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Islet-specific *Prmt5* excision leads to reduced insulin expression and glucose intolerance in mice

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Abstract

Protein arginine methyltransferase 5 (PRMT5), a symmetric arginine methyltransferase, regulates cell functions by influencing gene transcription through posttranslational modification of histones and non-histone proteins. PRMT5 interacts with multiple partners including menin, which controls beta cell homeostasis. However, the role of *Prmt5* in pancreatic islets, particularly in beta cells, remains unclear. A mouse model with an islet-specific knockout (KO) of the *Prmt5* gene was generated, and the influence of the *Prmt5* excision on beta cells was investigated via morphologic and functional studies. Beta cell function was evaluated by glucose tolerance test (GTT) and glucose-stimulated insulin secretion (GSIS) test. Beta cell proliferation was evaluated by immunostaining. Gene expression change was determined by real-time qPCR. Molecular mechanisms were investigated in beta cells *in vitro* and *in vivo* in *Prmt5* KO mice. The results show that islet-specific KO of *Prmt5* reduced expression of the insulin gene and impaired glucose tolerance and GSIS *in vivo*. The mechanistic study indicated that PRMT5 is involved in the regulation of insulin gene transcription, likely via histone methylation-related chromatin remodeling. The reduced expression of insulin in beta cells in the *Prmt5* KO mice may contribute to impaired glucose tolerance (IGT) and deficient GSIS in the mouse model. These results will provide new insights into exploring novel strategies to treat diabetes caused by insulin insufficiency.

Key Words

- ▶ PRMT5
- ▶ beta cell
- ▶ impaired glucose tolerance
- ▶ insulin transcription

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Introduction

Diabetes is a complex and heterogeneous disease accompanied by a deterioration of glycemic control (Ashcroft & Rorsman 2012, DeFronzo *et al.* 2015). Dysfunction of insulin-secreting beta cells, such as reduced insulin synthesis, reduces the amount of secreted insulin

or diminishes pulsatile secretion of insulin in response to a stimulus, contributing to the initiation of diabetes (Olson *et al.* 1993, Ashcroft & Rorsman 2012, Ferrannini & Mari 2014, Antonioli *et al.* 2015). Growing evidence suggests that epigenetic mechanisms are involved in regulating

beta cell dysfunction, beta cell age and the pathological process of diabetes (Pinney & Simmons 2010, Menzies *et al.* 2016, Bernstein *et al.* 2017). Several important epigenetic regulators, including DNA methyltransferases and histone-modifying enzymes (such as DNMT3A, EZH2, and p300), have been shown to regulate beta cell function (Chen *et al.* 2009, Fernandez-Zapico *et al.* 2009, Papizan *et al.* 2011, Dhawan *et al.* 2015, Rui *et al.* 2016).

Recently, a methyltransferase, protein arginine methyltransferase5 (PRMT5), was shown to regulate cells fate by diverse epigenetic means via influencing posttranslational modifications (Bedford & Clarke 2009, Yang & Bedford 2013). PRMT5, together with its cofactor MEP50, mediates the symmetric dimethylation of histones H2A and H4 at R3 and histone H3 at R8 (Pal *et al.* 2007, Girardot *et al.* 2014). The symmetric dimethylation of these histone residues generally generates repressive marks for gene transcription, and PRMT5 has been found in several transcriptional repressor complexes, including those containing SIN3A/HDAC, MBD2/NURD, and N-CoR/SMRT (Pal *et al.* 2003, Le Guezennec *et al.* 2006). Moreover, PRMT5 represses globin gene expression through recruitment of DNMT3A, indicating a potential crosstalk between histone arginine methylation and DNA methylation (Zhao *et al.* 2009, Rank *et al.* 2010). In addition to histones, PRMT5 regulates a number of non-histone proteins with functions in RNA processing, translation, signal transduction, apoptosis, and cell cycle regulation (Wei *et al.* 2014). Unexpectedly, PRMT5 has also been reported to activate the transcription of certain genes that are involved in adipogenic differentiation or skeletal muscle differentiation (Dacwag *et al.* 2007,

LeBlanc *et al.* 2012). We previously showed that menin, a crucial epigenetic regulator that controls islet homeostasis (Matkar *et al.* 2013, Feng *et al.* 2017a), interacts with PRMT5 to suppress cell proliferation, partly through regulating Hedgehog signaling (Gurung *et al.* 2013). However, the role of PRMT5 in beta cell proliferation and islet function has not yet been investigated *in vivo*.

Here, we developed *Prmt5* islet-specific conditional knockout (KO) mice and evaluated the impact of *Prmt5* KO on islets. Notably, we found that *Prmt5* KO impaired glucose tolerance (GT) and glucose stimulation-induced insulin secretion (GSIS). PRMT5 promotes insulin transcription and histone methylation at the insulin gene locus. The reduced insulin expression in beta cells in the *Prmt5* KO mice likely contributes to the impaired GT and defective GSIS in the mouse model.

