti-oxidative stress role in pancreatic β -cells is unclear.

Autophagy, a self-degradative process, has been shown to play a protective role in the PA-induced death of β -cells (Lee 2014, Watada & Fujitani 2015). Interestingly, several studies have demonstrated that

SIRT6 functions as a positive regulator of autophagy in some cell types, such as bronchial epithelial cells, cardiomyocytes and neurons. In these cells, SIRT6 regulates the autophagy process via inhibiting the AKT activity (Takasaka *et al.* 2014, Lu *et al.* 2016, Shao *et al.* 2016). Considering a critical role of autophagy in PA-induced β -cell dysfunction, it is reasonable to postulate that SIRT6 may exert a protective effect against the PA-induced β -cell dysfunction and apoptosis through modulation of autophagy.

In summary, our data suggest that SIRT6 plays a critical role in the protection of pancreatic β -cells from lipotoxicity-induced cellular dysfunction or even cell death. These findings are significant in T2DM as dyslipidemia is often associated with the pathogenesis of this type of diabetes. A decrease in SIRT6 protein or activity in β -cells may contribute to the T2DM development. Targeting SIRT6 may be useful for therapeutic development in the treatment of T2DM.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported in part by the grant R01DK091592 (XCD) from the National Institute of Diabetes and Digestive and Kidney Diseases and the Showalter Scholar Award (XCD) from the Ralph W and Grace M Showalter Research Trust.

Author contribution statement

X X designed and carried out the study, interpreted data, analyzed data and wrote the manuscript. X S, Q W, X Q, Y Z and X P contributed to data collection. X C D conceived the hypothesis, designed the experiments, analyzed and interpreted the data, and wrote the manuscript. All authors approved the manuscript.

Acknowledgements

We want to thank Dr Debbie Thurmond (Indiana University School of Medicine, USA) for providing the MIN6 cells.

References

- Biden TJ, Robinson D, Cordery D, Hughes WE & Busch AK 2004 Chronic effects of fatty acids on pancreatic beta-cell function: new insights from functional genomics. *Diabetes* **53** (Supplement 1) S159–S165. (doi:10.2337/diabetes.53.2007.S159)
- Choe M, Brusgard JL, Chumsri S, Bhandary L, Zhao XF, Lu S, Goloubeva OG, Polster BM, Fiskum GM, Girnun GD, *et al.* 2015 The RUNX2 transcription factor negatively regulates SIRT6 expression to alter glucose metabolism in breast cancer cells. *Journal of Cellular Biochemistry* **116** 2210–2226. (doi:10.1002/jcb.25171)
- Dong XC 2012 Sirtuin biology and relevance to diabetes treatment. *Diabetes Management* **2** 243–257. (doi:10.2217/dmt.12.16)

