Forkhead Box Protein 1 (FoxO1) Inhibits Accelerated β Cell Aging in Pancreas-specific SMAD7 Mutant Mice

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The mechanisms underlying the effects of exocrine dysfunction on the development of diabetes remain largely unknown. Here we show that pancreatic depletion of SMAD7 resulted in age-dependent increases in β cell dysfunction with accelerated glucose intolerance, followed by overt diabetes. The accelerated β cell dysfunction and loss of proliferation capacity, two features of β cell aging, appeared to be non-cell-autonomous, secondary to the adjacent exocrine failure as a “bystander effect.” Increased Forkhead box protein 1 (FoxO1) acetylation and secondary to the adjacent exocrine failure as a “bystander effect.” Increased Forkhead box protein 1 (FoxO1) acetylation and nuclear retention was followed by progressive FoxO1 loss in β cells that marked the onset of diabetes. Moreover, forced FoxO1 expression in β cells prevented β cell dysfunction and loss in this model. Thus, we present a model of accelerated β cell aging that may be useful for studying the mechanisms underlying β cell failure in diabetes. Moreover, we provide evidence highlighting a critical role of FoxO1 in maintaining β cell identity in the context of SMAD7 failure.

β Cell dysfunction and failure are hallmarks of type 2 diabetes (1–4). Oxidative stress, endoplasmic reticulum stress, hypoxic stress, and cytokine toxicity can all lead to β cell apoptosis, autophagy, and replication defects, resulting in global β cell failure (3, 4). Thus, β cell replacement may be an ideal strategy for treating diabetes (5). Although β cell neogenesis from non-β cell sources has been extensively studied, most evidence suggests that postnatal β cell expansion predominantly results from β cell replication (6–11). However, normally, the β cell replication ratio is fairly low in young adult mice and then gets progressively lower with increasing age (4, 12–16). Meanwhile, recent studies strongly suggest that protection of the differentiated phenotype of existing β cells is critical for the maintenance of a functional β cell mass and for the prevention of type 2 diabetes (2, 17–19), in which the transcription factor Forkhead box protein 1 (FoxO1) appears to play a key protective role against β cell senescence and failure.

TGFβ superfamily signaling pathways are essential for pancreas development (20), postnatal β cell homeostasis, and proper function (21–23). Binding of TGFβ superfamily ligands to a type II receptor catalyzes the phosphorylation of a type I receptor to trigger downstream signaling cascades. SMAD7 is a general inhibitor of all TGFβ superfamily pathways, and we have shown recently that SMAD7 plays a critical role during pancreas development and β cell replication (20, 22–24). Here we studied β cell function in a model of pancreatic SMAD7 deletion (SMAD7Ptf1a).

Results

Pancreas-specific (SMAD7Ptf1a) SMAD7 Knockout Mice—To characterize the role of SMAD7 in β cell development and postnatal β cell mass homeostasis, we deleted pancreatic SMAD7 by crossing SMAD7fx/fx mice with Ptf1a-Cre mice to generate Ptf1a-Cre; SMAD7fx/fx mice, simplified as SMAD7Ptf1a (supplemental Fig. 1, A and B). In Ptf1a-Cre mice, in which SMAD7 was deleted in the majority of the pancreatic cells (21, 25, 26), β cells were isolated by laser capture microdissection (LCM) after insulin staining, and the purity of the β cells was assured by analyzing gene transcripts (supplemental Fig. 2, A and B). The genomic DNA of total pancreata, isolated islets, or purified β cells from SMAD7Ptf1a mice was examined for the recombination efficiency of SMAD7fx using a specific primer pair in conventional PCR, showing nearly complete recombination in β cells in SMAD7Ptf1a mice (supplemental Fig. 2C).

Pancreatic SMAD7 Knockout Accelerates β Cell Dysfunction with Age—SMAD7Ptf1a mice developed late-onset, gradual loss of body weight (Fig. 1A) with age-dependent progressive glucose intolerance (detectable as early as 20 weeks of age), fol-

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This article contains supplemental Figures 1–3.

