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# Noninvasive Monitoring of $\beta$ -Cell Mass and Fetal $\beta$ -Cell Genesis in Mice Using Bioluminescence Imaging

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**Abstract:** Bioluminescence imaging (BLI) has been applied in gene therapy and research to screen for transgene expression, progression of infection, tumor growth and metastasis, and transplantation. It enables real-time and relatively noninvasive localization and serial quantification of biological processes in experimental animals. In diabetes research, BLI has been employed for the quantification of  $\beta$ -cell mass, monitoring of islet graft survival after transplantation, and detection of reporter gene expression. Here, we explore the use of BLI in a transgenic mouse expressing luciferase under the control of the mouse insulin 1 promoter (MIP-Luc-VU). A previous report on MIP-Luc-VU mice showed luminescence intensities emitted from the islets correlated well with the number of islets *in vitro* and *in vivo*. In this study, we showed MIP-Luc-VU mice fed a high fat diet for 8 weeks gave rise to a greater bioluminescent signal than mice fed a regular diet for the same period of time. Conversely, there was a strong reduction in the signal observed in diabetic *Mafa*-deficient/*Mafk*-transgenic mutant mice and streptozotocin-treated mice, reflecting the loss of  $\beta$ -cells. Furthermore, we were able to monitor fetal  $\beta$ -cell genesis in MIP-Luc-VU mice during the late gestational stage in a noninvasive and repetitive manner. In summary, we show that bioluminescence imaging of mice expressing a  $\beta$ -cell specific reporter allows detection of changes in  $\beta$ -cell mass and visualization of fetal  $\beta$ -cell neogenesis in uteri.

**Key words:**  $\beta$ -cell genesis, bioluminescence imaging, diabetes mellitus, MIP-Luc-VU

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## Introduction

Insulin is a 51-amino acid peptide hormone exclusively produced by pancreatic  $\beta$ -cells that plays a pivotal role in the maintenance of glucose homeostasis. Postnatal growth of the  $\beta$ -cell mass in mice derives from proliferation of preexisting  $\beta$ -cells rather than *de novo* differentiation of pluripotent stem cells [7]. In rodents, the number of  $\beta$  cells increases during the first year of life to compensate for increased metabolic needs [3]. However, the rate of  $\beta$ -cell replication decreases with

age from ~20% per day in pups to ~10% per day at weaning and 2–5% per day in young adults [3].

Type 1 or insulin-dependent diabetes mellitus results from a deficiency in insulin due to autoimmune-mediated destruction of  $\beta$ -cells [2]. In type 2 or non-insulin-dependent diabetes, which is characterized by chronic insulin resistance and a progressive decline in  $\beta$ -cell function, a decrease in the  $\beta$ -cell mass also contributes to disease initiation and progression [4]. Therefore, estimation of  $\beta$ -cell mass in both types of diabetes using positron emission tomography (PET) or nuclear mag-

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netic resonance imaging (MRI) could facilitate the study of disease pathophysiology and the effects of therapeutic interventions [10, 20].

The conventional method to assess  $\beta$ -cell mass in experimental animals involves analysis of histological sections over time. Recent progress in the development of noninvasive methods to monitor changes in  $\beta$ -cell mass has provided a powerful complement to this conventional assay. An increasing number of studies have shown that optical techniques such as bioluminescence imaging (BLI) can be used to quantify  $\beta$ -cell mass. The bioluminescent signal from the islets expressing a luciferase reporter has been shown to correlate with  $\beta$ -cell mass and can therefore serve as an indicator of changes in  $\beta$ -cell mass [8, 13].