- Doria A, Patti ME & Kahn CR 2008 The emerging genetic architecture of type 2 diabetes. *Cell Metabolism* **8** 186–200. (doi:10.1016/j.cmet.2008.08.006)
- Janikiewicz J, Hanzelka K, Kozinski K, Kolczynska K & Dobrzyn A 2015 Islet beta-cell failure in type 2 diabetes – within the network of toxic lipids. *Biochemical and Biophysical Research Communications* **460** 491–496. (doi:10.1016/j.bbrc.2015.03.153)
- Kahn SE, Hull RL & Utzschneider KM 2006 Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* **444** 840–846. (doi:10.1038/nature05482)
- Kanfi Y, Peshti V, Gil R, Naiman S, Nahum L, Levin E, Kronfeld-Schor N & Cohen HY 2010 SIRT6 protects against pathological damage caused by diet-induced obesity. *Aging Cell* **9** 162–173. (doi:10.1111/j.1474-9726.2009.00544.x)
- Kharroubi I, Ladrerie L, Cardozo AK, Dogusan Z, Cnop M & Eizirik DL 2004 Free fatty acids and cytokines induce pancreatic beta-cell apoptosis by different mechanisms: role of nuclear factor- κ B and endoplasmic reticulum stress. *Endocrinology* **145** 5087–5096. (doi:10.1210/en.2004-0478)
- Kugel S & Mostoslavsky R 2014 Chromatin and beyond: the multitasking roles for SIRT6. *Trends in Biochemical Sciences* **39** 72–81. (doi:10.1016/j.tibs.2013.12.002)
- Lee MS 2014 Role of islet beta cell autophagy in the pathogenesis of diabetes. *Trends in Endocrinology and Metabolism* **25** 620–627. (doi:10.1016/j.tem.2014.08.005)
- Lu J, Sun D, Liu Z, Li M, Hong H, Liu C, Gao S, Li H, Cai Y, Chen S, *et al.* 2016 SIRT6 suppresses isoproterenol-induced cardiac hypertrophy through activation of autophagy. *Translational Research* **172** 96–112 e116. (doi:10.1016/j.trsl.2016.03.002)
- Michishita E, McCord RA, Berber E, Kioi M, Padilla-Nash H, Damian M, Cheung P, Kusumoto R, Kawahara TL, Barrett JC, *et al.* 2008 SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. *Nature* **452** 492–496. (doi:10.1038/nature06736)
- Michishita E, McCord RA, Boxer LD, Barber MF, Hong T, Gozani O & Chua KF 2009 Cell cycle-dependent deacetylation of telomeric histone H3 lysine K56 by human SIRT6. *Cell Cycle* **8** 2664–2666. (doi:10.4161/cc.8.16.9367)
- Mostoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, Gellon L, Liu P, Mostoslavsky G, Franco S, Murphy MM, *et al.* 2006 Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell* **124** 315–329. (doi:10.1016/j.cell.2005.11.044)
- Muoio DM & Newgard CB 2008 Mechanisms of disease: molecular and metabolic mechanisms of insulin resistance and beta-cell failure in type 2 diabetes. *Nature Reviews Molecular Cell Biology* **9** 193–205. (doi:10.1038/nrm2327)
- Pan H, Guan D, Liu X, Li J, Wang L, Wu J, Zhou J, Zhang W, Ren R, Zhang W, *et al.* 2016 SIRT6 safeguards human mesenchymal stem cells from oxidative stress by coactivating NRF2. *Cell Research* **26** 190–205. (doi:10.1038/cr.2016.4)
- Prentki M & Nolan CJ 2006 Islet beta cell failure in type 2 diabetes. *Journal of Clinical Investigation* **116** 1802–1812. (doi:10.1172/JCI29103)
- Shao J, Yang X, Liu T, Zhang T, Xie QR & Xia W 2016 Autophagy induction by SIRT6 is involved in oxidative stress-induced neuronal damage. *Protein Cell* **7** 281–290. (doi:10.1007/s13238-016-0257-6)
- Sharma RB & Alonso LC 2014 Lipotoxicity in the pancreatic beta cell: not just survival and function, but proliferation as well? *Current Diabetes Reports* **14** 492. (doi:10.1007/s11892-014-0492-2)
- Shimabukuro M, Zhou YT, Levi M & Unger RH 1998 Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. *PNAS* **95** 2498–2502. (doi:10.1073/pnas.95.5.2498)
- Takasaka N, Araya J, Hara H, Ito S, Kobayashi K, Kurita Y, Wakui H, Yoshii Y, Yumino Y, Fujii S, *et al.* 2014 Autophagy induction by SIRT6 through attenuation of insulin-like growth factor signaling is involved in the regulation of human bronchial epithelial cell senescence. *Journal of Immunology* **192** 958–968. (doi:10.4049/jimmunol.1302341)
- Tasselli L, Xi Y, Zheng W, Tennen RI, Odrowaz Z, Simeoni F, Li W & Chua KF 2016 SIRT6 deacetylates H3K18ac at pericentric chromatin to prevent mitotic errors and cellular senescence. *Nature Structural and Molecular Biology* **23** 434–440. (doi:10.1038/nsmb.3202)
- Watada H & Fujitani Y 2015 Minireview: autophagy in pancreatic beta-cells and its implication in diabetes. *Molecular Endocrinology* **29** 338–348. (doi:10.1210/me.2014-1367)
- Weir GC & Bonner-Weir S 2004 Five stages of evolving beta-cell dysfunction during progression to diabetes. *Diabetes* **53** (Supplement 3) S16–S21. (doi:10.2337/diabetes.53.suppl_3.S16)
- Xiao C, Kim HS, Lahusen T, Wang RH, Xu X, Gavrilova O, Jou W, Gius D & Deng CX 2010 SIRT6 deficiency results in severe hypoglycemia by enhancing both basal and insulin-stimulated glucose uptake in mice. *Journal of Biological Chemistry* **285** 36776–36784. (doi:10.1074/jbc.M110.168039)
- Xiong X, Wang G, Tao R, Wu P, Kono T, Li K, Ding WX, Tong X, Tersey SA, Harris RA, *et al.* 2016 Sirtuin 6 regulates glucose-stimulated insulin secretion in mouse pancreatic beta cells. *Diabetologia* **59** 151–160. (doi:10.1007/s00125-015-3778-2)
- Yang B, Zwaans BM, Eckersdorff M & Lombard DB 2009 The sirtuin SIRT6 deacetylates H3 K56Ac in vivo to promote genomic stability. *Cell Cycle* **8** 2662–2663. (doi:10.4161/cc.8.16.9329)
- Zhong L, D'Urso A, Toiber D, Sebastian C, Henry RE, Vadysirisack DD, Guimaraes A, Marinelli B, Wikstrom JD, Nir T, *et al.* 2010 The histone deacetylase Sirt6 regulates glucose homeostasis via Hif1alpha. *Cell* **140** 280–293. (doi:10.1016/j.cell.2009.12.041)

Received in final form 16 August 2016

Accepted 6 September 2016

Accepted Preprint published online 6 September 2016