RESEARCH COMMUNICATION

Bmi-1 regulates the *Ink4a/Arf* locus to control pancreatic β -cell proliferation

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The molecular mechanisms that regulate the age-induced increase of p16^{INK4a} expression associated with decreased β -cell proliferation and regeneration are not well understood. We report that in aged islets, de-repression of the *Ink4a/Arf* locus is associated with decreased Bmi-1 binding, loss of H2A ubiquitylation, increased MLL1 recruitment, and a concomitant increase in H3K4 trimethylation. During β -cell regeneration these histone modifications are reversed resulting in reduced p16^{INK4a} expression and increased proliferation. We suggest that PcG and TrxG proteins impart a combinatorial code of histone modifications on the *Ink4a/Arf* locus to control β -cell proliferation during aging and regeneration.

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Emerging evidence indicates that proliferation of pancreatic β cells is an important mechanism not only to maintain homeostasis in the endocrine pancreas but also for adapting islet function to changes in metabolic demands (Dor et al. 2004; Kulkarni et al. 2004; Okamoto et al. 2006; Zhong et al. 2007). The inability of the β cells to expand and compensate for the changing insulin demand can contribute to the pathogenesis of diabetes. Several studies suggest that β -cell proliferation declines with age (Teta et al. 2005; Meier et al. 2008) and this age-dependent decline in the β -cell proliferation could curtail the ability of the endocrine pancreas to respond to metabolic changes. However, the cell-intrinsic genetic and epigenetic mechanisms regulating the age-dependent decline of β -cell proliferation are not very clear.

The products of the *Ink4a/Arf* (*Cdkn2a*) locus, p16^{INK4a} (a negative regulator of CDK4-D-type cyclins) (Kim and Sharpless 2006) and p19^{Arf} show increased expression with age (Nielsen et al. 1999; Krishnamurthy et al. 2004) and have been linked to reduction in the proliferative capacity of aged β cells (Krishnamurthy et al. 2006). Genome-wide studies have linked the *Ink4a/Arf* locus with type 2 diabetes and impaired insulin release, sug-

gesting that failure of islets to expand may play an important role in type 2 diabetes (for review, see Doria et al. 2008). Thus, understanding the regulation of *Ink4a/Arf* locus could reveal the molecular basis of reduced β -cell proliferation with aging and also be extremely useful in devising strategies to foster β -cell regeneration.

Several genetic studies show that a polycomb group (PcG) protein, Bmi-1, is essential for maintenance of adult stem cell self-renewal primarily due to its ability to repress the *Ink4a/Arf* locus (Jacobs et al. 1999; Lessard and Sauvageau 2003; Molofsky et al. 2003; Bruggeman et al. 2005). Bmi-1, along with Ring1 and Ring2, is a part of the polycomb-repressive complex 1 (PRC1), which possesses histone H2A-K119 ubiquitin E3 ligase activity, required for the repression of *Hox* genes (Wang et al. 2004). While Ring2 is the catalytic subunit, Bmi-1 maintains the integrity of the complex and is essential for enzymatic activity (Cao et al. 2005). Analysis in cancer cell lines and embryonic fibroblasts indicates that the ability of Bmi-1 to repress the *Ink4a/Arf* locus was dependent on the continued presence of another polycomb complex, PRC2, which possesses H3K27 methyltransferase activity mediated by Ezh2, at the *Ink4a/Arf* locus (Bracken et al. 2007; Kotake et al. 2007). However, the contribution of PRC1-dependent H2A ubiquitylation in the regulation of *Ink4a/Arf* expression has not been described. In addition, genetic studies show that the axial-skeletal transformations of *Bmi-1*^{-/-} mice were normalized by the loss of MLL1, a trithorax group (TrxG) protein and an H3K4 methyltransferase, suggesting that both PcG and TrxG proteins act to maintain proper spatial *Hox* gene expression in mice (Hanson et al. 1999). However, the role of TrxG proteins in regulating the *Ink4a/Arf* locus is not known.