Materials and methods

Mice and cell line

Cre/loxP strategy was used to develop *Prmt5* islet-specific conditional KO mouse (Fig. 1). The mice with *Prmt5^{tm1a}* (EUCOMM) *Wtsi* (from the Wellcome Sanger Institute, Cambridge, UK) were bred with FLPeR mice (B6.129S4-Gt (ROSA) 26Sortm1 (FLP1) Dym/Rain J (from the Jackson Laboratory), resulting in excision of the FRT-flanking lacZ and neomycin sequence. Subsequent breeding between F1 mice generated a population of progeny that included the desired *Prmt5^{loxP/loxP}* (l/l) genotype. The mice with *Prmt5^{l/l}* and the mice expressing Pdx1-driven Cre-ER recombinase

Figure 1

Diagrams of generation of *Pdx1-Cre-ER, Prmt5^{loxP/loxP}* mice. (A) The mice with *Prmt5^{tm1a}* (EUCOMM) *Wtsi* allele consisting of FRT sites followed by a LacZ sequence and loxP sites, were bred with FLPeR mice (B6.129S4-t (ROSA) 26Sortm1 (FLP1) Dym/Rain J), resulting in excision of the FRT-flanking lacZ and neomycin sequence. The F1 progeny were heterozygous for *Prmt5* (*Prmt5^{loxP/loxP}*). Subsequent breeding between F1 mice resulted in a population of progeny that included the desired genotype *Prmt5^{l/l}*. Then, *Pdx1-CreER; Prmt5^{l/l}* mice were generated by intercrossing *Pdx1-CreER* mice with *Prmt5^{l/l}* mice. (B, C) The genotypes were verified by PCR using tail DNA.

were intercrossed to generate homozygous *Pdx 1-CreER*; *Prmt5*^{fl} mice. The mice with *Pdx 1-CreER* genotype was initially obtained from Dr Melton's group (Gu *et al.* 2002). They were extensively used in our previously published work and shown tissue-specific and tamoxifen (TAM)-induced *Men1* deletion in islets (Yang *et al.* 2010). The pups from the breeding between *Prmt5*^{fl} and *Pdx 1-CreER* mice were genotyped routinely by PCR using the tail genomic DNA for the floxed *Prmt5* and the *Pdx1-Cre-ER* transgenes.

Pdx 1-CreER; *Prmt5*^{fl} mice and control mice were administrated with TAM (MP Biomedicals, Santa Ana, CA, USA) at 200mg/kg of body weight by gavage daily for two consecutive days, followed by 1 day off and then for another two consecutive days (Yang *et al.* 2010). Male mice with age-matched were used in this study, because male mice are more severely affected by type 2 diabetes than female mice generally. All mice were housed in a pathogen-free environment on a 12-h light/darkness cycle with *ad libitum* access to water and normal chow diet in the animal facility of University of Pennsylvania. All animal experiments and methods were approved by the Institutional Animal Care and Use Committee of University of Pennsylvania.

Rat insulinoma INS-1 cell line was obtained from Dr Xiaosong Ma (Kong *et al.* 2014), and the cells (passage 70–80) were maintained in RPMI-1640 medium (4.6mM glucose), supplemented with 10% (v/v) FBS (Sigma-Aldrich), 1mM sodium pyruvate, 10mM HEPES, 50μM β-mercaptoethanol, 100units/mL penicillin and 100μg/mL streptomycin at 37°C and 5% CO₂.

Physiological measurements

Blood glucose levels were assayed from tail vein blood. Glucose tolerance test (GTT; glucose at 2g/kg of body weight, i.p.) and GSIS (glucose at 3g/kg of body weight, i.p.) were performed on mice fasting overnight (16h) (Yang *et al.* 2010). In brief, for GTT, the mice were fasted for 16h and then injected intraperitoneally (i.p.) with glucose (2g/kg of body weight). Blood samples at 0, 15, 30, 60, 90, and 120min after the injection were obtained from tail-tip bleedings, and blood glucose levels were measured with a Glucometer (Lifescan, Wayne, USA). For GSIS, glucose (3g/kg of body weight) was injected i.p. and blood obtained by submandibular venipuncture was collected at 0, 15, and 30min in heparinized tubes. Serum insulin levels were measured by ELISA using a mouse insulin kit (Crystal Chem, Elk Grove Village, IL, USA).

Islet isolation and perfusion

Islets were isolated by pancreatic digestion, purified using a Ficoll-paque gradient (GE Healthcare). Immediately after collection, the purified islets were used for RNA extraction. For perfusion study, the islets need to be incubated 2 days at 37°C with 5% CO₂ before glucose stimulation.

Islets perfusion studies *in vitro* were carried out by Islet Cell Biology Core at University of Pennsylvania using an automated perfusion system. After 2-day incubation in PRMI1640 medium, islets were transferred into the perfusion system and subjected to 0, low (3mM) and high (16.7mM) glucose concentrations at 0min, 30th min and 50th min, and then switched back to 0mM glucose at 70min, and followed by a KCL (30mM) stimulation at 90th min for another 20min. Insulin in the medium was harvested automatically and measured with Radioimmunoassay and Biomarkers Core at University of Pennsylvania using ELISA.