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3 The abbreviations used are: FoxO1, Forkhead box protein 1; LCM, laser capture microdissection; α-SMA, α-smooth muscle actin; IPGTT, intraperitoneal glucose tolerance test; AAV, adeno-associated virus; RIP, rat insulin promoter; RT-qPCR, real-time quantitative PCR; NOD, nonobese diabetic.
FIGURE 1. β Cell dysfunction and aging in SMAD7Ptf1a mice. A–G, body weight (A), fasting blood glucose (B), IPGTT (C and D), serum insulin (E), β cell mass (F), and serum glucagon (G) at different ages in SMAD7Ptf1a mice compared with littermate control SMADfx/fx mice. H, 1 week of BrdU labeling followed by quantification of the percentage of β cells that incorporated BrdU. I and J, enrichment of gene transcripts of CyclinD1 (I) and CyclinD2 (J) in mouse islets of various ages. The values were normalized against CycloA, which is consistent among all conditions. K, islet perifusion study using islets from 20-week SMAD7Ptf1a and SMAD7fx/fx mice. Impaired glucose-stimulated insulin secretion was detected in SMAD7Ptf1a mice. *, p < 0.05 and n = 5 in all cases.
lowed by overt diabetes (after 30 weeks of age) (Fig. 1, B–D). Moreover, SMAD7Ptf1a mice also exhibit an age-dependent gradual loss of serum insulin (Fig. 1E) and β cell mass (Fig. 1F). The serum glucagon was unchanged (Fig. 1G). Impairment in β cell proliferation was detected (Fig. 1H and supplemental Fig. 3), seemingly resulting from decreases in the cell cycle activators CyclinD1 and CyclinD2 (Fig. 1, I and J). Moreover, glucose-stimulated insulin secretion by isolated islets was defective in SMAD7Ptf1a mice from 20 weeks on (Fig. 1K). These data suggest that pancreatic depletion of SMAD7 promotes β cell dysfunction and accelerates aging in SMAD7Ptf1a mice.

β Cell Dysfunction in SMAD7Ptf1a Mice Is Characterized by a Gradual Loss of β Cell Identity Genes—To confirm whether β cell dysfunction and accelerated aging are indeed the basis of the gradual loss of β cell mass and the development of glucose intolerance followed by overt diabetes in SMAD7Ptf1a mice, we examined the key β cell transcription factors Pdx1 (25), NeuroD1 (27), Nkx6.1 (28), and MafA (29) in isolated islets from different ages of SMAD7Ptf1a mice. These transcription factors seem to be required for β cells to be fully functional, whereas their loss has been correlated with β cell dysfunction and aging (2, 30). Our data show a clear decline in the expression of these genes from 20 weeks of age to 30 weeks of age in SMAD7Ptf1a mice by RT-qPCR (Fig. 2A), and by immunostaining (Fig. 2B).