Here we report that PcG and TrxG complexes play opposing roles in controlling the *Ink4a/Arf* locus to regulate the proliferative capacity of β cells during aging and regeneration. We show that decreased Bmi-1 binding to the *Ink4a/Arf* locus in aged islet led to reduced H2A ubiquitylation, which stimulated the recruitment of MLL1 and increased H3K4 trimethylation that augmented transcriptional activity from the *Ink4a/Arf* locus. During β -cell regeneration, increased Bmi-1 binding coincided with decreased H3K4 trimethylation, resulting in repression of the *Ink4a/Arf* locus and augmented β -cell proliferation. These results support the view that PcG and TrxG protein-dependent regulation of the *Ink4a/Arf* locus plays a critical role in regulating pancreatic β -cell proliferation during aging and regeneration.

Results and Discussion

To establish the compartment and age-dependent changes in the expression of Bmi-1, we analyzed the expression pattern of Bmi-1 in mouse pancreata at different ages. Immunohistochemical analysis on pancreatic sections from 2-wk-old mice revealed strong nuclear staining for Bmi-1 in the islets and ductal structures. Costaining with insulin showed that Bmi-1 was expressed primarily in β cells in the islets (Fig. 1A). Immunostaining of pancreatic sections from different ages showed that the expression of Bmi-1 in the islets and ducts declined with age, and in 10-wk-old pancreas, only a few β cells showed weak

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Figure 1. The expression of Bmi-1 declined with age and correlated with increased expression of *Ink4a/Arf* genes and reduced β-cell proliferation. (A) Expression pattern of Bmi-1 in the representative pancreatic sections from wild-type mice aged 2, 4, and 10 wk, respectively (20× magnification). (i) Islet cells; (d) ductal cells. (B) RNA levels of *Bmi-1*, *Ink4a*, and *Arf* in islets isolated from mice at different ages. (C) RNA levels of *Ink4a* and *Arf* and (D) Immunofluorescence for p16 and insulin, with DAPI, in pancreatic sections from 2-wk-old wild-type (WT) and *Bmi-1*^{-/-} mice (20× magnification). (E) Quantification of proliferating β cells at 2, 4, and 10 wk in wild-type and *Bmi-1*^{-/-} mice, as a percentage of Ki67⁺ insulin double-positive cells in islets. (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.005$.

staining (Fig. 1A). This age-related decline in the levels of Bmi-1 was reflective of the changes in the transcript levels of *Bmi-1* and correlated with a concomitant increase in the levels of transcripts from the *Ink4a/Arf* locus (Fig. 1B). This sharp decline in Bmi-1 expression early after birth may be unique to pancreatic β cells as previous reports indicate constant levels of Bmi-1 with respect to age (Krishnamurthy et al. 2004; Janzen et al. 2006; Molofsky et al. 2006; Bracken et al. 2007).

We next examined whether Bmi-1 regulated the expression of the *Ink4a/Arf* locus in pancreatic islets by analyzing *Bmi-1*^{-/-} mice (van der Lugt et al. 1994). Quantitative RT-PCR revealed increased transcript levels of *Ink4a* and *Arf* in islets isolated from *Bmi-1*^{-/-} mice compared with the wild-type littermates (Fig. 1C). Immunohistochemistry of pancreatic sections demonstrated strong expression of p16^{Ink4a} levels in the islets from 2-wk-old *Bmi-1*^{-/-} mice, while hardly any p16^{Ink4a} expression was discernable in the pancreas of wild-type littermates (Fig. 1D). The up-regulation of p16^{Ink4a} and p19^{Arf} proteins in *Bmi-1*^{-/-} mice was also confirmed by immunoblots (Supplemental Fig. S1A). However, other cell cycle regulators examined were unaffected in islets from *Bmi-1*^{-/-} mice (Supplemental Fig. S1B). Analysis of β-cell proliferation by staining pancreatic sections with Ki67 and insulin showed that the percentage of proliferating β cells was greatly reduced in *Bmi-1*^{-/-} mice compared with the wild-type littermates from 2 wk after birth (Fig. 1E), and confirmed by BrdU incorporation studies (Supplemental Fig. S1C). No difference in the percentage of proliferating exocrine cells between the *Bmi-1*^{-/-} mice and wild-type littermates was found (Supplemental Fig. S1D), indicating that there was no generalized decrease in proliferation throughout the pancreas. These observations suggested that loss of *Bmi-1* resulted in reduced β-cell proliferation due to the premature up-regulation of p16^{Ink4a}.