Gene expression analysis by quantitative real-time PCR (qPCR)

Total RNA was isolated from mouse islets and beta cell lines using RNA Mini Kit (Qiagen) and cDNA prepared was using Superscript III Reverse transcriptase (Invitrogen, Life Technologies) according to manufacturer's instructions. cDNA was used for qPCR with specific primers (Supplementary Table 1, see section on [supplementary materials](#) given at the end of this article) in the presence of Power SYBR Master Mix (Qiagen) using 7900HT qPCR machine (Applied Biosystems). All analyses were done in triplicate. Relative mRNA levels were analyzed using ΔΔCt method.

Western blot analysis

Cells were collected and lysed with RIPA lysis buffer (Sigma), followed by centrifugation at 20,000g for 10min at 4°C. Protein concentration was determined by BCA assay (Sigma-Aldrich). Cellular proteins were separated by 4.0–20.0% SDS-PAGE (Genscript, Piscataway Township, NJ, USA) and then transferred to a PVDF membrane. The membrane was blocked by TBST with 5% fat free milk, and incubated with antibodies directed against the following proteins: Insulin (1:1000) (Cell Signaling Technology, Cat # 8138S); PRMT5 (1:1000) (Cell Signaling Technology, Cat # ab31751); and β-actin (1:10000) (Sigma-Aldrich, Cat # A5441). Immunoreactive proteins were visualized by

exposure to X-ray film. Protein bands were analyzed by image scanning.

Chromatin immunoprecipitation (ChIP)

INS-1 cells were plated at a density of 2×10^6 cells/10 cm tissue culture plate, and cultured for 36 h with each treatment condition. After collection of cells, ChIP assays were performed according to the manufacturer's instructions using a QuickChIP kit (Novus Biologicals, Centennial, CO, USA), as previously reported (Matkar *et al.* 2015, Feng *et al.* 2017b). Briefly, cells were fixed with 1% formaldehyde and then lysed according to the protocol in a ChIP lysis buffer with protease inhibitors, and cellular DNA was sheared with sonication. After clearing, this lysate was incubated with either control IgG or a specific primary antibody (4 µg) at 4°C overnight, and collected with protein A/G agarose beads. The antibodies used here include anti-PRMT5 (Cell Signaling Technology, Cat # ab31751), anti-BRG1 (Abcam Cat # ab110641), anti-H3R8 (Abcam, Cat # ab130740), and anti-IgG (Abcam, Cat # ab46540). The protein-DNA complexes were eluted from the beads and DNA was amplified by qPCR using primer pairs (Supplementary Table 1) specific to the insulin promoter. Reactions were done in duplicate, and results are normalized to input chromatin and are reported as % input $\pm$ s.d.

shRNA, Lentivirus packaging and infection

Lentiviral packing plasmids pMD2.G and psPAX2 were purchased from Sigma-Aldrich. shRNA plasmids for *Prmt5* was pLKO.1-puromycin backbone-based and obtained from the University of Pennsylvania Perelman School of Medicine High-Throughput Screening Core. To produce lentivirus, as previously reported (Matkar *et al.* 2015), 293T cells were transfected with pMD2.G, psPAX2, and the plasmid of *Prmt5* shRNA using Fugene 6 (Promega) according to the manufacturer's instructions. For all virus production, after collecting and filtering the virus, cells were then transduced. Twenty-four hours after completion of transduction, cells were then selected with puromycin for 72 h.

Immunofluorescence (IF) and morphometric analysis

Mouse whole pancreas tissue was fixed in 4% formalin and processed for paraffin embedding. Antigen retrieval was performed by microwave heating in antigen retrieval solution-citrate buffered (Abcam). Detection was done using fluorochrome-conjugated secondary antibodies (Becton Dickinson) according to manufacturer's

instructions as previously reported (Yang *et al.* 2010). The following primary antibodies were used: anti-Insulin (1:200) (Cell Signaling Technology, Cat # 8138S), anti-Prmt5 (1:200) (Cell Signaling Technology, Cat # ab31751), and anti-Ki67 (1:200) (Abcam, Cat # ab15580). Cell nuclear staining was performed using DAPI (Sigma-Aldrich, Cat # 10236276001). The sections were visualized using a Nikon Eclipse E800 fluorescent microscope with a CCD digital camera. Whole pancreas scanning was implemented using a Leica DMI6000B fluorescent microscope. Quantification of insulin-positive areas and staining intensity was performed with ImageJ software.

Statistical analysis

Significant differences between two groups were determined by Tukey's test. The ANOVA method was used for statistical analysis on multiple groups. Analysis was performed using the Prism GraphPad v4.0 (GraphPad Software Inc.). Values are expressed as mean $\pm$ s.d., of which a representative experiment is depicted in the figures. *P* values <0.05 were considered statistically significant.

Results

Generation of Lox-P-mediated *Prmt5* islet-specific conditional knockout (KO) mouse model

To generate the mouse strains in which we can conditionally delete the *Prmt5* gene in islets in a time or tissue-specific manner, the mice with *Prmt5^{fl}* genotype were crossed with mice expressing Pdx1-driven Cre-ER, a fusion protein between CRE (loxP site-specific DNA recombinase) and part of the estrogen receptor, the fusion protein can cleave the loxP sites specifically when 4-hydroxy tamoxifen (TAM) binds the ER moiety of CRE-ER, as previously described (Yang *et al.* 2010) (Fig. 1A). Three groups of mice with genotypes including *Pdx1-Cre-ER; Prmt5^{fl}*, *Prmt5^{fl}* (Control1), and *Pdx1-Cre-ER; Prmt5^{+/+}* (Control2) were identified using PCR (Fig. 1C). Then, these mice were fed with TAM, and *Prmt5* excision in pancreatic islets was determined by qPCR and immunostaining of PRMT5.