A previous report on luminescence reporter mice that express luciferase under the control of the mouse insulin promoter [FVB/N-*Tg(Ins1-luc)VUPwrs/J*, named MIP-Luc-VU] showed luminescence intensities emitted from the islets correlated well with the number of islets *in vitro* and *in vivo* through islet transplantation into immunocompromised mice [21]. In this study, we measured the change in BLI signal intensity in response to augmentation or diminution of  $\beta$ -cell mass in MIP-Luc-VU mice. We also examined whether BLI could be used for identification of fetal  $\beta$ -cell genesis in uteri. We found that BLI could detect the increase in  $\beta$ -cell mass resulting from an 8-week high-fat diet. Furthermore, BLI could detect the reduction in  $\beta$ -cell mass resulting from streptozotocin (STZ) administration or generation of MIP-Luc-VU mice that were *Mafa*-deficient (ICR-*Mafa*<sup>sm1Staka</sup>/*Mafa*<sup>sm1Staka</sup>, *Mafa*<sup>-/-</sup>) on a *Mafr*-transgenic [ICR-*Tg(RIP-Mafr)1Staka*, *Mafr*<sup>+</sup>] background (*Mafa*<sup>-/-</sup>*Mafr*<sup>+</sup>). The transport of STZ into  $\beta$  cells by a glucose transporter, *Glut2*, leads to increased production of reactive oxygen species and cell death [5]. *MafA* is a transcription factor belonging to the large *Maf* family and is necessary for functional  $\beta$ -cell maturation through activation of the expression of *Ins1*, *Ins2*, *Pdx1*, *Glut2*, and *Beta2* [1, 23]. *MafK* is a transcription factor belonging to the small *Maf* family, whose members function as transcriptional repressors or form heterodimeric complexes that serve as transcriptional coactivators owing to a lack of distinct activation domains. Double mutant *Mafa*<sup>-/-</sup>*Mafr*<sup>+</sup> mice exhibit nearly complete lack of  $\beta$ -cells at birth and present with early onset of diabetes accompanied by a kidney disease that resembles human diabetic nephropathy [17]. Both models result in severe

$\beta$ -cell loss and dysfunction and insulin deficiency. Finally, we showed fetal  $\beta$ -cell neogenesis could be detected in uteri by BLI of pregnant wild-type mice that had been mated with MIP-Luc-VU mice.

## Materials and Methods

### Animals

Transgenic mice expressing luciferase under the control of the mouse insulin 1 promoter [FVB/N-*Tg(Ins1-luc)VUPwrs/J*, Stock number 007800, MIP-Luc-VU] were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) [21]. All mice used in the study was backcrossed to Jcl:ICR (CLEA Japan, Tokyo, Japan) more than four generations [ICR-*Tg(Ins1-luc)VUPwrs/J*]. *Mafa*<sup>-/-</sup> and *Mafr*<sup>+</sup> mice were generated as described previously [18, 23]. All mice were maintained in specific pathogen-free conditions in the Laboratory Animal Resource Center, University of Tsukuba. All experiments were performed according to the Guide for the Care and Use of Laboratory Animals at the University of Tsukuba and approved by the Institutional Review Board of the University.

### Bioluminescence imaging of MIP-Luc-VU mice

To detect bioluminescence of MIP-Luc-VU mice using an IVIS Spectrum (Caliper, Alameda, CA, USA), 50  $\mu$ g of D-luciferin (Promega, Madison, WI, USA) per kilogram of body weight was injected intraperitoneally (i.p.) or intravenously (i.v.) and imaged for 1 min 10 min later. Equal area regions of interest (ROI) were centered over the bioluminescent region, and bioluminescence intensity was quantified as the sum of all detected photon counts per second within an ROI by using the Living Image software (Caliper). The *Mafa*<sup>-/-</sup> MIP-Luc-VU mice were obtained from interbreeding of ICR-*Mafa*<sup>sm1Staka</sup>/*Mafa*<sup>+</sup> (*Mafa*<sup>+/-</sup>) and *Mafa*<sup>+/-</sup> MIP-Luc-VU mice, and *Mafa*<sup>-/-</sup>*Mafr*<sup>+</sup> MIP-Luc-VU mice were generated from interbreeding of *Mafa*<sup>+/-</sup>*Mafr*<sup>+</sup> mice and *Mafa*<sup>+/-</sup> MIP-Luc-VU mice. For imaging of fetal  $\beta$ -cell development, pregnant wild-type ICR mice fertilized by MIP-Luc-VU mice were injected with 5  $\mu$ g per weight of D-luciferin intravenously at embryonic days (E) 14.5, 16.5, and 18.5 and imaged for 3 min 20–30 min later.