β Cell Dysfunction and Aging in SMAD7Ptf1a Mice Likely Results from an Environment of Exocrine Atrophy and Fibrosis—We then examined possible mechanisms underlying the β cell dysfunction and aging in SMAD7Ptf1a mice. We saw an age-dependent progressive exocrine atrophy and fibrosis in SMAD7Ptf1a mice (Fig. 3, A and B), associated with a significant decrease in postnatal acinar cell proliferation (Fig. 3C), and increases in the fibrosis markers α-SMA (Fig. 3D) and collagen I (Fig. 3, E and F). Thus, we hypothesize that SMAD7 knockout in the exocrine pancreas may secondarily affect endocrine β cells, similar to the development of insulin insufficiency in patients with severe exocrine defects such as chronic pancreatitis (31–33). To test this hypothesis, we transplanted 300 islets from 20-week-old SMAD7Ptf1a mice under the kidney capsule of alloxan-treated diabetic NOD/SCID mice and followed the graft function for 20 more weeks (until 40 weeks of age for the graft). If a defect in β cell progenitors led to a β cell-autonomous defect and, thus, β cell senescence in SMAD7Ptf1a mice,
FIGURE 3. β Cell dysfunction in SMAD7Ptf1a mice likely results from an environment of exocrine atrophy and fibrosis. A, gross images showing age-dependent exocrine atrophy and fibrosis in SMAD7Ptf1a mice. Arrows point to the pancreas. w, weeks. B, pancreas weights in differently aged SMAD7Ptf1a mice were compared with littermate control SMAD7fx/fx mice. C, 1-week BrdU labeling followed by quantification of the percentage of acinar cells that incorporated BrdU in differently aged SMAD7Ptf1a mice compared with littermate control SMAD7fx/fx mice. D and E, RT-qPCR for pancreas α-SMA (D) and collagen I (E) transcripts. F, representative Collagen I staining images in the pancreata of SMAD7Ptf1a and control SMAD7fx/fx mice at different ages. Scale bars = 50 μm. *, p < 0.05 and n = 5 in all cases.
these transplanted islets should still gradually lose function (as they would have if they had not been transplanted), resulting in glucose intolerance and hyperglycemia in the recipient mice. However, if the β cell defect in SMAD7^{Ptf1a} mice is due to bystander effects on β cells from the exocrine pathology (non-β cell-autonomous), then the islets should function normally after isolation from the original environment (Fig. 4A).

We found no difference in blood glucose levels and glucose responses in alloxan-treated mice that received either type of islet (Fig. 4, B and C). Immunostaining for insulin in grafts and recipient pancreata confirmed loss of recipient β cells but β cell persistence in the graft (Fig. 4D). These results suggest that the age-dependent β cell dysfunction in SMAD7^{Ptf1a} mice likely results from the effects of the exocrine atrophy and fibrosis rather than from a β cell-specific developmental defect.

**Nuclear Translocation and Subsequent Loss of FoxO1 Precedes the Onset of Diabetes in SMAD7^{Ptf1a} Mice—FoxO1, a key transcription factor in insulin signaling, has been shown to play an essential inhibitory role during β cell dedifferentiation, dysfunction, and failure. FoxO1 in the adult pancreas is predominantly expressed by β cells, and its nuclear translocation may lead to down-regulation of Pdx1 expression and decreased β cell replication. However, FoxO1 nuclear translocation has also been shown to improve NeuroD1, MafA, and Nkx6.1 expression in β cells, contributing to the maintenance of a functional differentiated phenotype to resist stress-induced dedifferentiation.
tion, dysfunction, and failure (2, 30). We found a significant and progressive age-dependent decrease in FoxO1 mRNA in the islets of SMAD7Ptf1a mice (Fig. 5A). Moreover, nuclear translocation of FoxO1 was clearly detected at 20 weeks of age, followed by nearly complete loss of FoxO1 at 30 weeks of age in SMAD7Ptf1a mice, whereas littermate control SMAD7fx/fx mice showed consistently detectable cytoplasmic FoxO1 (Fig. 5B). Of note, analyses of FoxO1 acetylation on isolated islets suggested increases in FoxO1 acetylation at 20 weeks in SMAD7Ptf1a mice, which may explain the nuclear retention of FoxO1 at this time point (Fig. 5C). These data suggest that FoxO1 may protect against β cell dysfunction and aging in SMAD7Ptf1a mice but then also suggest that the loss of FoxO1 function may portend β cell loss and the onset of diabetes.