The islet-specific conditional KO of *Prmt5* leads to IGT and defective GSIS

PDX1 expression was shown to be generally limited to pancreatic islets and duodenal epithelium in the adult,

especially in beta cells and some delta cells (Miller *et al.* 1994, Peers *et al.* 1994, Peshavaria *et al.* 1994, Guz *et al.* 1995, Wu *et al.* 1997, Fu *et al.* 2013). Thus, the deletion of *Prmt5* induced by Pdx1-driven Cre-ER is supposed to occur in mouse islets. We set up three groups of mice: *Prmt5*^{+/l} (Control1), *Pdx1-Cre-ER; Prmt5*^{+/+} (Control2), and *Pdx1-Cre-ER; Prmt5*^{+/l}, started to gavage them with TAM, and monitored the blood glucose level as shown in Fig. 2A. After TAM treatment, the *Prmt5*^{+/l} (Control1) mice were designated as *Prmt5*^{+/l}, the *Pdx1-Cre-ER; Prmt5*^{+/+} (Control2) mice were designated as *Cre-ER*, and *Pdx1-Cre-ER; Prmt5*^{+/l} mice were designated as *Prmt5*^{Δ/Δ}, respectively. The bodyweight of the three groups of mice was measured weekly up to the ninth week post TAM administration, and no obvious difference in body weight was observed among the groups during the period of time (Fig. 2B). Histological examination of PRMT5 expression level in pancreas sections from the three different groups of mice using IF staining indicates an obvious deletion of PRMT5 in islets of the *Prmt5*^{Δ/Δ} mice compared to those in the other two groups of control mice (Fig. 2C). The staining result of PRMT5 on pancreas section from *Pdx1-Cre-ER; Prmt5*^{+/l} mice without TAM administration is similar with the PRMT5 staining of two control group mice (data not show), indicating no leaky effect of Pdx1driven Cre-ER on *Prmt5* excision. Consistently, PRMT5 mRNA level in purified mouse islets also showed a significant decrease in islets of the *Prmt5*^{Δ/Δ} mice as compared to that in control mice (Fig. 2D). Certain level (~ 20%) of the remaining *Prmt5* mRNA in islets from *Prmt5*^{Δ/Δ} mice was still detectable by qPCR, suggesting that in some islet cells from mice treated with TAM may still have one or

two alleles of the floxed *Prmt5*. Nevertheless, these results indicate that the Pdx-1-Cre-ER system we used here is a TAM inducible and effective to *Prmt5* excision.

To determine the effect of *Prmt5* KO on beta cell function *in vivo*, we performed the glucose tolerance test (GTT) on the mice at week 3, 6, and 9 post TAM administration. The result showed that no obvious change in glucose tolerance (GT) was observed among the three groups 3 weeks after TAM treatment. However, GT was gradually impaired in *Prmt5*^{Δ/Δ} mice from 6 to 9 weeks after *Prmt5* excision (Fig. 3A, B and C). Approximately two-fold increase in area under curve (AUC) was observed in *Prmt5* KO mice as compared to control mice at ninth week (Fig. 3C). In addition, fasting blood glucose level of the *Prmt5*^{Δ/Δ} group was only moderately higher than that in the control groups at ninth week (Fig. 3D), whereas random blood glucose number of the *Prmt5*^{Δ/Δ} group of mice was significantly higher than other groups of mice (Fig. 3E). To further examine whether PRMT5 deletion affects the GSIS, the above three groups of mice were injected with glucose, followed by detection of the blood insulin level. The results indicate that the control mice (i.e. *Prmt5*^{+/l} and *Cre-ER*) showed enhanced level of serum insulin following glucose uptake (Fig. 3F). However, *Prmt5* KO mice (*Prmt5*^{Δ/Δ}) showed markedly defective GSIS (Fig. 3F), concomitant with the marked IGT (Fig. 3B and C).

To further clarify the time course of the defect observed in *Prmt5* KO mice involving impaired insulin secretion, *in vitro* perfusion studies were performed in *Prmt5* KO and control islets isolated at the first and sixth weeks post TAM administration (Fig. 4A). The isolated islets were also used to extract RNA and do qPCR to test mRNA

Figure 2

Acute *Prmt5* excision in mice islets was induced by TAM treatment. (A) A schema of experimental design. *Prmt5*^{+/l}, *Pdx1-Cre-ER; Prmt5*^{+/+}, and *Pdx1-Cre-ER; Prmt5*^{+/l} mice (male mice, *n* = 5 for each group) were fed TAM at the age of 10 weeks. After TAM treatment, the *Prmt5*^{+/l} (Control1) mice were designated as *Prmt5*^{+/l}, the *Pdx1-Cre-ER; Prmt5*^{+/+} (Control2) mice were designated as *Cre-ER*, and *Pdx1-Cre-ER; Prmt5*^{+/l} mice were designated as *Prmt5*^{Δ/Δ}, respectively. Mice were euthanized at 10th week after TAM feeding. GTT were done before and at third, sixth, and ninth week post TAM treatment. (B) Body weight change for all experimental group mice after TAM treatment. (C) Immunostaining for PRMT5 and insulin on pancreas samples of three different groups of mice. Scale bar, 25 μm. (D) Comparison of PRMT5 mRNA level between isolated islets of control mice (*Prmt5*^{+/l} and *Cre-ER*) and *Prmt5* excision mice (*Prmt5*^{Δ/Δ}). ***P* < 0.01.

