Deletion of PTEN in Pancreatic β Cells Protects Against Deficient β Cell Mass and Function in Mouse Models of Type 2 Diabetes

Running title: Beta cell PTEN deletion protects against type 2 diabetes

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Submitted 10 December 2009 and accepted 5 September 2010.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes. The American Diabetes Association, publisher of Diabetes, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes in print and online at http://diabetes.diabetesjournals.org.

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Objective: Type 2 diabetes is characterized by diminished function in pancreatic β cells. Insulin signaling within the β cells has been shown to play a critical role in maintaining its essential function. Under basal conditions, enhanced insulin-PI3K signaling via deletion of PTEN, a negative regulator of this pathway, leads to increased β cell mass and function. In this study, we investigated the effects of prolonged β cell-specific PTEN deletion in models of type 2 diabetes.

Research Design and Methods: Two models of type 2 diabetes were employed: high fat diet and db/db model which harbors a global leptin signaling defect. Cre-loxP system, driven by the rat insulin promoter, was employed to obtain mice with β cell-specific PTEN deletion (RIPcre+ Pten<sup>fl/fl</sup>).

Results: Islet PTEN expression was increased in type 2 diabetes. RIPcre+ Pten<sup>fl/fl</sup> mice were completely protected from hyperglycemia and glucose intolerance in both models. These mice exhibited similarly increased β cell mass under basal and diabetic conditions. Their β cell function and islet PI3K signaling remained intact, in contrast to HFD-fed wildtype and db/db islets that exhibited diminished β cell function and attenuated PI3K signaling. These protective effects in β cells occurred in the absence of compromised response to DNA-damaging stimuli.

Conclusion: PTEN exerts a critical negative effect on both β cell mass and function. Alleviating the brake through PTEN inhibition in β cells can be a novel therapeutic target to prevent the decline of β cell mass and function in type 2 diabetes.

The quintessential defects in type 2 diabetes are the development of peripheral insulin resistance and β cell dysfunction (1-3). In fact, the loss of insulin secretion in β cells in response to glucose occurs before the emergence of insulin resistance and hyperglycemia (4-6). Once insulin resistance develops, hyperglycemia, high circulating free fatty acids and inflammatory cytokines further abrogate glucose responsiveness to insulin secretion (2; 7-9). It is becoming increasingly clear that insulin/IGF-1 signaling plays an important role in the maintenance of β cell function under both basal and diabetic conditions. Mice with β cell-specific deletion of insulin-like growth factor 1 (IGF-1) receptor exhibit a defect in glucose-stimulated insulin secretion (10; 11), whereas insulin receptor deletion in β cells results in both attenuated insulin secretion in response to glucose, in addition to reduced β cell mass with aging (12; 13). Thus β cells are not only an essential source of the hormone insulin, but are also a critical target of insulin action in the maintenance of β cell function.

Phosphoinositide 3-kinase (PI3K) signaling cascade is one of the major intracellular signaling pathways through which insulin and IGF-1 mediate their effects (14). Phosphatase with tensin homology (PTEN) is a dual-specific phosphatase and a potent negative regulator of this pathway by its ability to dephosphorylate phosphatidylinositol-3,4,5-triphosphate (PIP3) to phosphatidylinositol-4,5-bisphosphate (PIP2), thereby effectively removing the critical secondary messenger of this signaling cascade (15; 16). Although PTEN was first discovered as a tumor suppressor, recent studies have highlighted the important physiological role of PTEN in metabolism (16-18). Tissue-targeted deletion
of PTEN in liver, fat or muscle lead to improved insulin sensitivity in these insulin-responsive tissues and protects mice from HFD-induced diabetes (19-22). Additionally, we and others have reported that mice with PTEN deletion in pancreatic β cells show increased β cell mass due to both increased proliferation and reduced apoptosis without compromising β cell function under basal condition (23; 24).