**Forced Expression of FoxO1, but Not SMAD7, in β Cells Inhibits β Cell Aging**

To confirm the hypothesis that FoxO1 accelerates β cell dysfunction and aging in SMAD7Ptf1a mice, we generated an AAV-RIP-FoxO1 viral vector to specifically express FoxO1 in β cells. The RIP-GFP virus and AAV-RIP-SMAD7 virus were also generated to be used as controls. We then used our recently developed intraductal virus delivery system (23, 34–36) to efficiently express FoxO1 or SMAD7 in β cells in vivo. SMAD7Ptf1a mice received either AAV-RIP-FoxO1 or an identical titer of AAV-RIP-GFP as one control or an identical titer of AAV-RIP-SMAD7 as another control at 20 weeks of age when glucose intolerance was present. After viral infusion, the mice were monitored for 10 more weeks (30 weeks of age), a time when these mice would normally be diabetic (Fig. 6A). Western blotting for FoxO1 or SMAD7 was performed on isolated islets, confirming the efficacy of the viral infusion (Fig. 6, B and C). We found that the SMAD7Ptf1a mice that received FoxO1 viral infusion had significantly improved fasting blood glucose (Fig. 6D), enhanced glucose tolerance (Fig. 6E), and increased β cell mass by 34.5% ± 4.3% (p < 0.05) compared with mice that received either of the two control viruses, suggesting that forced expression of FoxO1 inhibited β cell dysfunction. Messenger RNA was then analyzed by RT-qPCR on islet samples, showing a significant increase in NeuroD1, MafA, and Nkx6.1 but not Pdx1 or cell cycle activators (Fig. 6F). Immunostaining further showed significant recovery of expression of MafA and NeuroD1 in β cells from SMAD7Ptf1a mice that received FoxO1 virus (Fig. 6G). Moreover, the glucose-stimulated insulin secretion of the FoxO1-expressing islets from SMAD7Ptf1a mice 10 weeks after viral infusion was significantly improved (Fig. 6H). These data highlight a pivotal protective role for FoxO1 during β cell failure. However, administration of AAV-RIP-SMAD7 virus into 20 weeks of age SMAD7Ptf1a mice rescued neither FoxO1 nor loss of β cells despite increased β cell replication (Fig. 6, I and J). These data are consistent with our conclusion.

**FIGURE 5.** Nuclear translocation and subsequent loss of FoxO1 precedes the onset of diabetes in SMAD7Ptf1a mice. A, FoxO1 gene expression was analyzed in isolated islets from differently aged SMAD7Ptf1a and littermate control SMAD7fx/fx mice, which was normalized against CycloA. B, representative images of co-immunostaining for insulin (INS) and FoxO1 at 20 and 30 weeks (w) of age. HO, Hoechst. C, Western blotting for FoxO1 acetylation in isolated β cells. *, p < 0.05 and n = 5 in all cases. Scale bar = 50 μm.
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that β cell failure in SMAD7Ptf1a mice is non-β cell-autonomous and further suggest that SMAD7 is critical for normal postnatal β cell replication.

**Discussion**

Here we detected an age-dependent decline in β cell mass in SMAD7Ptf1a mice resulting from β cell dysfunction and, apparently, accelerated senescence. Of note, a gradual loss of β cell identity genes in β cells concomitantly occurred during this accelerated aging process, consistent with recent reports that β cell dedifferentiation occurs prior to dysfunction and failure (2, 30, 37).

According to previous reports on pancreatic development, Ptf1a is expressed in the lineage of both endocrine and exocrine cells (21, 25, 26). Thus, SMAD7 should be knocked out in both endocrine and exocrine cells in SMAD7Ptf1a mice. Knockout of SMAD7 in the exocrine pancreas resulted in an age-dependent progressive acinar atrophy and pancreatic fibrosis, whereas increased progressive β cell dysfunction and aging may be either cell-autonomous or secondary to exocrine defects in SMAD7Ptf1a mice. Thus, islets were moved from SMAD7Ptf1a mice into an environment devoid of the nearby atrophic exocrine pancreas, and these islets did not progress to failure in the new location. These data strongly suggest that the β cell dysfunction and aging in SMAD7Ptf1a mice is specifically due to the overall SMAD7 knockout pancreatic environment rather than the result of a β cell-autonomous defect.