Figure 3

Islet-specific *Prmt5* KO in mice impaired GT and GSIS. (A, B, C) Glucose tolerance test (GTT, glucose at 2 g/kg of body weight, i.p.). All mice tested in experiment have been starved overnight (16 h) at third, sixth, and ninth week post TAM treatment ($n = 5$ for each group). (D, E) Fasting glucose number and random glucose number were tested on the three groups of the mice at ninth week post TAM administration. (F) *In vivo* glucose-stimulated insulin secretion (GSIS, glucose at 3 g/kg of body weight, i.p. $n = 5$ for each group) test. All mice tested in experiment have been starved overnight (16h) at 10th week after TAM treatment. ** $P < 0.01$.

level of PRMT5 and insulin. The results showed that the expression of *Prmt5* was reduced to about 50% relative to control mice at the first week after TAM treatment (Fig. 4B). Unexpectedly, insulin mRNA level was declined by approximately 50% compared to control mice (Fig. 4C). For the perfusion results, insulin release in response to 16.8mM glucose was markedly reduced in *Prmt5* KO mice (Fig. 4D). For the islets isolated from the mice at

sixth week post TAM administration, the mRNA level of PRMT5 and insulin decreased even further to about 25% of the control (Fig. 4E and F). The insulin secretion was also obviously reduced as compared with the control mice (Fig. 4G).

As *Prmt5* KO led to significant reduction of insulin synthesis in beta cells, one may expect to observe marked IGT and quite low fasting insulin level.

Figure 4

Islet-specific *Prmt5* KO in mice downregulated the insulin expression in islets and impaired the glucose-stimulated insulin release in perfusion studies. (A) schema of experimental design. *Prmt5*^{fl/fl} and *Pdx-1-Cre-ER; Prmt5*^{fl/fl} mice (male mice, $n = 8$ for each group) were administered with TAM by gavage at the age of 10 weeks. Islets were isolated from the mice at first week and sixth week post TAM administration. Three mice were euthanized for each of these two different time points. The islets used for doing qPCR and perfusion studies were a pool of three individual mice. (B, C) mRNA level of PRMT5 and insulin in islets isolated from *Prmt5*^{fl/fl} and *Pdx-1-Cre-ER; Prmt5*^{fl/fl} mice at first week post TAM administration. (D) *In vitro* perfusion studies on the islets isolated at first week post TAM administration. (E, F) mRNA level of *Prmt5* and insulin in islets isolated from *Prmt5*^{fl/fl} and *Pdx-1-Cre-ER; Prmt5*^{fl/fl} mice at sixth week post TAM administration. (G) *In vitro* perfusion studies on the islets isolated at sixth week post TAM administration. * $P < 0.05$; ** $P < 0.01$.

Although we did observe significantly impaired GT, we did not see dramatically reduced fasting insulin level in the *Prmt5* KO mice. We predicted that some compensatory mechanisms such as beta cell proliferation may occur *in vivo* as a response to the reduced insulin release. To test this, we performed histological studies to verify whether beta cell proliferation was induced after *Prmt5* excision. The pancreas samples were selected from the mice treated with TAM at variant time (i.e. first, sixth, and ninth post TAM administration) and were fixed to make tissue sections. IF staining results showed that a very clear increasing of Ki67 staining, a biomarker for cell proliferation, on the pancreatic islets from *Prmt5* KO mice treated with TAM (Supplementary Fig. 1). However, the increased Ki67 expression did not occurred immediately following TAM-induced *Prmt5* excision in islets, but observed at and after sixth week. The quantification of the percentage of insulin staining area over whole pancreas area on the pancreas tissue sections from the mice at the ninth week post TAM treatment also indicated an increasing beta cell mass in *Prmt5* KO mice relative to control mice (Supplementary Fig. 2). Collectively, the islet-specific conditional KO of *Prmt5* leads to IGT and defective GSIS. But the beta cell proliferation we found here seems a result of compensation to impaired insulin release and glucose intolerance when deleted PRMT5 in islets. This compensatory proliferation of beta cell would contribute to antagonize insulin expression decrease-mediated IGT.