### High-fat diet

Male MIP-Luc-VU mice were fed either a control regular diet (RD) or a high-fat diet (HFD) consisting

62.2% fat, 19.6% carbohydrates, and 18.2% protein on a calorie basis (Oriental Yeast, Tokyo, Japan) for 8 weeks beginning at 6 weeks of age. Fasting blood glucose levels in both groups of mice were measured using a DRI-CHEM 3500 (Fujifilm, Tokyo, Japan).

#### *Histological analysis*

Mice fed either a RD or a HFD for 8 weeks were euthanized, and pancreatic tissues were removed. Tissues fixed in 10% formalin were embedded in paraffin, and each section was stained with hematoxylin and eosin. Islet area and total pancreas area of randomly chosen cross sections were measured using a Biorevo BZ-9000 microscope (Keyence, Osaka, Japan) and the BZ-II Analyzer software (Keyence). Relative islet area (%) was determined as the islet:pancreas ratio. For immunohistochemical analyses, tissue sections were incubated with guinea pig anti-insulin antibody (Abcam, Cambridge, MA, USA) and rabbit anti-glucagon antibody (Millipore, Billerica, MA, USA), anti-somatostatin antibody (Invitrogen, Camarillo, CA, USA), and anti-pancreatic polypeptide antibody (Dako, Glostrup, Denmark) for 8 h at 4°C. The antigens were visualized using appropriate secondary antibodies conjugated with Alexa488 and Alexa594 with nuclear staining using diaminidino-2-phenylindole (DAPI) (Invitrogen).

#### *Diabetes induction*

MIP-Luc-VU mice at 8 weeks of age were rendered diabetic by an intraperitoneal injection of streptozotocin (STZ, Sigma, St. Louis, MO, USA) at a dose of 200 mg/kg body weight in 0.1 M citrate buffer (pH 4.5).

#### *Statistical analysis*

Results are expressed as means  $\pm$  standard error of the mean. Statistical significance was calculated by paired or unpaired Student's *t*-test. *P* values of less than 0.05 were considered significant.