To assess whether increased levels of p16^{Ink4a} were responsible for the reduced proliferative capacity of pancreatic β cells in *Bmi-1*^{-/-} mice, isolated islets were treated with siRNA targeting *Ink4a*. Islets isolated from

Bmi-1^{-/-} mice displayed reduced proliferation compared with islets isolated from wild-type littermates. Strikingly, treatment with *Ink4a* siRNA, which effectively reduced *Ink4a* expression, was sufficient to rescue β-cell proliferation in *Bmi-1*^{-/-} islets (Fig. 2A). Similarly, knockdown of *Bmi-1* in the pancreatic β-cell line Min6 resulted in decreased cell growth kinetics that were rescued by the simultaneous knockdown of both *Bmi-1* and *Ink4a* (Fig. 2B). These data indicate that repression of p16^{Ink4a} is the major mechanism by which Bmi-1 regulates β-cell proliferation. We next assessed the physiological consequences of loss of *Bmi-1* by carrying out morphometric and metabolic analyses of *Bmi-1*^{-/-} mice. Examination of pancreatic sections from 10-wk-old mice revealed a dramatic reduction in the size of islets in the pancreas from *Bmi-1*^{-/-} mice as compared with their littermates (Fig. 2C). The size of individual β cells did not appear to differ in the *Bmi-1*^{-/-} and wild-type littermates, indicating that the reduced islet size was likely due to a decrease in the number of β cells. The β-cell mass in *Bmi-1*^{-/-} mice was similar to wild-type littermates at birth (Supplemental Fig. S2A,B) but was severely reduced by 10 wk (Fig. 2D), suggesting a postnatal role for *Bmi-1* in β-cell proliferation. The reduced β-cell mass in the *Bmi-1*^{-/-} mice resulted in abnormal glucose homeostasis, as measured by glucose tolerance tests (GTTs) at 10 wk (Fig. 2E). Wild-type mice had the highest glucose levels 15 min after injection and reached baseline by the end of the 120-min testing period. In contrast, *Bmi-1*^{-/-} mice displayed elevated blood glucose levels that failed to return to baseline levels during the testing period. The serum insulin levels after overnight fasting failed to increase in *Bmi-1*^{-/-} mice in response to glucose challenge (Fig. 2F). Insulin tolerance test showed no difference between *Bmi-1*^{-/-} and wild-type littermates, indicating that changes in islet physiology in the *Bmi-1*^{-/-} mice were not due to effects of insulin resistance (Supplemental Fig. S2C).

To understand the mechanism by which Bmi-1 regulates the *Ink4a/Arf* locus in β cells, we first examined the binding of Bmi-1 across the entire *Ink4a/Arf* locus by chromatin immunoprecipitation (ChIP) assays (Fig. 3A).

Figure 2. *Bmi-1*^{-/-} mice display diminished β -cell mass, hypoinsulinemia, and glucose intolerance. (A) Quantification of proliferating islet cells (left panel) and levels of *Ink4a* (right panel) in cultured islets isolated from 4-wk-old wild-type (WT) and *Bmi-1*^{-/-} mice, transfected with *Ink4a* or scrambled siRNAs. (B) Growth profiles (left panel) and corresponding levels of *Bmi-1* and *Ink4a* (right panel) in Min6 cells transfected with *Bmi-1*, *Ink4a*, *Bmi-1* + *Ink4a*, or scrambled siRNAs. *P*-value symbols indicated are for comparison of *Bmi-1* and *Bmi-1* + *Ink4a* samples. (C) Pancreatic sections from 10-wk-old wild-type and *Bmi-1*^{-/-} mice stained with insulin (Ins) and glucagon (Glu) with DAPI (5 $\times$ magnification). (D) β -Cell mass in wild-type and *Bmi-1*^{-/-} mice at 2, 4, and 10 wk of age (*n* = 5 for each phenotype and age). (E) GTT for wild-type and *Bmi-1*^{-/-} mice at 10 wk (*n* = 5 for each phenotype). (F) Plasma insulin levels in 10-wk-old wild-type and *Bmi-1*^{-/-} mice (fasted overnight) at 0 and 30 min, after glucose injection (*n* = 5 for each phenotype). (*) *P* < 0.05; (**) *P* < 0.01; (***) *P* < 0.005.