PTEN has been shown to be upregulated in models of insulin resistance, including a genetic model of combined ablation of insulin/IGF-1 signaling in β cells (25-27). Furthermore, in vitro over-expression of PTEN in pancreatic β cell lines showed impaired insulin secretion in response to ambient glucose (28). However, the regulation of PTEN expression in β cells in models of type 2 diabetes in vivo was unknown. We show here that PTEN expression was increased in islets of both high fat diet (HFD)-fed and db/db mice, which was accompanied by attenuation in PI3K signaling, suggesting the potential causal role of PTEN in the pathogenesis of β cell dysfunction in type 2 diabetes. In this report, we investigated the essential role of PTEN in β cells in the context of type 2 diabetes models. We used the rat insulin promoter (RIP) to drive deletion of PTEN in the Cre-loxP system (RIPcre+ Ptenfl/fl). RIPcre+ Ptenfl/fl mice were protected from HFD-induced type 2 diabetes due to their increased islet mass and proliferation with intact β cell function. β cells from RIPcre+ Ptenfl/fl mice were protected against HFD-induced β cell dysfunction both in vitro and in vivo, which can be attributed to the constitutively active PI3K signaling in their islets. Furthermore, RIPcre+ Ptenfl/fl mice in the db/db background still remained euglycemic, despite being severely insulin resistant. Interestingly, their β cell mass was not significantly different from db/db littermates. However, their β cell function and islet PI3K signaling remained intact. Together, our data highlight the critical role of β cell PTEN in the development of β cell dysfunction in type 2 diabetes and support PTEN as a potential therapeutic target for β cell growth and the preservation of its function.

**RESEARCH DESIGN AND METHODS**

Ptenfl/fl mice (exons 4 and 5 of Pten flanked by loxP sites by homologous recombination) were mated with RIPcre mice (Cre transgene under the control of the rat insulin 2 promoter TgN[ins2-cre]25Mgn from Jackson Laboratories). RIPcre+ Ptenfl/fl mice were intercrossed to generate RIPcre+ Pten+/+, RIPcre+ Pten+fl and RIPcre+ Ptenfl/fl mice. RIPcre+ Pten+/+ mice were used as controls. RIPcre+ Ptenfl/fl Lepr db/db mice were generated by breeding RIPcre+ Pten+fl mice with Lepr-db/db mice (Jackson Laboratories) to obtain RIPcre+ Ptenfl/fl Lepr+db/db mice. RIPcre+ Ptenfl/fl Lepr+db/db mice were then intercrossed to generate RIPcre+ Pten+/+ Lepr+/+ (wildtype), RIPcre+ Pten+/+ Lepr+db/db (db/db), and RIPcre+ Ptenfl/fl Lepr+db/db (-/-; db/db) mice. Only male mice were used for experiments and only littermates were used as controls. Genotypes for Cre and Pten gene were determined with PCR using ear clip DNA as described previously (19; 23). All mice were maintained on a mixed 129J-C57BL/6 background and housed in pathogen-free facility on a 12-hr light-dark cycle and fed a libitum with standard irradiated rodent chow (5% fat; Harlan Tecklad, Indianapolis, IN) in accordance with Ontario Cancer Institute Animal Care Facility protocol, without restriction on the animal activity.

**High fat diet feeding.** HFD (Dyets lard Surmwit Mouse Diet DYET# 182084; Fyets Inc., Bethlehem, PA) for RIPcre+ Ptenfl/fl and RIPcre+ Pten+/+ mice were started at 2 months of age and continued for 7 months. **Metabolic studies.** All overnight fasts were carried out between 5 pm to 10 am. All blood glucose, glucose tolerance tests, insulin
tolerance tests and glucose stimulated insulin secretion tests were performed on overnight-fasted animals as previously described (23).

**Islet perfusion assay.** Detailed perfusion protocol was described in previous publication (29). In brief, 60 islets were placed in perfusion chamber at 37°C with capacity of 1.3 ml, and perifused with Kreb-Ringer bicarbonate buffer at 1 ml/min. Islets were equilibrated with Kreb-Ringer bicarbonate HEPES buffer (2.8 mmol/l glucose) for 30 min. They were then stimulated with 2.8 mmol/l glucose for 10 min, followed by a 40 min incubation with 16.7 mmol/l glucose. Fractional insulin content was determined with radioimmunoassay kit (Linco Research, St. Louis, MO). At the end of each perfusion, islets were collected and lysed with acid ethanol for assessment of insulin content. First phase secretion was 10-25 min, second phase secretion was 25-50 min. Results were presented as insulin secreted normalized to 60 islets and to total insulin content.

**Immunohistochemistry and immunofluorescence.** Pancreas was fixed in 4% paraformaldehyde in 0.1M phosphate-buffered saline (pH 7.4) as previously described (25). For immunohistochemical and immunofluorescent staining antibodies against PTEN (NeoMarker, Fremont, CA), Akt (Cell Signaling Technology, Beverly, MA), p-Akt (Ser473) (Cell Signaling Technology, Beverly, MA), GAPDH (Cell Signaling Technology, Beverly, MA), GLUT-2, mTOR (Santa Cruz Biotechnology, Santa Cruz, CA), p-mTOR (Cell Signaling Technology, Beverly, MA), p-p53 (R&D systems), p53 (Santa Cruz Biotechnology, Santa Cruz, CA), PDX-1, and PTEN were used (antibody sources for Akt, p-Akt, GLUT-2, PTEN refer to immunohistochemistry and immunofluorescence).