## **Results**

#### *Noninvasive monitoring of changes in $\beta$ -cell mass*

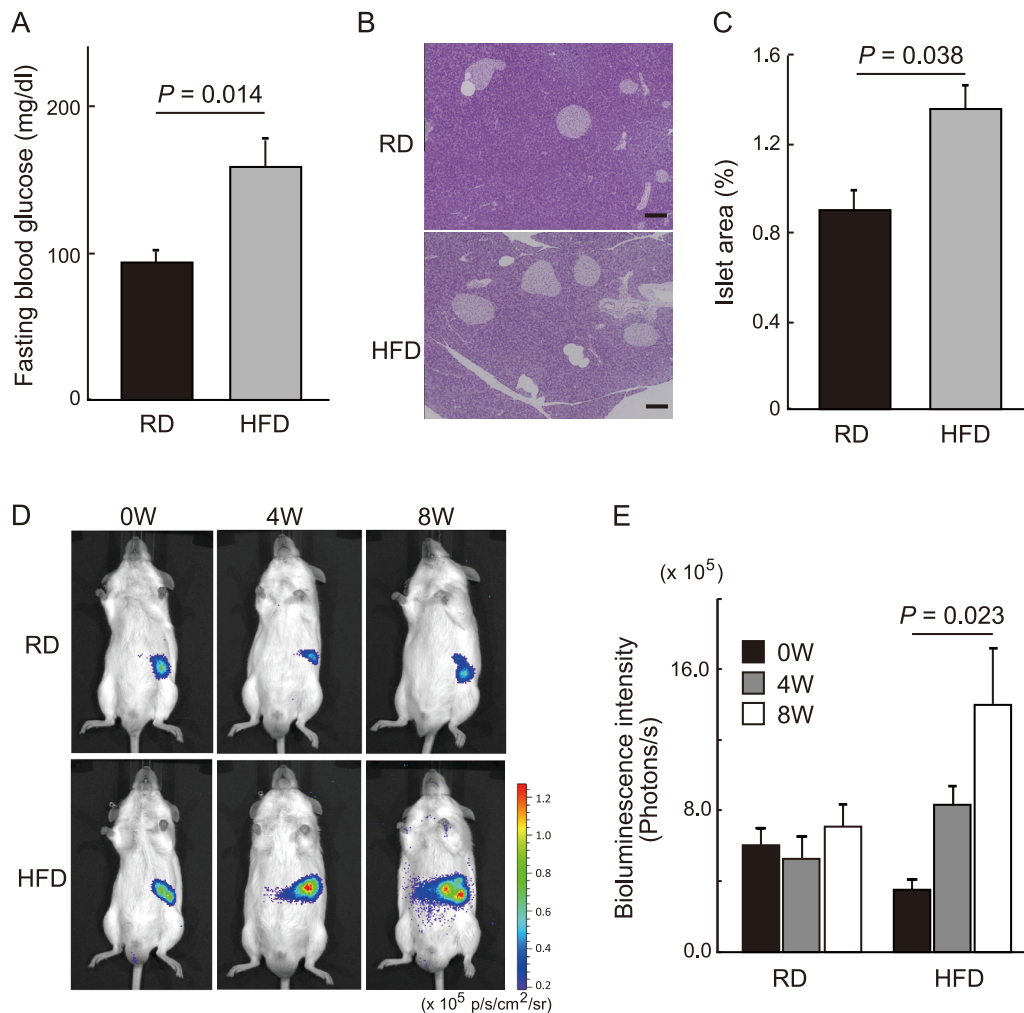
Male MIP-luc-VU mice fed a HFD for 8 weeks developed hyperglycemia (RD and HFD:  $95 \pm 7.2$  and  $157 \pm 18.4$  mg/dl, respectively,  $n=7$  in each group,  $P=0.014$ ) (Fig. 1A). After 8 weeks on a HFD, histological sections of pancreata also showed islet hypertrophy and a significant increase in relative islet area in HFD-fed mice

( $1.37 \pm 0.12\%$ ,  $n=3$ ) compared with RD-fed mice ( $0.88 \pm 0.12\%$ ,  $n=4$ ,  $P=0.038$ ) (Fig. 1B and 1C). The bioluminescence intensity observed in the HFD group ( $n=7$ ) increased from  $3.53 \pm 0.58 \times 10^5$  photons/s at week 0 to  $1.41 \pm 0.32 \times 10^6$  photons/s following 8 weeks of HFD administration, likely reflecting an increase in islet area (paired *t*-test,  $P=0.023$ ) (Fig. 1D and 1E). In contrast, there was little change in bioluminescence intensity observed in the control RD group over this period ( $6.06 \pm 0.96 \times 10^5$  photons/s at week 0 to  $7.11 \pm 3.13 \times 10^5$  photons/s following 8 weeks).