ChIP assays demonstrated the binding of Bmi-1 throughout the *Ink4a/Arf* locus in the islets from 4-wk-old wild-type mice, with age-matched *Bmi1*^{-/-} islets serving as binding controls (Fig. 3B). We next assessed whether loss of Bmi-1 in islets resulted in changes in polycomb-dependent histone modifications on the *Ink4a/Arf* locus. Besides analyzing the levels of H2A ubiquitylation (H2A-ub), we also examined the levels of trimethylation at H3K27 (H3K27me3), a histone modification mediated by Ezh2 that has been linked with silencing of the *Ink4a/Arf* locus (Bracken et al. 2007; Kotake et al. 2007). Islets from *Bmi1*^{-/-} mice showed a significant decrease in H2A ubiquitylation levels (Fig. 3B). Surprisingly, however, the levels of H3K27me3 at *Ink4a/Arf* locus were unaffected (Fig. 3B). Further, the binding and levels of Ezh2 were unchanged in the absence of *Bmi-1*, suggesting that the Ezh2-dependent H3K27me3-repressive histone modification mark alone was not sufficient for repression of the *Ink4a/Arf* locus (Fig. 3B; Supplemental Fig S3A). To address how the Ezh2-mediated methylation of H3K27 contributed to the regulation of the *Ink4a/Arf* locus, we examined islets in which Ezh2 expression was knocked down by specific siRNA (Supplemental Fig. S3B). The knock-down of Ezh2 not only led to reduced binding of Ezh2 and trimethylation of H3K27, but Bmi-1 binding as well as H2A ubiquitylation levels were also reduced at the *Ink4a/Arf* locus (Fig. 3C), suggesting that Ezh2 is required for the binding of Bmi-1. These data are consistent with a sequential recruitment mechanism of polycomb action (Hernandez-Munoz et al. 2005) to regulate the *Ink4a/Arf* locus, in which Ezh2-mediated H3K27 methylation enables the binding of Bmi-1, leading to H2A ubiquitylation and repression of the *Ink4a/Arf* locus.

As aging islets are also characterized by increased p16^{Ink4a} expression (Supplemental Fig. S4A,B), we compared the binding of Bmi-1 and Ezh2 and histone modifications within the *Ink4a/Arf* locus in young (2 wk) and old (24 wk) islets. The binding of both Bmi-1 and Ezh2 at the *Ink4a/Arf* locus declined in the old islets and resulted in reduced levels of H3-K27me3 and H2A-ub modifications at the *Ink4a/Arf* locus (Fig. 4A). The fact that the expression from *Ink4a/Arf* locus increased with aging even in the absence of *Bmi-1* (as shown in Fig. 1C) suggested additional levels of regulation of the *Ink4a/Arf*

Figure 3. *Bmi-1* plays a key role in regulation of the *Ink4a/Arf* locus through modulation of histone modifications. (A) Schematic representation of the *Ink4a/Arf* locus, with blue regions marked 1–4 indicating the amplified regions in the ChIP studies. ChIP analysis for the indicated antibodies at the *Ink4a/Arf* locus in islets isolated from wild-type (WT) and *Bmi-1*^{-/-} mice (4 wk old) (B) and upon treatment of islets from 4-wk-old mice (C), with siRNA targeting Ezh2 or control, scrambled siRNA. Primer set 5 indicates negative control [Exon 2 of *HoxC13* locus] (Cao et al. 2005). (*) *P* < 0.05; (**) *P* < 0.01; (***) *P* < 0.005.

locus. We examined whether other histone modifications typically associated with transcriptional activation were changed in aged islets. We compared the status of H3K4 trimethylation (H3K4me3) at the *Ink4a/Arf* locus in young and old islets and found increased levels of H3K4 trimethylation in the old islets (Fig. 4A). These changes in H3K4 trimethylation prompted us to compare the binding of H3K4 methyltransferase MLL1 to *Ink4a/Arf* locus in young and old islets. We found that the increased levels of H3K4me3 at the *Ink4a/Arf* locus with islet aging coincided with increased binding of MLL1 (Fig. 4A). These results suggested that polycomb and MLL1 have opposing roles in the regulation of the *Ink4a/Arf* locus and that the loss of *Bmi-1* could initiate mechanisms that enhance transcriptional activation from the *Ink4a/Arf* locus. To test this, we compared the levels of MLL1 binding and H3K4 trimethylation modifications at the *Ink4a/Arf* locus in the wild-type and *Bmi-1*^{-/-} islets. In the absence of *Bmi-1*, we found increased binding of MLL1 and increased levels of H3K4me3 modification at the *Ink4a/Arf* locus (Supplemental Fig. S4C). Our observations suggest that in the young islets, high levels of *Bmi-1* would ensure increased H2A-ubiquitylation (Fig. 4B), repression of *Ink4a/Arf* expression and greater β -cell proliferative capacity. With aging, reduced *Bmi-1* binding would facilitate H3K4 trimethylation, increased *Ink4a/Arf* expression, and reduced β -cell proliferative capacity.

