Bioluminescence Imaging of Mouse Pancreas

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1 Introduction

The mortality rate due to diabetes significantly correlates with the economic prosperity of a country. In particular, differences in diet and life style associated with the degree of economic prosperity may be a key factor in the diabetes mortality rate of a country. Type 2 diabetes (T2D), which accounts for almost 90% of diabetes cases, is the leading cause of blindness, kidney failure, and amputation of hand and foot in adults (Scully, 2012). Patients with diabetes have a notably high risk of developing heart disease, stroke, high blood pressure, blindness, kidney failure, nervous system disease, and other chronic complications that severely affect their quality of life. The disease results from the development of insulin resistance in various tissues and a concomitant impairment of insulin secretion in pancreatic β cells (Orme et al., 2009). A progressive decrease in β cell mass of up to 60% has been reported in T2D (Butler et al., 2003), which parallels the extent of reduction in glucose-induced insulin secretion (Del Guerra et al., 2005). However, in another study, considerably smaller decrements of β cell mass were observed in patients with T2D (Rahier et al., 2008). Because there are no reported longitudinal studies on β cell mass in living organisms, it is still unclear whether loss of β cell mass and dysfunction of the pancreas are involved in the various symptoms of this disease of the pancreas (Rhodes, 2005).

Because the relationship between β cell mass and disease is unclear, monitoring the β cell mass and pancreatic function in living organisms may be instrumental in deciding the therapeutic approaches that best facilitate recovery of β cell mass and regeneration of pancreatic function in patients with pancreatic diseases such as T2D, acute pancreatitis and type 1 diabetes that is caused by autoimmune to own β cell. With recent technical innovations in various imaging modalities, molecular imaging is gaining more attention in the basic biomedical sciences and in clinical research and practice (Paty et al., 2004). Indeed, non-invasive imaging techniques are revolutionizing the understanding of diseases at the cellular and molecular level. The ability to non-invasively visualize β cells would greatly assist the development of new methods in the prevention and treatment of diabetes (Paty et al., 2004). In this review, we summarized overall technologies for in vivo imaging of mouse pancreas, and followed sections were particularly focused on bioluminescence imaging with MIP-luc and Bmp4-Luc.

2 Non-invasive Imaging Methods for in vivo Detection and Quantification of β cell Mass and Pancreatic Function

Beta cell mass and pancreatic function are most commonly estimated from measurements of insulin secretion or from morphometric analysis of histological sections. The β cell mass is not stationary but varies in response to the requirement for insulin secretion under different physiological status such as pregnancy, and under pathophysiological conditions including obesity and insulin resistance (Bouwens et al., 2005). Currently, advances in whole body molecular imaging are occurring in:

- Magnetic resonance imaging (MRI),
- Positron emission tomography-computed tomography (PET/CT),
- Fluorescence imaging (FLI),
- Bioluminescence imaging (BLI).
2.1 Magnetic Resonance Imaging (MRI)

MRI is a technique capable of delivering high-resolution quantitative information about local tissue accumulation of specific cell types and can be useful to accurately estimate the pancreatic volume by separating the fat component. However, tissue containing β cells accounts for only 1-2% of the pancreas and due to a lack of contrast with the surrounding tissue in the pancreas, MRI cannot distinguish between the exocrine and endocrine pancreas without specific contrast reagents. Super paramagnetic iron oxide nanoparticles (SPIO) have been used to label β cell islet transplants and longitudinally monitor their fate non-invasively. Saudek and coworkers labeled β cell islets with commercial SPIO and transplanted them into livers of experimental rats via the portal vein (Koblas et al., 2005). The SPIO-labeled islets were clearly visible in the liver of both healthy and diabetic rats for up to 22 weeks using MRI.

It has also been observed that blood glucose levels in diabetic rats were significantly decreased after transplantation with SPIO-labeled islets, indicating that the SPIO particles did not destroy the functionality of β cell islets. This method was further used to longitudinally monitor in vivo tolerance of transplanted pancreatic islets in both syngeneic and allogeneic rat models (Kriz et al., 2005). Within seven days post-transplantation, the allogeneic animals exhibited similar MRI islet patterns to the animals in the syngeneic group; all animals remained normoglycemic. By day 12, all the allogeneic animals turned diabetic and the detectable transplanted islet spots on MR images became significantly fewer at 14 days post-transplantation.