Next, we examined the ability of BLI to monitor the decrease in  $\beta$ -cell mass in two different models of  $\beta$ -cell destruction. We first observed a decline in BLI intensity following STZ injection into MIP-Luc-VU mice (Fig. 2A and 2B). By the fourth day after STZ treatment, signal intensity was reduced by 18.7% (from  $4.06 \pm 0.49 \times 10^5$  photons/s to  $7.60 \pm 0.24 \times 10^4$  photons/s,  $n=5$ ). Comparing STZ-treated mice with control mice, BLI signal was significantly lower in STZ-treated mice at day 4 (Control:  $4.34 \pm 1.12 \times 10^5$  photons/s,  $P=0.014$ ). In the second set of experiments, we generated *Mafa*-deficient and *Mafk*-transgenic (*Mafa*<sup>-/-</sup>*Mafk*<sup>+</sup>) mice that expressed the MIP-Luc transgene to monitor the changes in  $\beta$ -cell mass in a genetic diabetes models (Fig. 2C and 2D). We measured the intensity of the BLI signal in 6 week-old MIP-Luc-VU ( $n=8$ ), *Mafa*<sup>-/-</sup>MIP-Luc-VU ( $n=5$ ), and *Mafa*<sup>-/-</sup>*Mafk*<sup>+</sup>MIP-Luc-VU ( $n=6$ ) mice following intravenous injection of luciferin. Bioluminescence intensity in MIP-Luc-VU mice and *Mafa*<sup>-/-</sup>MIP-Luc-VU mice was  $3.81 \pm 0.75 \times 10^6$  and  $2.75 \pm 0.11 \times 10^6$  photons/s, respectively, whereas the signal observed in the *Mafa*<sup>-/-</sup>*Mafk*<sup>+</sup>MIP-Luc-VU mice was significantly reduced ( $2.89 \pm 0.99 \times 10^5$  photons/s,  $P=0.0006$  vs. wild-type). Immunohistochemical analysis of pancreata from the three genotypic groups of mice using anti-hormone antibodies was performed at 20 weeks of age. Consistent with our previous findings, there was a reduction in the ratio of  $\beta$  to  $\alpha$  cells in *Mafa*<sup>-/-</sup> mice accompanied by an abnormal islet architecture characterized by the presence of several non  $\beta$ -cells in the core of the islets. We further observed atrophic islets in *Mafa*<sup>-/-</sup>*Mafk*<sup>+</sup> mice owing to the loss of  $\beta$ -cells, which were replaced by glucagon-positive cells (Fig. 2E) [17, 23].

#### *BLI of embryonic $\beta$ -cell neogenesis*

We examined whether embryonic  $\beta$ -cell neogenesis could be detected noninvasively by BLI in MIP-Luc-VU



**Fig. 1.** (A) Fasting blood glucose levels of male MIP-Luc-VU mice following 8 weeks of a regular diet (RD) or a high fat diet (HFD) ( $n=7$  in each group). (B) Hematoxylin and eosin staining of pancreatic sections from mice fed the RD or HFD for 8 weeks. Scale bars indicate  $100 \mu\text{m}$ . (C) Islet area relative to total pancreas of mice fed the RD ( $n=4$ ) or HFD ( $n=3$ ). (D) Representative bioluminescence images of MIP-Luc-VU mice fed the RD or HFD. (E) Quantification of signal intensity in the RD ( $n=7$ ) and HFD groups ( $n=7$ ) at 0, 4, and 8 weeks.