The islet-specific conditional *Prmt5* KO reduced intracellular insulin expression in beta cells

Several conditions have been implicated in impaired insulin response under glucose stimulation, such as low level of insulin synthesis, inefficiency of insulin secretion, and even non-response to glucose stimulation (Fu *et al.* 2013). Here, we found that insulin mRNA level was downregulated in mouse islets after *Prmt5* excision (Fig. 4C and F). However, whether PRMT5 regulates insulin transcription is not clear. To this end, we investigated the effect of *Prmt5* KO in influencing intracellular synthesis of insulin in the mouse islets using qPCR, Western blot, and immunostaining. Notably, qRT-PCR showed a significant decline of insulin mRNA level in mouse islets with *Prmt5* KO (Figs 4C and F, 5A). Consistent with reduced insulin mRNA, the proinsulin protein level was also downregulated as compared with the control groups (Fig. 5B, C and D). Further, the insulin IF staining also showed lower insulin expression in the pancreatic islets from *Prmt5* KO mice (Fig. 5E). The quantification with ImageJ showed that the insulin level was moderately, yet significantly lower in *Prmt5* KO mice than that in the control mice (Fig. 5F), correlating with the lower mRNA and protein levels in the islets (Fig. 5A, B, C and D). These results indicate that the *Prmt5* excision led to reduced insulin expression in islets.

To further confirm these results, the *Prmt5* expression was downregulated by specific shRNA in INS-1 cells. After PRMT5 knockdown (KD), the expression level of insulin

Figure 5

Islet-specific *Prmt5* deletion in mice reduced intracellular insulin level in beta cells. (A) Insulin mRNA level in islets of control and *Prmt5* KO mice was determined by qPCR. (B) Western blot for detecting the protein level changes of PRMT5 and proinsulin in islets of *Prmt5* KO mice and control mice. (C, D) Quantification of PR5 and proinsulin expression levels in Western blot assay. (E) Immunostaining for insulin and nucleus (DAPI) (Scale bar, 25 μm) and representative images from control and *Prmt5* KO groups. (F) Quantitation and comparison of insulin fluorescence density between control and *Prmt5* excision mice groups. It was calculated from the integrated density data of insulin fluorescence signals measured using ImageJ software. (G, H) *Prmt5*-specific shRNA treatment on INS-1 cells suppresses the intracellular expression of PRMT5 and insulin in mRNA level (qPCR) and protein levels (Western blot). (I, J) PRMT5 enzyme activity specific inhibitor EPZ015666 treatment decreases the intracellular mRNA and protein levels of proinsulin in INS-1 cells. **P* < 0.05; ***P* < 0.01.

was decreased sharply in the mRNA level (Fig. 5G) and also in protein level (Fig. 5H). Consistent with these findings, treating the INS-1 cells by a small molecular compound, EPZ015666, a specific inhibitor of PRMT5 enzyme activity (Duncan *et al.* 2016, Jin *et al.* 2016, Scaglione *et al.* 2018), also caused a reduced expression of insulin at both the mRNA level (Fig. 5I) and protein level (Fig. 5J). These results suggest that the PRMT5 deletion or inhibition in islets reduced insulin synthesis.

PRMT5 binds to the promoter of insulin gene and correlates with dimethylation of target histones and binding of an ATP-dependent chromatin remodeling enzyme

It is well known that PRMT5 functions in transcriptional repression (Karkhanis *et al.* 2011, Tae *et al.* 2011). However, some previous reports also suggested that PRMT5 works as a cofactor to activate gene transcription, and this effect is closely linked to the methylation of histone H3 on Arg8 and chromatin remodeling (Dacwag *et al.* 2007, LeBlanc *et al.* 2012). To test how PRMT5 upregulates the insulin gene transcription, we performed chromatin immunoprecipitation (ChIP) with control or PRMT5 specific shRNA-treated INS-1 cell lines. The results showed that PRMT5, dimethylated H3R8, and BRG1 were enriched at the insulin gene promoter in INS-1 cells treated with control-shRNA (Fig. 6A). In contrast, in *Prmt5* KD cells, the bindings of PRMT5 and BRG1, as well as the level of dimethylated H3R8 at the insulin promoter were significantly reduced (Fig. 6A). Likewise, knockdown of PRMT5 in INS-1 cells led to the significant reduction of polymerase II (Pol II) binding, which is crucial for gene transcription (Fig. 6B). As a control, PRMT5 KD did not significantly affect the level of total histone 3 level at the insulin gene promoter (Fig. 6C). These results indicate that PRMT5 binds to the promoter of the insulin gene, and this binding is crucial for demethylation of H3R8 and BRG1 binding to the insulin promoter. It is likely that PRMT5 directly promotes insulin gene transcription by dimethylating H3R8 and regulating the binding level of ATP-dependent chromatin remodeling enzymes (Fig. 6D), as PRMT5 promotes gene transcription involved in adipogenic differentiation or skeletal muscle differentiation (Dacwag *et al.* 2007, LeBlanc *et al.* 2012).