**Gamma irradiation and qPCR.** Isolated islets are incubated in RPMI (10% FBS) at 37°C overnight, then irradiated with 30Gy of gamma irradiation the next day. Islets were harvested after overnight in culture. RNA was extracted with RNeasy Plus Mini Kit (Qiagen, Valencia, CA) according to protocol provided. cDNA was synthesized according to protocol published elsewhere (30). PCR was monitored in real-time using the ABI Prism 7900HT Real-time PCR system (Applied Biosystem). Experiments were performed in triplicate for each sample. Primer sequence for Mdm2, Bax, and p21 are available upon request.

**Statistics.** Data are presented as means ± standard errors of the mean and were analyzed by one-sample t test, independent-sample t test, and one-way analysis of variance with the post-hoc Tukey least significant difference test, where appropriate.
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All data were analyzed using the statistical software package SPSS (version 16.0) for Macintosh.

RESULTS

Increased PTEN expression with attenuated PI3K signaling in islets of HFD-fed and db/db mice. PTEN transcript and protein levels were measured in islets of mice with type 2 diabetes. In both HFD-induced and db/db mice, PTEN transcripts in islets were significantly increased as assessed by qRT-PCR (Fig. 1A). PTEN protein expression was also increased as demonstrated by immunohistochemistry of pancreatic sections and western blotting of isolated islet lysates (Fig. 1B and C). The increase in islet PTEN expression in both of these type 2 diabetes models was accompanied by attenuated PI3K signaling, as demonstrated by the reduction of p-Akt, p-mTOR, and p-FoxO-1 expression (Fig. 1C).

RIPcre+Ptenfl/fl mice were protected against HFD-induced diabetes. We have previously shown that RIPcre+Ptenfl/fl mice exhibit an increase in β cell mass and function under basal conditions (23). To investigate whether these positive attributes of PTEN deletion in pancreatic β cells conferred protection against type 2 diabetes, we fed these mice a prolonged HFD for 7 months. Efficient PTEN deletion in β cells, along with partial deletion in the hypothalamus, persisted in the RIPcre+Ptenfl/fl mice on prolonged HFD (Fig. 2A and B). Despite their increased weight gain upon HFD feeding, RIPcre+Ptenfl/fl mice remained remarkably euglycemic throughout the duration of prolonged HFD, in contrast to the gradual increase in blood glucose levels in control littersmates (Fig. 3A and B). They also exhibited improved glucose tolerance (Fig. 3C). The attenuation of insulin secretion in response to glucose is a characteristic β cell defect in type 2 diabetes (1-3). Indeed, this attenuation was observed in both the first and second phases of insulin secretion after in vivo glucose challenge in wildtype mice after a prolonged HFD. In contrast, insulin secretion in response to glucose was preserved in RIPcre+Ptenfl/fl mice (Fig. 3D).

Increased β cell mass and β cell size in islets of HFD-fed RIPcre+Ptenfl/fl mice. During HFD-induced diabetes, development of peripheral insulin resistance leads to a compensatory increase in β cell mass to meet the increasing demands for insulin. This compensatory proliferation was observed in RIPcre+Ptenfl/+ islets on HFD (Fig. 4A and B). RIPcre+Ptenfl/fl islets showed an already increased β cell mass under chow-fed conditions and we observed no further increase in β cell mass on HFD. The increased β cell mass was due to both an increase in proliferation and β cell size in RIPcre+Ptenfl/fl mice under both chow and HFD conditions, which likely reflects the direct effects of PTEN deletion in their β cells (Fig. 4C, E and F). Furthermore, age-matched chow- and HFD-fed RIPcre+Ptenfl/fl mice showed similarly increased proportion of large islets (Fig. 4D). Despite the increased proliferation and cellular growth in RIPcre+Ptenfl/fl islets, their architectures were maintained (Fig. 5A).