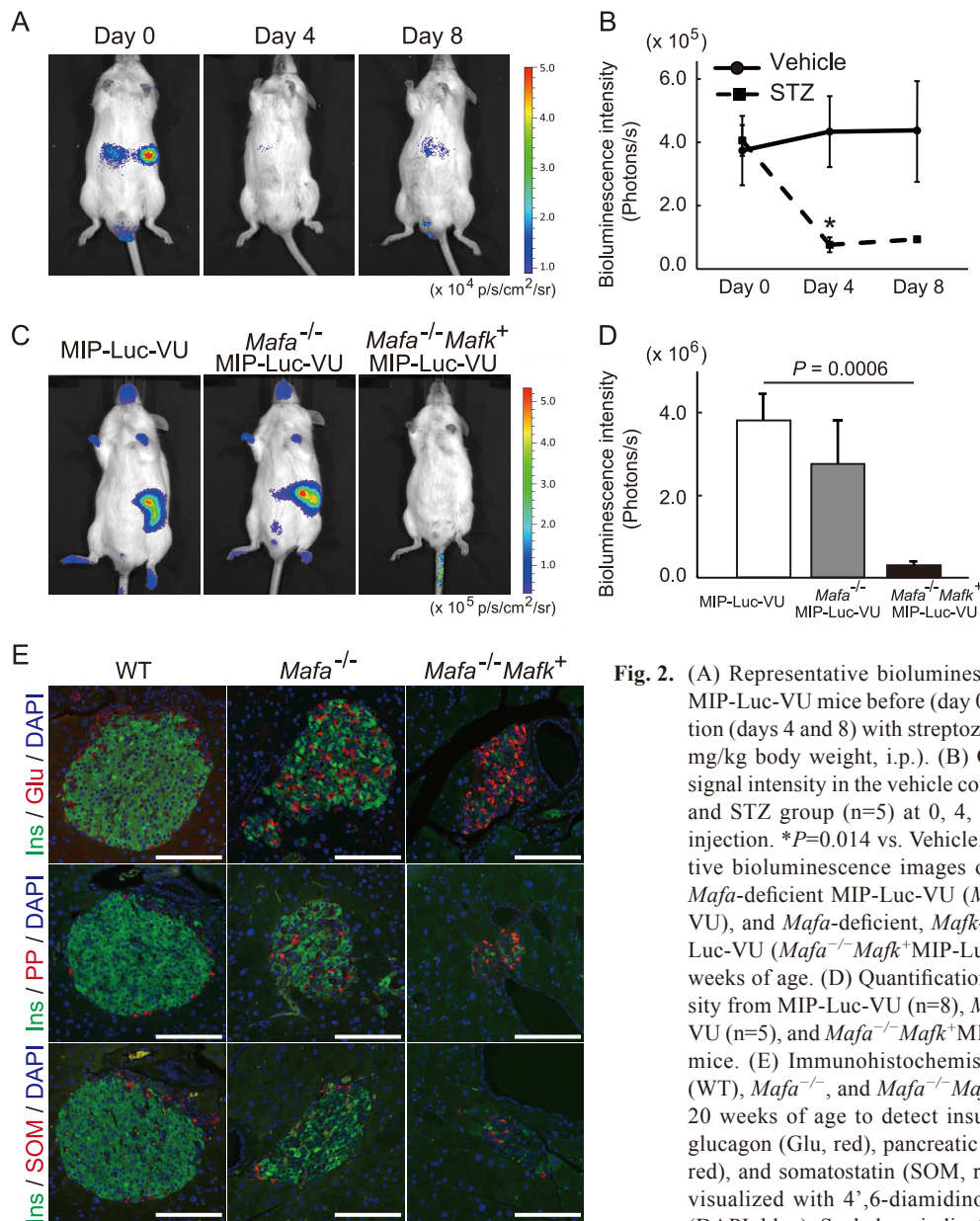
mice in utero. BLI of pregnant wild-type ICR females fertilized by MIP-Luc-VU males first revealed an in utero signal at E16.5 (Fig. 3A). Although the number of individual signals increased during the late gestational stage, the signal intensity of the signals did not. In addition, the signal intensity of individual spots differed significantly from  $2.7 \times 10^4$  to  $2.4 \times 10^5$  photons/s. The luminescence observed in pregnant mice was derived solely from the fetuses as demonstrated through the peritoneum (Fig. 3B). We observed a total of 15 bioluminescence signals in 20 transgene-positive fetuses identified

by PCR-based genotyping in three pregnant mice, suggesting that the level of detection of fetal pancreas by BLI was about 75.0% at E18.5.