The onset of the diabetic phenotype in SMAD7Ptf1a mice occurred relatively later, and the glucose response in young SMAD7Ptf1a mice seemed to be better than in control wild-type littermates. This phenomenon may be due to the effects of SMAD7KO on the early development of pancreatic cells, e.g. the speed of maturation of β cells, or the effects of SMAD7KO on insulin secretion by the newly differentiated β cells.

**β Cell dedifferentiation occurs prior to β cell dysfunction and aging,** during which FoxO1 has been shown to play a pivotal inhibitory role (2, 30). Here we show that FoxO1 nuclear exclusion occurs early during progressive β cell dysfunction, followed by complete loss of FoxO1, loss of the β-cell identity genes NeuroD1, MafA, and Nkx6.1, and onset of diabetes. Of note, increased FoxO1 acetylation was detected at 20 weeks in β cells in SMAD7Ptf1a mice as a likely basis for the FoxO1 nuclear retention (39). Forced sustained expression of FoxO1 in β cells not only rescued the expression of the β cell identity genes NeuroD1, MafA, and Nkx6.1 but also significantly improved β cell function, again highlighting the anti-aging role of FoxO1 in pancreatic β cells. Moreover, the effect of FoxO1 on NeuroD1 and MafA is consistent with previous reports (2, 30). Interestingly, when the AAV-RIP-SMAD7 virus was infused into 20-week-old SMAD7Ptf1a mice, although it neither rescued FoxO1 nor prevented β cell dysfunction and aging, it indeed significantly increased β cell replication. These data are consistent with our previous findings in which inhibition of TGFβ signaling in an non-inflammatory environment led to enhanced β cell replication (21–23). Moreover, these new data suggest that the loss of FoxO1 mRNA in β cells may be regulated indirectly by SMAD7 because the islets from SMAD7Ptf1a mice behaved normally after removal from the pancreatic milieu. Thus, it is expected that loss of FoxO1 mRNA is due to bystander effects from the progression of acinar atrophy.

SMAD7 loss in the exocrine pancreas may augment TGFβ receptor signaling, which may lead to epithelial-mesenchymal transition by acinar cells, resulting in exocrine fibrosis and atrophy. The pathology in the exocrine pancreas may produce inflammatory cytokines as an insult to β cells. β Cells modulate the cellular localization of FoxO1 to resist insulin-induced damage. However, in the end, the eventual loss of FoxO1 results in β cell senescence and failure. Because a proportion of patients develop β cell insufficiency because of chronic pancreatitis (31–33), which is similar to what we have observed in SMAD7Ptf1a mice, our data also suggest that maintenance of FoxO1 expression in β cells in these patients may substantially protect β cells and improve their function. Previous studies have shown a coordinated role for SMAD and FoxO proteins in the regulation of gene expression in non-β cells (40). Future experiments may be applied to study the molecular interaction of SMAD7 and FoxO1 in primary β cells.

A previous study by Smart et al. (24) showed that overexpression of SMAD7 in β cells leads to loss of β cell identity and function. Recently, we showed that a modest up-regulation in SMAD7 increases β cell proliferation (23). Here we show that pancreatic depletion of SMAD7 resulted in a progressive exocrine defect and β cell loss. In line with these findings, an elegant study recently showed that β cell dedifferentiation could be reversed by a small-molecule inhibitor of TGFβ receptor signaling (41). Together, all of these studies highlight a pivotal role for TGFβ receptor signaling in the regulation of pancreas homeostasis. Hence, extensive dissection of TGFβ receptor signaling may substantially improve our understanding of β cell biology and β cell therapy for diabetes.