In contrast, the syngeneic animals remained normoglycemic and the number of transplanted islets detected by MRI remained relatively constant over the entire six week study period (Kriz et al., 2005; Medarova and Moore; 2009; Antkowiak et al., 2012). Furthermore, SPIO-labeled islet transplanted was used to trace in mouse models (Juang et al., 2010, Jung et al., 2011.) This clearly shows that MRI methods using SPIO-labeled islets can be used to non-invasively detect in vivo tolerance and rejection of islet transplants. Therefore, when considering the potential of MRI for this purpose, researchers should also take into account approaches to enhance the sensitivity and specificity of the imaging probes. Moreover, novel MRI sensors were developed to trace the transplanted islets. Zn2+-responsive T1 agent, GdDOTA-diBPEN was used to monitor β cell function (Lubag et al., 2011) and small interfering RNA (siRNA)-nanoparticle probe targeting apoptotic related gene caspase-3 (MN-siCaspase-3) was used for dual-purpose therapy/imaging by transplanted islet (Wang et al., 2011).

2.2 Positron Emission Tomography-Computed Tomography (PET/CT)

PET/CT techniques provide good resolution, high sensitivity, and accurate quantification of the physiological, biochemical, and pharmacological processes in living subjects. PET imaging requires the use of radioactive probes to assess β cell mass and pancreatic function, and 18F-FDG (2-deoxy-2-[18F]fluoro-D-glucose) is the most commonly used PET imaging probe for quantification of the rat endocrine pancreas (Malaise et al., 2000), as well as for determination of the in vivo distribution of dendritic cells (Prince et al., 2008).

PET imaging of transplanted islets of Langerhans was first reported using 18F-FDG -labeled islet cells in vivo with rat models (Toso et al., 2005). Since then, PET/CT imaging of human islet transplantation using 18F-FDG -labeled islets has been performed in five patients (Eriksson et al., 2009). Recently, the endogenous pancreatic β cell mass was quantitatively evaluated in healthy and type 1 diabetic patients using 18F-Fluoropropyl-Dihydrotetabenazine (18F-FP-(+)-DTBZ) (Notmandin et al., 2012). Thus, the 18F-FDG technique has the potential to evaluate and monitor islet survival and engraftment in the
clinical transplantation setting; however, there are several limitations of this method. There is a requirement for pre-treatment of β cells, the technique is non-quantitative, is not sufficiently sensitive in cases where there are relatively few β cell losses, and tracking is limited by the half-life of the radioisotope. Furthermore, novel tracers were developed using $^{64}$Cu-DO3A-VS-Cys$^{40}$-Exendin-4 and $^{68}$Ga-DOTA-(PEG)$_2$-biotin for in vivo imaging of transplanted islets (Wu et al., 2011; Eriksson et al., 2012).

With respect to advantages and disadvantages as non-invasive imaging applications, MRI technology currently has higher spatial resolution and lower sensitivity, whereas PET/CT has poorer spatial resolution but higher sensitivity. Thus, a combined PET/CT and MRI technique may be very promising to obtain more complete functional and anatomical imaging of pancreatic β cells.

2.3 Fluorescence Imaging (FLI)

FLI uses red or green fluorescent protein (RFP or GFP) as a reporter molecule. Signal is generated by excitation of the fluorescent proteins by light of a given wavelength and subsequent emission of light at another wavelength, which is detected with a charge-coupled camera. The light signal from the RFP or GFP provides information on histological features, while the fluorescence signals are also able to be used for cell sorting. However, to date FLI has proven to be technically challenging in its application to in vivo imaging in living animals. Recently, non-invasive imaging approaches have emerged as a method to validate the behavior of labeled agents in the pancreas in vivo (Kelly et al., 2008; Mukai et al., 2009; Gotthardt et al., 2006; Martinic et al., 2008).