## Discussion

In this study, we present two novel findings. First, diabetic *Mafa*<sup>-/-</sup>*Mafk*<sup>+</sup> mice expressing a bioluminescent reporter under the control of the insulin promoter exhibited a reduction in signal, reflecting the impaired  $\beta$ -cell development in these mice. Second, we were able to use





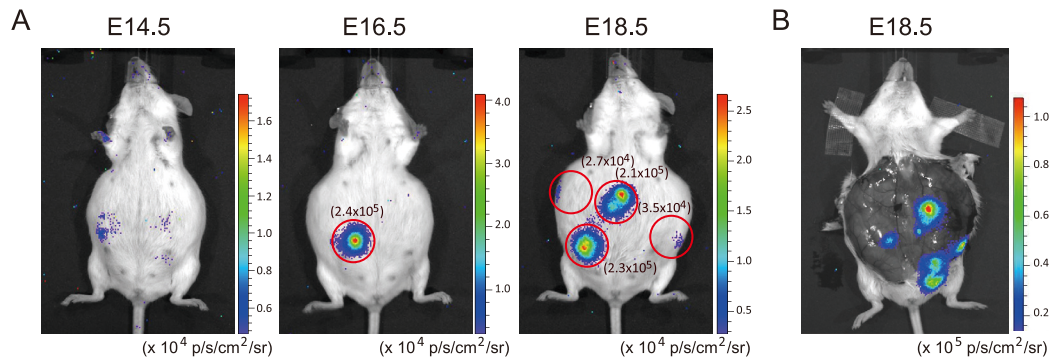
**Fig. 2.** (A) Representative bioluminescence images of MIP-Luc-VU mice before (day 0) and after injection (days 4 and 8) with streptozotocin (STZ, 200 mg/kg body weight, i.p.). (B) Quantification of signal intensity in the vehicle control group ( $n=5$ ) and STZ group ( $n=5$ ) at 0, 4, and 8 days after injection. \* $P=0.014$  vs. Vehicle. (C) Representative bioluminescence images of MIP-Luc-VU, *Mafa*-deficient MIP-Luc-VU (*Mafa*<sup>-/-</sup> MIP-Luc-VU), and *Mafa*-deficient, *Mafk*-transgenic MIP-Luc-VU (*Mafa*<sup>-/-</sup> *Mafk*<sup>+</sup> MIP-Luc-VU) mice at 6 weeks of age. (D) Quantification of signal intensity from MIP-Luc-VU ( $n=8$ ), *Mafa*<sup>-/-</sup> MIP-Luc-VU ( $n=5$ ), and *Mafa*<sup>-/-</sup> *Mafk*<sup>+</sup> MIP-Luc-VU ( $n=6$ ) mice. (E) Immunohistochemistry of wild-type (WT), *Mafa*<sup>-/-</sup>, and *Mafa*<sup>-/-</sup> *Mafk*<sup>+</sup> mice islets at 20 weeks of age to detect insulin (Ins, green), glucagon (Glu, red), pancreatic polypeptide (PP, red), and somatostatin (SOM, red). Nuclei were visualized with 4',6-diamidino-2-phenylindole (DAPI, blue). Scale bars indicate 50  $\mu$ m.

BLI to image fetal  $\beta$ -cell genesis in a noninvasive manner. Other researchers have developed different strains of mice carrying a  $\beta$ -cell-specific luciferase reporter that permit BLI to quantitate  $\beta$ -cell mass [19, 22]. In contrast to other  $\beta$ -cell imaging modalities such as PET and MRI, BLI provides quantifiable data with high throughput and inherently low background [14].

We also performed BLI of MIP-Luc-VU mice on ICR background following Virostko's protocols, in which *in vivo* BLI images were obtained in the right lateral position 10 min after intraperitoneal injection of luciferin with a dose of 150 mg/kg [21]. Signal intensities of the

ICR transgenic mice at 10 weeks of age showed  $1.92 \pm 0.43 \times 10^6$  photons/s ( $n=7$ ). Furthermore, signal intensities of 25 isolated islets ( $n=7$ ) following overnight culture in 11.1 mM glucose showed  $6.2 \pm 0.21 \times 10^6$  photons/s. These results are quite comparable with previous results obtained from FVB transgenic mice, suggesting the difference between the ICR and FVB genetic background has no significant impact on the bioluminescence intensities in MIP-Luc-VU mice [21].