RIPcre+Ptenfl/fl islets were protected against HFD-induced β cell dysfunction. In order to assess the direct effects of PTEN deletion specifically in β cells, we measured insulin secretion during perifusion assay on isolated islets ex vivo. Under basal condition, both RIPcre+Ptenfl/+ and RIPcre+Ptenfl/fl islets demonstrated similar insulin release after glucose stimulation (Fig. 4G and H). However, after HFD feeding, RIPcre+Ptenfl/fl mice maintained robust insulin secretion in response to glucose in contrast to islets of HFD-fed RIPcre+Ptenfl/+ mice, which showed attenuation in both phases of insulin secretion (Fig. 4I and J). These data suggest that diabetes protection in HFD-fed RIPcre+Ptenfl/fl mice are likely due to both preserved β cell function and mass.
Beta cell PTEN deletion protects against type 2 diabetes

RIP<sup>cre</sup> Pten<sup>fl/fl</sup> Lepr<sup>db/db</sup> mice remained euglycemic despite severe insulin resistance. Both RIP<sup>cre</sup> Pten<sup>fl/fl</sup> Lepr<sup>db/db</sup> mice and RIP<sup>cre</sup> Pten<sup>+/+</sup> Lepr<sup>db/db</sup> littermates exhibited similar degree of weight gain and insulin resistance (Fig. 6A and D). However, despite severe insulin resistance in RIP<sup>cre</sup> Pten<sup>fl/fl</sup> Lepr<sup>db/db</sup> mice, they continued to remain remarkably euglycemic and showed normal glucose tolerance (Fig. 6B and C). Furthermore, in vivo GSIS experiment showed robust insulin secretion in response to glucose stimulation in RIP<sup>cre</sup> Pten<sup>fl/fl</sup> Lepr<sup>db/db</sup> mice (Fig. 6E). Interestingly, islets of RIP<sup>cre</sup> Pten<sup>fl/fl</sup> Lepr<sup>db/db</sup> mice demonstrated similar degrees of hypertrophy and proliferation to those of RIP<sup>cre</sup> Pten<sup>+/+</sup> Lepr<sup>db/db</sup> islets and did not show a further increase in β cell mass compared to db/db controls (Fig. 7B and C). Islets of RIP<sup>cre</sup> Pten<sup>fl/fl</sup> Lepr<sup>db/db</sup> mice showed no signs of disorganized architecture (Fig. 7A).

RIP<sup>cre</sup> Pten<sup>fl/fl</sup> Lepr<sup>db/db</sup> islets demonstrated increased β cell function. To assess the direct effects of PTEN deletion on β cell function in the absence of leptin signaling, we examined islet function ex vivo by perifusion. RIP<sup>cre</sup> Pten<sup>+/+</sup> Lepr<sup>db/db</sup> islets showed diminished insulin secretion, whereas robust first phase insulin secretion was observed in RIP<sup>cre</sup> Pten<sup>fl/fl</sup> Lepr<sup>db/db</sup> islets after glucose stimulation, further confirming the preserved glucose responsive insulin secretory function in PTEN-deficient β cells in db/db mice (Fig. 6F and G). Thus in contrast to the HFD-fed RIP<sup>cre</sup> Pten<sup>fl/fl</sup> mice, where the combination of increased β cell mass and function likely contributed to the protective effect against diabetes, increased β cell function and not mass in RIP<sup>cre</sup> Pten<sup>fl/fl</sup> Lepr<sup>db/db</sup> mice was likely responsible for their diabetes protection.

Enhanced PI3K signaling in RIP<sup>cre</sup> Pten<sup>+/+</sup> Lepr<sup>db/db</sup> islets. We next determined the effects of PTEN deletion on PI3K signaling in β cells in the db/db model.
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RIPcre^+ Pten^+/+ Lepr^{db/db} islets showed reduced levels of p-Akt, while PTEN deletion completely rescued this defect (Fig. 7A and D). Phosphorylation of mTOR and FoxO-1 also remained high in RIPcre^+ Pten^fl/fl Lepr^{db/db} islets consistent with constitutively active PI3K cascade (Fig. 7D). In keeping with their normal insulin secretion in response to glucose, GLUT2 was highly expressed in islets of RIPcre^+ Pten^fl/fl Lepr^{db/db} mice in contrast to the near-complete loss of GLUT2 expression in islets of Lepr^{db/db} mice (Fig. 7A and D). PDX-1 was also downregulated in RIPcre^+ Pten^+/+ Lepr^{db/db} islets, while the RIPcre^+ Pten^fl/fl Lepr^{db/db} islets continued to show intense expression of PDX-1 and nuclear localization of the protein to a similar degree as the wildtype islets on chow diet (Fig. 7A and D).