To study the role of *Bmi-1* in regulating the regenerative capacity of β cells, we used a model of islet regeneration after streptozotocin (STZ)-induced β -cell destruction. Four-week-old mice were subjected to a single dose of STZ (90 mg/kg), and pancreata were harvested at different time points. This low-dose treatment did not lead to any significant DNA damage and islet morphology was maintained (Supplemental Fig. S5A). Increased β -cell proliferation was observed after STZ treatment, consistent with regeneration following β -cell destruction (Supplemental Fig. S5B). Immunoblots of islets isolated after 4 d of STZ treatment showed increased *Bmi-1* and decreased p16^{Ink4a} levels (Supplemental Fig. S5C). Immunohistochemistry revealed that STZ treatment resulted in increased number of *Bmi-1*-expressing β cells that often coincided with proliferating islet cells (Fig. 5A; Supplemental Fig. S5D). Increased *Bmi-1* expression was

consistent with increased ubiquitylated H2A and reduced levels of p16^{Ink4a} in the β cells (Fig. 5 B,C). The lower levels of p16^{Ink4a} were sustained 15 d after the treatment (Supplemental Fig. S5E). ChIP analysis on isolated islets showed that up-regulation of *Bmi-1* levels correlated with increased *Bmi-1* binding, H2A ubiquitylation, and a concomitant decrease in H3K4 trimethylation at the *Ink4a/Arf* locus, upon STZ treatment (Fig. 5D). These results indicate that during regeneration increased *Bmi-1* binding to the *Ink4a/Arf* locus led to a decrease in p16^{Ink4a} levels, resulting in increased proliferation of β cells. To confirm the requirement of *Bmi-1* for β -cell proliferation during regeneration, *Bmi-1*^{-/-} mice and wild-type littermates were subjected to STZ treatment. *Bmi-1*^{-/-} mice after STZ treatment did not show any changes in β -cell proliferation (Fig. 5E). These results suggest a strong link between *Bmi-1*-dependent H2A ubiquitylation of *Ink4a/Arf* locus and β -cell regenerative capacity. While the *Ink4a/Arf* locus is clearly regulated in a *Bmi-1*-dependent fashion during β -cell regeneration, the global appearance of changes in H2A ubiquitylation with aging and/or regeneration may be indicative of the existence of other potential chromatin targets for the polycomb complexes in the β cells. Our studies, along with recent observations in liver regeneration models, suggest that H2A ubiquitylation may be a key phenomenon governing gene silencing during tissue regeneration (Nakagawa et al. 2008).

Even though *Bmi-1* has been widely implicated in the regulation of *Ink4a/Arf* locus, the precise molecular mechanisms have not been reported previously. We show here that *Bmi-1* is critical for the H2A ubiquitylation and repression of the *Ink4a/Arf* locus. Loss of H2A ubiquitylation promotes MLL-mediated H3K4 trimethylation and transcriptional activation from the *Ink4a/Arf* locus. While a role for *Bmi-1* in promoting self-renewal of stem cells and regulation of senescence via repression of p16^{Ink4a} has been described previously, our study shows that self-renewal and regeneration of pancreatic β cells, a terminally differentiated cell type, are also governed by *Bmi-1*-dependent regulation of the *Ink4a/Arf* locus.

Our work suggests that levels of *Bmi-1* are critical in determining the proliferative potential of β cells. A decline in the levels and binding of *Bmi-1* to *Ink4a/Arf* locus with aging translates into reduced proliferative potential and consequently reduced self-renewal of β cells. Experiments with STZ-induced β -cell regeneration suggest that the modulation of *Ink4a/Arf* expression levels by *Bmi-1* is critical to the β -cell regeneration process. It will be interesting to examine if similar changes in the levels of *Bmi-1* and histone modifications of the *Ink4a/Arf* are a prerequisite for β -cell mass expansion in physiological contexts, such as dietary stress and obesity. Our study shows a requirement for polycomb proteins in the regulation of pancreatic β -cell self-renewal via *Ink4a/Arf* locus with aging. However, our work does not rule out the involvement of other targets of PcG and/or TrxG in β -cell aging. In this context, it is noteworthy that rescue of β -cell proliferation by inactivation of p16^{Ink4a} in the *Bmi-1*^{-/-} islets and *Ezh2* β -cell knockout mice (H. Chen and S.K. Kim, pers.