**Experimental Procedures**

**Mouse Manipulation**—All mouse experiments were approved by the Animal Research and Care Committee at the Children’s Hospital of Pittsburgh and the University of Pittsburgh Institutional Animal Care and Use Committee. Bacterial artificial chromosome transgenic Ptf1a promoter Cre reporter (Ptf1a-Cre) mice (21, 25) have a C57BL/6J background, as has...
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been described before. C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). SMAD7fx/fx was generated as described before (20). In Ptf1a-Cre; SMAD7fx/fx (SMAD7pTfla) mice, analysis of purified β cells that were prepared by LCM showed nearly complete recombination in β cells. Only male mice were used in the current experiments. Pancreatic intraductal virus infusion was performed as described before (36). AAV8 viruses (titration of 1012 genome copy particles/ml and in a volume of 150 μl) were delivered via catheter at a rate of 6 μl/min. The fasting blood glucose test and intraperitoneal glucose tolerance test (IPGTT) have been described before (21).

Virus Production—The generation of adeno-associated virus (AAV) serotype 8 carrying SMAD7 or FoxO1 under the rat insulin promoter (RIP) has been described before (23). Both constructs have a GFP reporter. The ORF of the mouse FoxO1 gene was amplified from cDNA of 8-week-old mouse islets. RIP was amplified from rat genomic DNA. GFP was amplified from pLVX-IRES-ZsGreen (Clontech, Mountain View, CA). Titration of viral vectors was determined by dot plot assay. Purified AAV vectors were filtered and stored at −80 °C.

Islet and β Cell Isolation—Pancreas digestion and islet isolation have been described previously (42). β Cells in SMAD7pTfla mice were isolated by LCM as described before (34, 43). Isolation of Genomic DNA and PCR for the SMAD7 Mutant—Genomic DNA extraction and conventional PCR have been described previously (21). The primers for examining SMAD7 mutants were 5′-GAGGACAGGGGATCGATGG-3′ (sense) and 5′-GACACGCTGCAATGCGACG-3′ (antisense), which amplify a genomic DNA PCR product of 1.7 kb from wild-type mice, 2.2 kb from SMAD7 floxed knockin mice, and 0.7 kb from SMAD7 floxed knockin mice after Cre recombination.

Isolation of RNA and RT-qPCR—RNA extraction and RT-qPCR have been described previously (10, 44). All primers were purchased from Qiagen (Hilden, Germany). They were CycloA (QT00247709), FoxO1 (QT00116186), Smad7 (QT00124607), CyclinD1 (QT00154595), CyclinD2 (QT00170618), Insulin (QT00114289), Pdx1 (QT00102235), NeuroD1 (QT00251265), Mafa (QT001037638), Nkx6.1 (QT00143318), Ck19 (QT00156667), Amylase (QT00179242), Cd31 (QT01052044), Vimentin (QT01059670), Glucagon (QT00124033), Synaptophysin (QT01042314), Somatostatin (QT01046528), Pancreatic polypeptide (QT00103999), Collagen I (QT00162204), and α-smooth muscle actin (α-SMA) (QT00140119). RT-qPCR values were normalized against CycloA, which proved to be stable across the samples.

Immunohistochemistry and Western Blotting—Western blotting and immunohistochemistry were performed as described before (21, 23). Nuclear staining was performed with Hoechst 33342 (HO, Sigma-Aldrich, St. Louis, MO). Primary antibodies were as follows: guinea pig polyclonal insulin-specific (Dako, Carpinteria, CA); goat polyclonal NeuroD1-specific (Santa Cruz Biotechnology, Dallas, TX); rabbit polyclonal SMAD7-, CyclinD1-, and CyclinD2-specific (Santa Cruz Biotechnology); FoxO1- and GAPDH-specific (Cell Signaling Technology, San Jose, CA), Collagen I- and Pdx1-specific (Abcam, Cambridge, MA, USA), MafA-specific (Bethyl Laboratories, Inc.); Nkx6.1-specific (a kind gift from Dr. Maike Sander, University of California, San Diego, CA); and rat CD31-specific (BD Biosciences) and BrdU-specific (Abcam). Secondary antibodies were Cy2-, Cy3-, Cy5-, or HRP-conjugated goat-, rat-, rabbit-, and guinea pig-specific (Jackson ImmunoResearch Laboratories, West Grove, PA).