In addition to the direct regulation on insulin gene by PRMT5, we also examined the impact of *Prmt5* excision on transcription level of some insulin secretion-related genes in mouse islets. The results indicated that *Prmt5* excision downregulated gene transcription level of *Pdx1*

Figure 6

PRMT5 binds to the insulin promoter, and PRMT5 KD reduces dimethylation of H3R8 at the promoter and represses insulin transcription. ChIPs were performed using antibodies against PRMT5, dimethylated (diMe) H3R8, BRG1 and Polymerase II in control and PRMT5 KD INS-1 cell lines treated by *Prmt5* specific shRNA. (A) ChIP results demonstrate that binding of PRMT5 and dimethylated H3R8 at the insulin promoter in control and PRMT5 KD INS-1 cells. IgG-binding level to the insulin promoter was used as a negative control. (B) ChIP assay detects Pol II binding at the insulin promoter in control and PRMT5 KD INS-1 cells. (C) Binding of the total histone 3 levels at insulin promoter was found. (D) A suggested model for regulation of insulin transcription by PRMT5 in beta cells. * $P < 0.05$; ** $P < 0.01$.

and *Foxa2*, but did not influence gene transcription of *Gck*, *Glut2*, *Sur 1* and *Kir 6.2*, the genes that affects glucose sensing or insulin secretion (Supplementary Fig. 3). This result implies that some other players, which were also regulated by PRMT5, are possibly involved in impaired insulin secretion besides the directly epigenetic-regulated insulin gene transcription.

Discussion

Epigenetic regulation plays a crucial role in regulating the homeostasis and function of islets. However, it has been unclear how PRMT5 regulates islets *in vivo*. Our current studies revealed that targeted *Prmt5* excision in islets led to IGT and defective GSIS. Moreover, PRMT5 is essential for optimal insulin expression *in vitro* and *in vivo*, and PRMT5 binds the insulin promoter. To our knowledge, this is the first time that definitive evidence showed PRMT5 directly promotes insulin synthesis in beta cells, and PRMT5 is enriched at the insulin promoter. Thus, PRMT5-mediated

optimal insulin expression may be an important factor that contributes to IGT and defective GSIS in *Prmt5* islets-specific conditional KO mice model.

In current study, *Pdx-1* promoter driven Cre-ER system was used to develop an islet-specific KO of *Prmt5*. *Pdx 1*-CreER-containing mice were initially developed by Dr. Melton's group and used to explore the role of *Pdx 1* in islets development (Gu *et al.* 2002). This system was extensively used in our previously published work and shown tissue-specific and TAM-induced *Men1* deletion in islets of adult mice (Yang *et al.* 2010). Here, this system also showed an effectively KO of target gene, i.e., *Prmt5* in islet. It provided a valid platform to help us explore the functions of *Prmt5* particularly in pancreatic islets.

PRMT5 promotes insulin transcription in beta cells

Although emerging evidence suggests that PRMT5 functions as an oncogene and is well-recognized as a therapeutic target in cancer treatment (Morettin *et al.* 2015, Zhang *et al.* 2015, Tarighat *et al.* 2016, Yap *et al.* 2017), its role in regulating metabolism *in vivo* is not well defined (Tsai *et al.* 2013). PRMT5 was found to stimulate CREB phosphorylation via increases in histone H3R2 methylation that enhanced chromatin accessibility at gluconeogenic promoters in hepatocytes (Tsai *et al.* 2013). A recent study also shows that dysfunction of PRMT5 in mice with HFD-induced hepatic steatosis upregulates the expression of the master transcription factors, PPAR α and PGC-1 α , which are involved in fatty acid metabolism and mitochondrial biogenesis (Huang *et al.* 2018). We successfully knocked out the *Prmt5* gene in mouse islets and observed the IGT and defective GSIS response in mice. These findings indicate that PRMT5 is involved in regulating glucose metabolism *in vivo*, at least partly via regulating the synthesis of insulin in islets beta cells.

To investigate the mechanism underlying the PRMT5-mediated regulation of insulin transcription, we found PRMT5, dimethylated H3R8, and BRG1 enriched at the promoter of insulin using ChIP assay. Notably, *Prmt5* KO led to the reduction of the amount of PRMT5, dimethylated H3R8 and BAG1 at the promoter. These findings indicate that PRMT5 may directly promote insulin gene transcription by dimethylating H3R8 and increase the binding level of BRG1, a component of an ATP-dependent chromatin remodeling enzyme, to insulin promoter to remodel chromatin structure there. This is consistent with previous reports that PRMT5 activated genes transcription which involved in adipogenic differentiation or skeletal muscle differentiation

(Dacwag *et al.* 2007, LeBlanc *et al.* 2012). However, more detail mechanism should be elucidated in further experiments to support PRMT5 epigenetically regulating insulin transcription in pancreatic beta cells.

Besides the direct regulation of insulin transcription by PRMT5, it is well known that the insulin mRNA is also impacted by various diabetogenic stressors, for example, glucotoxicity, oxidative/ER stress, and inflammation. Here, as we observed an obvious IGT in *Prmt5* KO mice, the decreased insulin mRNA level may be one result of the IGT. However, about 50% decline of the insulin expression level in islets isolated at the first week post TAM treatment help us to exclude that IGT is a cause to the downregulated insulin transcription in *Prmt5* KO mice, as the IGT in *Prmt5* KO mice just took place at the sixth week after TAM administration, but the reduction of insulin expression occurred within 1 week following *Prmt5* deletion (Fig. 4). Nevertheless, it remains to be further investigated on how PRMT5 deletion leads to IGT.