Tumorigenic response to DNA-damaging stimuli is not compromised in islets of RIPcre^+ Pten^fl/fl or RIPcre^+ Pten^fl/fl Lepr^{db/db} mice. Given PTEN’s role as a tumor suppressor in various cancer-prone tissues (16-18), we assessed for changes suggestive of cellular transformation and response to DNA-damaging stimuli such as gamma irradiation. Despite the significant increase in islet mass in both HFD-fed RIPcre^+ Pten^fl/fl and RIPcre^+ Pten^fl/fl Lepr^{db/db} islets, intact islet integrity was observed even in the aged mice at 9 months of age either fed HFD or on db/db background. Intact laminin staining in the basement membrane, as well as localization of β-catenin to the plasma membrane both demonstrated absence of deregulated growth (Fig. 8A). Furthermore, induction of DNA damage with gamma irradiation showed a similar degree of DNA-repair and apoptosis response in both RIPcre^+ Pten^+/+ and RIPcre^+ Pten^fl/fl islets, including phosphorylation of p53 and cleavage of MDM2 (Fig. 8C). The transcript levels of p53 target genes, including Bax, Mdm2, and p21 also showed similar changes in response to gamma irradiation between RIPcre^+ Pten^+/+ and RIPcre^+ Pten^fl/fl islets (Fig. 8B). Interestingly RIPcre^+ Pten^fl/fl islets exhibited significantly reduced expression of pro-apoptotic gene Bax under basal condition, which is consistent with their constitutively active PI3K signaling (Fig. 8B). Thus PTEN deletion in β cells protects against β cell dysfunction in both HFD and db/db models of type 2 diabetes without any finding suggestive of deregulated growth. Furthermore, PTEN deleted β cells still maintained intact response to DNA-damaging stimuli, which further suggest that they are not more prone to tumor formation.

DISCUSSION

In pre-diabetic individuals, insulin resistance in the classic metabolic tissues including the liver, muscle and fat is present; however glucose homeostasis can be maintained as long as pancreatic β cells are able to increase insulin production to compensate for the increased insulin demands (7; 34; 35). Accumulating evidence shows that a defect in insulin-PI3K signaling in pancreatic β cells may contribute to the development of β cell dysfunction leading to the onset of type 2 diabetes. Indeed, mice with genetic ablation of insulin/IGF-1 receptors specifically in the β cells demonstrate reduced β cell function (10; 12; 13). Therefore, a new paradigm of type 2 diabetes pathogenesis suggests that diminished insulin responsiveness specifically in the pancreatic β cells plays a central role in the disease development.

Interestingly, PTEN, a critical negative regulator of the PI3K signaling pathway, has been shown to be upregulated in β cells that have complete absence of insulin/IGF-1 signaling (25), leading to a hypothesis that PTEN may play a causal role in the pathogenesis of β cell dysfunction. Here, we have shown that when type 2 diabetes was induced either by HFD or global leptin signaling deficiency, increased PTEN expression was observed in islets along with diminished PI3K signaling, suggesting that β
cell PTEN may indeed play a critical role in the development of type 2 diabetes. We and others have already shown that tissue-specific increase in PI3K signaling in pancreatic β cells resulting from deletion of PTEN leads to increased β cell mass and function under basal conditions (23; 24). However, the biological outcome from continued deletion of PTEN in β cells under metabolically stressed conditions was elusive. In this report, we show that after prolonged HFD, RIP<sup>cre</sup><sup>+</sup> <i>Pten<sup>fl/fl</sup></i> mice continued to exhibit increased islet mass, and were protected against the loss of glucose-stimulation insulin secretion. The enhanced β cell function was demonstrated not only <i>in vivo</i> but also <i>in vitro</i> which support the direct effect of PTEN in β cells. However, RIP<sup>cre</sup><sup>+</sup> <i>Pten<sup>fl/fl</sup></i> mice exhibited enhanced insulin sensitivity, which may have masked the full potential effects of PTEN deletion in pancreatic β cells against metabolic stress in the HFD model.