Data Analysis—Quantification of gene expression, β cell proliferation, and β cell mass has been described before (21, 23). All values are depicted as mean ± S.E. Five repeats were analyzed in each condition. All data were statistically analyzed by one-way analysis of variance with a Bonferroni correction, followed by Fisher’s exact test to compare two groups. Significance was considered when p < 0.05.

Author Contributions—The study was conceived and designed by X. X. and G. K. G. Acquisition of data was performed by X. X., C. C., P. G. T. Z., S. F., J. F., C. S., and K. P. Analysis and interpretation of data were carried out by X. X., H. D., and G. K. G. X. X. drafted the article, and all authors revised the article and approved the final version to be published.

References
1. Pipeleers, D., Chintinie, M., Denys, B., Martens, G., Keymeulen, B., and Gorus, F. (2008) Restoring a functional β-cell mass in diabetes. Diabetes Obes. Metab. 10, 54–62
2. Talchai, C., Xuan, S., Lin, H. V., Sussel, L., and Accili, D. (2012) Pancreatic β cell differentiation as a mechanism of diabetic β cell failure. Cell 150, 1223–1234
3. Kitamura, T. (2013) The role of FOXO1 in β-cell failure and type 2 diabetes mellitus. Nat. Rev. Endocrinol. 9, 615–623
4. Gunasekaran, U., and Gannon, M. (2011) Type 2 diabetes and the aging pancreatic β cell. Aging 3, 565–575
5. Gaglia, J. L., Shapiro, A. M., and Weir, G. C. (2005) Islet transplantation: progress and challenge. Arch. Med. Res. 36, 273–280
6. Dor, Y., Brown, J., Martinez, O. I., and Melton, D. A. (2004) Adult pancreatic β-cells are formed by self-duplication rather than stem-cell differentiation. Nature 429, 41–46
7. Tata, M., Rankin, M. M., Long, S. Y., Stein, G. M., and Kushner, J. A. (2007) Growth and regeneration of adult β cells does not involve specialized progenitors. Dev. Cell 12, 817–826
8. Meier, J. J., Butler, A. E., Saisho, Y., Monchamp, T., Galasso, R., Bhushan, A., Rizza, R. A., and Butler, P. C. (2008) β-Cell replication is the primary mechanism subserving the postnatal expansion of β-cell mass in humans. Diabetes 57, 1584–1594
9. Georgia, S., and Bhushan, A. (2004) β Cell replication is the primary mechanism for maintaining postnatal β cell mass. J. Clin. Invest. 114, 963–968
10. Xiao, X., Chen, Z., Shiota, C., Prasadian, K., Guo, P., El-Gohary, Y., Paredes, J., Welsh, C., Wiersch, J., and Gittes, G. K. (2013) No evidence for β cell neogenesis in murine adult pancreas. J. Clin. Invest. 123, 2207–2217
11. Xiao, X., and Gittes, G. K. (2015) Concise review: new insights into the role of macrophages in β-cell proliferation. Stem Cells Transl. Med. 4, 655–658
12. Kushner, J. A. (2013) The role of aging upon β cell turnover. J. Clin. Invest. 123, 990–995
13. Rankin, M. M., and Kushner, J. A. (2009) Adaptive β-cell proliferation is severely restricted with advanced age. Diabetes 58, 1365–1372
14. Tata, M., Long, S. Y., Wartschow, L. M., Rankin, M. M., and Kushner, J. A. (2005) Very slow turnover of β-cells in aged adult mice. Diabetes 54, 2577–2587
15. Stolovich-Rain, M., Hija, A., Grimsby, J., Glaser, B., and Dor, Y. (2012) Pancreatic β cells in very old mice retain capacity for compensatory proliferation. J. Biol. Chem. 287, 27407–27414