Prmt5 KO influences expression of the insulin secretion-related genes

In addition to inadequate synthesis of insulin, reduced uptake of glucose and impaired insulin secretion are also involved in reduced insulin secretion after glucose stimulation (Fu *et al.* 2013). Previous studies have shown that glucose sensors including glucose transporter 2 (GLUT2) and glucokinase (GCK) play crucial roles in response to blood glucose uptake and utilization (Janssen *et al.* 1994, Thorens 2015). As to the regulation of insulin secretion, the priming and fusion of insulin-containing granules precede insulin exocytosis and secretion. This event is triggered by elevation of intracellular [Ca²⁺] (Gauthier & Wollheim 2008, Ashcroft & Rorsman 2012). Sulfonylurea receptor1 (SUR1) was involved in the regulation of Ca²⁺-induced exocytosis by interacting with a Ca²⁺ sensor, Piccolo (Fujimoto *et al.* 2002) and a cAMP-binding protein called CAMPS (Ozaki *et al.* 2000). Previous studies also showed that the ATP-sensitive potassium channel (KATP channel) is an essential ion channel involved in glucose-induced insulin secretion. The deletion of the components of KATP channel, SUR 1 and KIR6.2, will lead to a defective GT (Winarto *et al.* 2001). In addition, FOXA2 has been identified as an essential activator of genes that function in multiple pathways governing insulin secretion (Lantz *et al.* 2004, Wolfrum *et al.* 2004).

Our results show that *Prmt5* KO did not reduce the expression of GLUT2 and GCK in islets. The transcription

factors that upregulate GLUT2 and GCK expression, PDX1 and PPAR- γ (Im *et al.* 2006), was not significantly changed when downregulated PRMT5 expression. These results suggest that *Prmt5* KO may not cause IGT and defective GSIS via influencing glucose uptake and phosphorylation by glucose kinase in beta cells. Nevertheless, we could not rule out the possibility that *Prmt5* deletion influences insulin secretion through regulating other processes of beta cell biology such as ion-channel-regulated insulin secretion, even though our results show that *Prmt5* KO did not downregulate the *Kir6.2* and *Sur-1* gene (Supplementary Fig. 3). And further studies are also required to determine whether *Prmt5* KO regulated FOXA2 expression influences insulin secretion as we found that the *Foxa2* gene mRNA level was reduced after *Prmt5* KO (Supplementary Fig. 3).

Beta cell proliferation following *Prmt5* excision may be a compensatory mechanism to control blood glucose level against impaired insulin synthesis

As *Prmt5* KO led to significant reduction of insulin synthesis in beta cells, marked IGT and quite low fasting insulin level should be observed in *Prmt5* KO mice. In this study, we did observe significantly IGT, but we did not see dramatically reduced fasting insulin level in the *Prmt5* KO mice. In this regard, we found that *Prmt5* excision resulted in a gradually increased proliferation of beta cells in mouse islets. In our previous study, we found that PRMT5 interacts with menin to epigenetically suppress Hedgehog signaling by imparting the repressive H4R3m2s mark at the *Gas1* promoter (Gurung *et al.* 2013). It suggests that PRMT5 is a suppressor to beta cell proliferation. In the current study, our *in vivo* data did not show an immediately proliferation of beta cell after PRMT5 excision, rather a gradual and late increase in proliferation following development of IGT. The qPCR data did not show that PRMT5 deletion increases the expression level of *Gas-1* in islets as observed in the insulinoma cells (Gurung *et al.* 2013) (Supplementary Fig. 3). Thus, the beta cell proliferation after *Prmt5* KO in mouse islets seems a compensatory response to defective insulin secretion. On the other hand, we cannot rule out the possibility that acute *Prmt5* excision may lead gradual and slow epigenetic changes in chromatin that results in enhanced beta cell proliferation.

In addition, some studies recently proved an inverse relationship between beta cell proliferation and function (Klochender *et al.* 2016, Puri *et al.* 2018). The data we obtained in this study, that is, the early inhibition

of insulin transcription and late happen of beta cell proliferation in *Prmt5* KO mice, may help us rule out the possibility for that the defective insulin secretion is a direct result of beta cell proliferation.

Collectively, we evaluated the effect of PRMT5 deletion in beta cells on beta cell function *in vivo*. Slight increase in fasting blood glucose level and markedly IGT in mice with *Prmt5* excision in islets were observed. This phenotype may reflect a compensatory outcome of enhanced beta cell numbers as a result of deficient insulin synthesis in *Prmt5* KO mice. The net effect of *Prmt5* KO may explain that the *Prmt5* KO mice showed IGT and insufficient GSIS due to reduced insulin synthesis in beta cells; yet, the relatively normal fasting blood insulin level resulting from the increased islet mass to maintain background level of insulin. Given the important effect of PRMT5 on insulin synthesis by these studies, these findings will enhance the understanding of beta cell biology. Moreover, these results will likely provide new insights into exploring novel strategies to treat diabetes caused by insulin insufficiency.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/JOE-19-0268>.

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.

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Author contribution statement

J M, X H, and X H conceived and designed the studies, and performed the analysis and interpretation of the data. J M, X H, Y C, K O, K M S, Y W, B G, Z F, and B W K performed the experiments. J M and X H drafted the manuscript and all other authors revised it. All authors approved the final version of the paper.

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