To monitor β cell mass and pancreatic function, GFP-transgenic mice were generated. These transgenic mice express GFP under the control of the mouse insulin I gene promoter (MIP), and the GFP in the transgenic mice is specifically expressed in β-cells in islets from embryonic day E13.5 through adulthood (Hara et al., 2003). However, when non-obese diabetic MIP-GFP mice were subjected to non-invasive imaging under rigorous conditions, the results suggested that the majority of the fluorescence from MIP-GFP mice failed to show the required target-to-background ratio (Hutteman et al., 2011). Furthermore, Rat insulin promoter (RIP)-GFP was used to sort human β cell transduced by adenovirus vector (Meyer et al., 1998). Imaging of the pancreas from MIP-red fluorescent protein (RFP) transgenic mice was used to study on pancreatic development and function in normal and disease states (Hara et al., 2006).

Increasing evidence indicates that endoplasmic reticulum (ER) stress is associated with a variety of diseases including diabetes, neurodegenerative disease, cancer, bipolar disease, liver disease, cardiac disease, muscle degeneration, and autoimmune disease (Kim et al., 2008). Several scientists have found evidence that T2D may be an example of an important human disease caused by ER stress (Laybutt et al., 2007; Marchetti et al., 2007). T2D occurs in patients who fail to compensate for insulin resistance by increasing insulin secretion. Therefore, pancreatic β cell dysfunction and apoptosis are central to T2D pathogenesis.

Moreover, complex ER stress responses are involved in β cell protection, as well as cell dysfunction and death during T2D. A fusion protein of XBP-1 and Venus, a variant of GFP, has proven to be successful as a stress indicator. XBP-1 is a key transcription factor for many unfolded protein response genes important in protein folding. The indicator mRNA, spliced as a result of the ER stress response, is translated into an XBP-1-Venus fusion protein, resulting in fluorescence (Iwawaki et al., 2004). As expected, pancreas tissue exposed to high levels of ER stress showed intense fluorescence in XBP1-venus transgenic mice, suggesting that ER stress has an important role in the pancreas in vivo.
2.4 Bioluminescence Imaging (BLI)

BLI is based on the detection of light produced by cells tagged with luciferase, which catalyzes the emission of light by converting the substrates luciferin, oxygen, Mg2+ and adenosine triphosphate (ATP) to oxy-luciferin, carbon dioxide and adenosine diphosphate (ADP) (Figure 1). The expression of luciferase is detected by cooled charge coupled device (CCD) cameras in luciferase transgenic animals or in grafted, luciferase transgenic cells. The imaging procedure is technically simple and highly quantifiable. Because luciferase expression can be put under the control of tissue-specific regulatory elements and factors, BLI allows non-invasive imaging of gene expression. In addition, BLI is a non-radioactive imaging modality, in contrast to other imaging systems such as PET/CT. BLI has traditionally provided two-dimensional planar images with limited spatial and depth resolution (Arvanitis et al., 2005). Recent advances have enabled the translation of BLI data into three-dimensional tomographic imaging, thereby theoretically providing better quantification and signal localization (Slavine et al., 2006; Dikmen et al., 2005). Green light is more absorbed than red light depending on depth of the living tissue (Zhao et al. 2005); therefore, signal depth can be estimated by measuring the ratio of red to green emitted light using different multi filters on the surface of the animal and computing its likely origin (O’Neill et al., 2010).

![Figure 1: Schematic representation of the reporter gene technology relating to luciferase.](image)

Recently, green- and red-emitting beetle luciferases, which were developed by mutation, have been shown to act on a single, commonly used, D-luciferin substrate and emit green and red light by separate enzymatic reactions. In this system, any two or more luciferases that stably emit separable emission spectra can be combined to monitor the expression of multiple genes simultaneously in a single assay (Nakajima et al., 2010).
In addition, naturally secreted luciferases from marine copepods such as *Gaussia princeps* have been cloned (Gluc) and optimized for mammalian gene expression (Tannous *et al*., 2005). Moreover, a secreted type of luciferase, *Cypridina noctiluca* luciferase (Cluc) has also been developed (Kanjou *et al*., 2007). This secreted bioluminescence reporter protein enables luciferase activity in the blood of luciferase transgenic animals to be measured, and in vivo determination of target gene expression in a living organism to be assessed. This system allows measurement of the amount of functional secreted protein quantitatively and exactly using a simple assay system. It is less time-consuming and more cost-effective than conventional methods, and will accelerate the improvement of in vivo monitoring systems. Secreted reporters are detected in body fluids such as blood and urine, and are thus shown to be simple and useful tools for ex vivo real-time monitoring of in vivo biological processes (Tannous *et al*., 2011).