In the db/db model, RIP<sup>cre</sup><sup>+</sup> <i>Pten<sup>fl/fl</sup></i> <i>Lepr<sup>db/db</sup></i> mice developed obesity with significantly diminished insulin sensitivity to a similar degree as the RIP<sup>cre</sup><sup>+</sup> <i>Pten<sup>+/+</sup></i> <i>Lepr<sup>db/db</sup></i> littermate controls (Fig. 6A and D). Yet, RIP<sup>cre</sup><sup>+</sup> <i>Pten<sup>fl/fl</sup></i> <i>Lepr<sup>db/db</sup></i> mice remained completely glucose tolerant and euglycemic throughout the period of seven months. Islets from RIP<sup>cre</sup><sup>+</sup> <i>Pten<sup>fl/fl</sup></i> <i>Lepr<sup>db/db</sup></i> mice demonstrated an increase in islet area to a similar degree as those of RIP<sup>cre</sup><sup>+</sup> <i>Pten<sup>+/+</sup></i> <i>Lepr<sup>db/db</sup></i> mice. However RIP<sup>cre</sup><sup>+</sup> <i>Pten<sup>fl/fl</sup></i> <i>Lepr<sup>db/db</sup></i> islets remained responsive to glucose-stimulated insulin secretion. This is likely attributed by the PI3K signaling which remained activated in these islets. As such GLUT-2 and PDX-1, which are important in glucose sensing and cell differentiation, are maintained. These data illustrate the importance of the negative regulation that PTEN exerts on β cells, whereupon PTEN deletion leads to enhanced PI3K signaling and protection against β cell dysfunction that occurs in type 2 diabetes.

Given the well-known tumorigenic effects of PTEN deletion in some tissues, we assessed for evidence of deregulated growth (16-18). PTEN deficient islets showed intact architecture and demonstrated intact DNA repair response to gamma irradiation as wildtype counterparts. Furthermore, previously we have shown that PTEN deletion in combination with the activation of an oncogene, cMyc, is still not able to lead to tumor formation (36). However, more extensive analysis and longitudinal observation are required to conclusively demonstrate the full impact of PTEN deletion in pancreatic β cells on tumorigenesis. It is worth noting that PTEN deleted β cells behave differently than β cells that express constitutively active Akt. Although the RIP-Akt transgenic mice exhibit increased islet mass, these mice have compromised β cell function (37; 38). PTEN deficient β cells, in contrast, do not show loss of function even after a prolonged exposure to HFD, despite the continued proliferation and PI3K signaling within these cells. This important distinction between PTEN deleted and Akt over-expressing β cells illustrates the fundamental difference between removing a physiological brake, which would allow for PI3K signaling to return to physiological levels, whereas forced expression of Akt may lead to a supra-physiological levels of PI3K signaling which can lead to cell proliferation and dedifferentiation. Further investigations to delineate other important genes or pathways that distinguish between these two modes of enhancing PI3K signaling may unveil clinically safe therapeutic targets for β cells.

In summary, our findings show that PTEN plays a critical role in the pathogenesis of β cell dysfunction in type 2 diabetes, and highlight the tissue-specific role of PTEN in attenuating PI3K signaling in β cells. Our results show that PTEN deletion in β cells leads to an increase in β cell mass and
function as a result of restoring PI3K signaling to a physiological level in diabetic condition. Indeed, PTEN deletion leads to a complete protection against β cell defects even in the face of severe insulin resistance, providing a genetic evidence of the critical negative regulation that PTEN exerts in impeding β cell function and growth that occurs in type 2 diabetes. Our results highlight opportunities for therapeutic targeting of PTEN in β cells for treatment or prevention of type 2 diabetes.

Author contributions. L. W. research data, contributed to discussion, wrote manuscript, reviewed/edited manuscript. Y. L. research data. S. Y. L. research data. K. T. N. research data. S. A. S. research data. A. S. research data. T. W. M. contributed to discussion. H. G. contributed to discussion. M. W. contributed to discussion, reviewed/edited manuscript.

ACKNOWLEDGMENT
L.W. is supported by Doctoral Research Award from the Canadian Institutes of Health Research (CIHR). This work is supported by grants to M.W. from CIHR and the Canadian Diabetes Association, as well as CIHR grant MOP-64464 to H.Y.G.

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FIGURE LEGENDS

FIG. 1. Islet PTEN up-regulation with concomitant attenuation of PI3K signaling in models of type 2 diabetes. A and B: PTEN transcript levels by qPCR (A) and immunohistochemical staining (B) of in chow- and HFD-fed (7 months on HFD); wildtype (+/+ ) and db/db islets (7 months of age) (n=3). C: Western blot (left panel) and quantification (right panel) of PTEN, p-Akt (Ser473), total Akt, GLUT2 and PDX-1 in chow- and HFD-fed (7 months on HFD); wildtype and db/db islets (7 months of age) (n=3). *P<0.05; **P<0.005. Scale bar, 50 µm. The results are presented as average ± standard error.

FIG. 2. PTEN deletion in RIPcre Ptenfl/fl islets. A: Immunohistochemical staining of PTEN in RIPcre Pten+/+ (+/+) and RIPcre Ptenfl/fl (-/-) islets of pancreas sections. B: Western blot (left panel) and quantification (right panel) of PTEN expression in RIPcre Ptenfl/fl islets and hypothalamus (Hyp) (n = 3). *P<0.05; **P<0.005. Scale bar, 50 µm. The results are presented as average ± standard error.