As shown in Fig. 1, MIP-Luc-VU mice fed a HFD for only 8 weeks exhibited a significant increase in bioluminescence; however feeding of a HFD for 2 months



**Fig. 3.** (A) Sequential bioluminescence images of pregnant wild-type females that had been fertilized by MIP-Luc-VU males at embryonic days (E) 14.5, 16.5, and 18.5. The numbers indicate bioluminescence intensity (photons/s) from each region of interest. (B) Abdominal skin was removed to expose the fetuses to verify that all light emission was emanating from the fetuses.

had no substantial effect on bioluminescence in a previous study [21]. The difference probably results from the fat content in the diet; the content used in this study was twice that of the previous study. Meanwhile, the increase in BLI was substantially more than the increase in  $\beta$ -cell area (197 vs. 155%), suggesting that BLI might be influenced by other factors, including chronic hyperglycemia and inflammatory mediators [16, 21].

Noninvasive imaging and quantification of the  $\beta$ -cell mass *in vivo* has been considered important in basic science and clinical studies. A number of human studies have shown that both type 1 and type 2 diabetes are associated with a significant deficit in  $\beta$ -cell mass [11]. *Mafa*<sup>-/-</sup>*Mafk*<sup>+</sup> mice display nearly complete lack of  $\beta$  cells at birth and have fasting blood glucose levels that are more than 500 mg/dl [17]. As shown in Fig. 2, the reduction of the BLI signal to background levels in the mutant mice was consistent with the lack of insulin-positive cells observed in this strain.

It has been shown that STZ administration induces *ins1* transcription in extrapancreatic organs, which could be monitored by BLI using mice expressing the luciferase gene under the control of rat insulin promoter (RIP-Luc) [6, 9]. We observed here that some but not all MIP-Luc-VU mice treated with STZ generated a bioluminescent signal from the region of the liver [6]. Although *Mafa*<sup>-/-</sup>*Mafk*<sup>+</sup> mice develop a hyperglycemia that is comparable to or more severe than that observed in the STZ model, these mice did not show any extrapancreatic insulin gene expression. The difference between these results and those of Chen *et al.* might be explained by the plausible contribution of large Maf

transcription factors to hyperglycemia-induced insulin activation in extrapancreatic regions. Indeed, MafA regulates *ins2* transcription in thymic medullary epithelial cells (mTEC), which are necessary for tissue-specific antigen presentation and negative selection of autoreactive T cells [12].

In the process of  $\beta$ -cell development, fully differentiated  $\beta$  cells first appear around E13 at the start of a massive wave of endocrine differentiation in the pancreas known as the “secondary transition” [15]. In this study, we could detect a BLI signal only after the secondary transition during E16.5 and E18.5, probably due to an insufficient  $\beta$ -cell number and low insulin gene activity before E16.5. Visualization of  $\beta$ -cell development in primary and early developmental stages *in vivo* will require improvement of the imaging device to enable detection of very faint signals in addition to mice expressing a more intense bioluminescent reporter. Furthermore, we found wide variations between the signal intensities from the pregnant animals. This is probably due to the position and direction of the fetuses and not to individual differences in fetal development.

In summary, we demonstrated that alterations in  $\beta$ -cell mass and fetal  $\beta$ -cell neogenesis in uteri in MIP-Luc-VU mice could be monitored by BLI in a noninvasive manner. BLI using  $\beta$ -cell-specific reporter mice should facilitate the study of diabetes to gain a better understanding of its pathophysiology and should also be useful for drug development and for strategies aiming at  $\beta$ -cell regeneration.

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