Figure 4. Polycomb-mediated regulation of *Ink4a/Arf* locus during aging by histone modification (A) ChIP analysis for the indicated antibodies at the *Ink4a/Arf* locus with aging, with IgG as control, Young indicates 2 wk; old, 24–30 wk of age. Time points chosen to reflect very high (2 wk) and low (24–30 wk) levels of PcG proteins. (B) Coimmunostaining of ubiquitinated H2A (H2A-ub) and insulin (Ins) with DAPI, in pancreatic sections from 2-wk-old and 24-wk-old wild-type mice. (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.005$.

Figure 5. Bmi-1-dependent regulation of *Ink4a/Arf* locus plays a critical role in β -cell regeneration. Coimmunostaining for Bmi-1 and Ki67 (A) and coimmunostaining for H2A-ub and insulin (Ins) with DAPI (B) in pancreatic section from vehicle or STZ-treated 4-wk-old wild-type (WT) mice. Arrows indicate cells that are positive for both Bmi-1 and Ki67 (20 $\times$ magnification). (C) Immunofluorescence for p16^{Ink4a} and insulin (Ins) with DAPI on pancreatic sections from 4-wk-old mice treated with control vehicle or a single dose of STZ (20 $\times$ magnification). (D) ChIP analysis for the indicated antibodies at the *Ink4a/Arf* locus on islets from 4-wk-old wild-type mice injected with STZ or control vehicle. Analyses A–D were performed on mice 4 d after injections. (E) Proliferation of β cells in wild-type and *Bmi-1*^{−/−} mice after STZ or vehicle treatment, measured as a percentage of Ki67⁺ insulin double-positive islet cells. Analysis performed 15 d after STZ or control injections. $n = 5$ mice per treatment. (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.005$.

comm.) indicates that *Ink4a/Arf* might be the primary PcG target in maintenance of β -cell proliferative potential.

Although we focused on pancreatic β cells, the regulation of the *Ink4a/Arf* locus by PcG and TrxG proteins is likely to be a general mechanism utilized by other cell types. Moreover, our data also provide a molecular basis for the previously reported genetic antagonism between PcG and TrxG regulatory modules (Hanson et al. 1999). Finally, our results suggest that the epigenetic “histone code” in pancreatic β cells is dynamic and that reprogramming of the β -cell epigenome by members of the polycomb gene family could be an effective strategy in promoting β -cell regeneration in diabetes.

Materials and methods

Animals, islets and cell lines, and metabolic and regeneration analyses

Targeted disruption of the *Bmi-1* allele has been described before (van der Lugt et al. 1994). The animals were maintained by mating *Bmi-1*^{+/−} males and females on a C57BL/6J background. Min6 cells were maintained in DMEM containing 10% FBS and 25 mM glucose at 37°C in 5% CO₂ environment. Islets were isolated using the Liberase enzyme blend (Roche), and cultured as described before (Zhong et al. 2007). GTT and plasma insulin measurements were performed following overnight fasting of mice, as described previously (Zhong et al. 2007). Four-week-old *Bmi-1*^{−/−} mice and wild-type littermates were given single intraperitoneal injection of 90 mg/kg STZ (Sigma-Aldrich) or vehicle, and the pancreata were harvested.

Histology and morphometry

Standard immunofluorescence staining protocol was used (Zhong et al. 2007). Details of the antibodies, proliferation, and morphometric analyses are described in the Supplemental Material.

Gene expression (targeting and analysis) and ChIP

RNA and Western blot analyses were performed according to the Supplemental Material. The knockdown of *Ezh2*, *Bmi-1*, and *Ink4a* in Min6 cells or mouse islets was done by transfection with a specific targeting siRNA listed in the Supplemental Material. ChIP analysis was performed using the Millipore ChIP kit (Millipore 17-295) according to the manufacturer's instructions with minor modifications. RT-PCR and ChIP primers are listed in Supplemental Tables 1 and 2, respectively.

Detailed experimental procedures are described in the Supplemental Material.

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