FIG. 3. RIPcre Ptenfl/fl mice showed maintained glucose metabolism and in vivo glucose stimulated insulin secretion after prolonged HFD while demonstrating drastic weight gain. A: Weight of RIPcre Pten+/+ (+/+) and RIPcre Ptenfl/fl (-/-) mice at the start of HFD (2 month of age) and after HFD (9 month of age) with chow-fed RIPcre Pten+/+ and RIPcre Ptenfl/fl mice alive at the same time points. B: Fasting blood glucose of RIPcre Pten+/+ and RIPcre Ptenfl/fl mice fed either chow or HFD for 7 months (n > 7). C: Glucose tolerance tests of RIPcre Pten+/+ and RIPcre Ptenfl/fl mice after 7 months of either chow or HFD feeding (n > 7). D: in vivo glucose stimulated insulin secretions of RIPcre Pten+/+ and RIPcre Ptenfl/fl mice after 7 months of either chow or HFD feeding (n > 3). *P<0.05. The results are presented as average ± standard error.

FIG. 4. RIPcre Ptenfl/fl mice showed maintained high islet mass and β cell size with protection against HFD-induced β cell dysfunction. A and B: Insulin staining (A) and quantification (B) of pancreas sections of RIPcre Pten+/+ (+/+) and RIPcre Ptenfl/fl (-/-) mice fed either chow or HFD (n = 3), Scale bar, 500 µm. C: Percentage of Ki67 positive cells in islets from RIPcre Pten+/+ and RIPcre Ptenfl/fl mice fed either chow or HFD (n = 3). D: proportion of small (<10 cells), medium (10-200 cells) and large (>200 cells) islet in pancreas from RIPcre Pten+/+ and RIPcre Ptenfl/fl mice fed either chow or HFD (n = 3). E and F: Immunofluorescent staining of insulin/DAPI (E) and quantification of β cell size (F) of pancreas from RIPcre Pten+/+ and RIPcre Ptenfl/fl mice fed either chow or HFD (n=3), Scale bar, 50 µm. G and H: Insulin secretion per 60 islets during perfusion analysis (G) and quantification of area under the curve (H) of chow-fed RIPcre Pten+/+ and RIPcre Ptenfl/fl islets (n=3). I and J: Insulin secretion per 60 islets during perfusion analysis (I) and quantification of area under the curve (J) of HFD-fed RIPcre Pten+/+ and RIPcre Ptenfl/fl islets (n=3). *P<0.05. The results are presented as average ± standard error.

FIG. 5. RIPcre Ptenfl/fl mice showed maintained islet PI3K signaling after prolonged HFD. A: Immunofluorescent staining of insulin/glucagon, and immunohistochemical staining of p-Akt (Ser473), total Akt, GLUT2 and PDX-1 in chow-fed, HFD-fed RIPcre Pten+/+ (+/+) and HFD-fed RIPcre Ptenfl/fl (-/-) mice. B: Western blot (left panel) and quantification (right panel) of p-Akt (Ser473), total Akt, p-mTOR (Ser2448), total mTOR, p-FoxO-1 (Ser253) and total FoxO-1,
GLUT-2 and PDX-1 of islets from RIPcre+ Pten+/+ and RIPcre+ Ptenfloflo mice fed either chow or HFD (n = 3). *P<0.05. Scale bar, 50 µm. The results are presented as average ± standard error.

**FIG. 6.** RIPcre+ Ptenfloflo Leprdb/db mice exhibit comparable weight gain and normal glucose tolerance despite being insulin resistant with normal β cell function. A: Weight of wildtype (WT), RIPcre+ Pten+/+ Leprdb/db (db/db), and RIPcre+ Ptenfloflo Leprdb/db (-/-; db/db) mice at 2 and 7 month of age (n > 7). B: Fed blood glucose of wildtype, RIPcre+ Pten+/+ Leprdb/db, and RIPcre+ Ptenfloflo Leprdb/db mice from 2 to 7 month of age (n > 7). C: Glucose tolerance test of wildtype, RIPcre+ Pten+/+ Leprdb/db, and RIPcre+ Ptenfloflo Leprdb/db mice at 7 month of age (n > 7). D: Insulin tolerance test of wildtype, RIPcre+ Pten+/+ Leprdb/db, and RIPcre+ Ptenfloflo Leprdb/db mice at 7 month of age (n > 7). E: in vivo glucose stimulated insulin secretion of wildtype, RIPcre+ Pten+/+ Leprdb/db, and RIPcre+ Ptenfloflo Leprdb/db mice at 7 month of age (n = 3). F and G: Insulin secretion per 60 islets during perifusion analysis (F) and quantification of area under the curve (G) of wildtype, RIPcre+ Pten+/+ Leprdb/db, and RIPcre+ Ptenfloflo Leprdb/db mice (n=3). *P<0.05 for RIPcre+ Ptenfloflo Leprdb/db mice compared to RIPcre+ Pten+/+ Leprdb/db mice or as indicated; φP<0.05 for both RIPcre+ Pten+/+ Leprdb/db and RIPcre+ Ptenfloflo Leprdb/db mice compared to wildtype mice. The results are presented as average ± standard error.

**FIG. 7.** RIPcre+ Ptenfloflo Leprdb/db mice exhibit islet hypertrophy with normal islet architecture and enhanced PI3K signaling. A: Immunofluorescent staining of insulin/glucagon, and immunohistochemical staining of synaptophysin, p-Akt (Ser473), total Akt, GLUT2 and PDX-1 of islets from wildtype, RIPcre+ Pten+/+ Leprdb/db, and RIPcre+ Ptenfloflo Leprdb/db mice at 7 month of age. B: Quantification of islet area in wildtype, RIPcre+ Pten+/+ Leprdb/db, and RIPcre+ Ptenfloflo Leprdb/db at 7 month of age (n = 4). C: Percentage of Ki67 positive cells in wildtype, RIPcre+ Pten+/+ Leprdb/db, and RIPcre+ Ptenfloflo Leprdb/db mice at 7 month of age (n = 4). D: Western blot (left panel) and quantification (right panel) of p-Akt (Ser473), total Akt, p-mTOR (Ser2448), total mTOR, p-FoxO-1 (Ser253), total FoxO-1, GLUT-2 and PDX-1 of islets from wildtype, RIPcre+ Pten+/+ Leprdb/db, and RIPcre+ Ptenfloflo Leprdb/db mice at 7 month of age (n=3). *P<0.05. Scale bar, 500µm (A, top panel); 50 µm (A, row 2-6). The results are presented as average ± standard error.

**FIG. 8.** HFD-fed RIPcre+ Ptenfloflo and RIPcre+ Ptenfloflo Leprdb/db mice showed intact islet integrity and uncompromised response to gamma irradiation. A: Laminin and β-catenin staining demonstrated intact basement membrane and cell-to-cell adhesion in HFD-fed (9 months of age) RIPcre+ Ptenfloflo (-/-) and RIPcre+ Ptenfloflo Leprdb/db (-/-; db/db) (9 months of age) mice. B: Transcripts levels of p53 related gene including Bax, Mdm2, and p21 during qPCR of RIPcre+ Pten+/+ (+/+), and RIPcre+ Ptenfloflo islets with or without 30Gy gamma irradiation (n=2). C: Western blotting (left panel) and quantification (right panel) of p-p53 (Ser392), total p53, cleaved MDM2, and total MDM2 in RIPcre+ Pten+/+ and RIPcre+ Ptenfloflo islets with or without 30Gy gamma irradiation (n=2). *P<0.05. Scale bar, 50 µm. The results are presented as average ± standard error.
Beta cell PTEN deletion protects against type 2 diabetes

Figure 1

A

B

C
Beta cell PTEN deletion protects against type 2 diabetes

Figure 2

A

PTEN

B

Hyp
Islet

PTEN expression (fold-change from control)

Figure 3

A

Chow +/+
Chow -/-
HFD +/+ 
HFD -/-

Weight (g)

Time after birth (month)

B

Chow +/+
Chow -/-
HFD +/+ 
HFD -/-

Fasting blood glucose (mmol/L)

Time after birth (month)

C

Blood Glucose (mM)

Time after glucose injection (min)

D

Percentage of baseline serum insulin

Time after glucose injection (min)
Figure 4
Figure 5

Beta cell PTEN deletion protects against type 2 diabetes
Figure 6

Beta cell PTEN deletion protects against type 2 diabetes
Beta cell PTEN deletion protects against type 2 diabetes

Figure 7

A

B

C

D

Protein expression (fold-change from control)
Beta cell PTEN deletion protects against type 2 diabetes

Figure 8

A

HFD -/+  HFD -/-  db/db  -/- db/db
Laminin
β-catenin

B

Protein expression (fold-change from control)

Bax  Mdm2  p21

C

p-p53  p53  MDM2 (cleaved)  MDM2 (total)  Actin