BLI is currently undergoing significant development, and non-invasive techniques such as this allow repeated measures of pancreatic β cell mass and function in living mice and may thereby reduce the cost and improve the efficiency of studies in mice. In this chapter, we present two approaches to detect pancreatic β cell mass and function. One is the MIP-luciferase transgenic mouse model, in which the transgenic mice express firefly luciferase under the control of the mouse insulin 1 (Ins1) promoter (MIP-luc) (Park *et al*., 2005; 2009). The other is the Bmp4-Luciferase transgenic mouse model, in which transgenic mice express firefly luciferase under the control of the mouse bone morphogenetic protein 4 (Bmp4) promoter (Yasunaga *et al*., 2011).

### 3 Bioluminescent Imaging in MIP-luc Transgenic Mice

MIP-luc transgenic mice allow real-time imaging of insulin-secreting pancreatic β cells in living mice. The β cells of MIP-luc transgenic mice emit a light signal that can be visualized externally by bioluminescent imaging using specialized equipment. In order to determine whether the intensity of the bioluminescent signal accurately reflects changes in β cell mass, or whether the system simply monitors transcriptional modulation of the mouse insulin 1 gene, the correlation between the bioluminescent signal and the β cell mass was investigated in MIP-luc transgenic mice fed a regular diet, or a high-fat Western diet. When male MIP-luc transgenic mice were fed a standard rodent diet or a high-fat Western diet beginning at 4 weeks of age, and the bioluminescent signal and β cell mass were measured after 6 and 10 weeks on each diet, the body weight, β cell mass and bioluminescent signal were greatly increased in mice fed the high-fat diet (Park and Bell, 2009). There was a statistically significant correlation between β cell mass and bioluminescent signal. Thus, in vivo bioluminescent imaging can be used to non-invasively monitor changes in β cell mass in living MIP-luc transgenic mice, and it complements other approaches for monitoring β cell mass in states of insulin resistance, obesity, and diabetes.

#### 3.1 The Function of Insulin in the Pancreatic β Cell

Insulin regulates blood glucose levels and body adiposity. Insulin reduces the blood glucose concentration by suppressing gluconeogenesis in the liver and by promoting glucose uptake into muscle and fat. Under normal conditions, the pancreatic islet β cells increase insulin release sufficiently to compensate for reduced insulin function, thereby maintaining normal glucose homeostasis (Kahn *et al*., 1993). However, in the case of obesity and insulin resistance associated with T2D, β cells are unable to compensate fully for decreased insulin sensitivity (Kahn *et al*., 2001). However, β cell dysfunction exists in individu-
als who are at high risk of developing the disease even when their glucose levels are still normal (Kahn et al., 2001). The ability of the β cell to adapt to changes in insulin sensitivity seems to result from two parameters: the functional responsiveness of the cell, and β cell mass. In response to the insulin resistance observed in obesity, human β cells can increase insulin release to levels four to five-fold higher than in insulin-sensitive individuals, whereas β cell volume is only enhanced by approximately 50% (Butler et al., 2003). These observations suggest that interpretation of the secretory response of β cells to a given stimulus must take into account the prevailing degree of insulin sensitivity. The insulin level simultaneously indicates pancreatic β cell function and mass and, while it is an important marker to monitor, it is unable to distinguish between function and mass. Assessment of β cell mass by bioluminescent imaging adds an important dimension to the study of pancreatic function.

3.2 Generation of MIP-luc Transgenic Mice

MIP-luc transgenic mice were generated using an 8.3-kb fragment of the Ins1 promoter to target luciferase expression to the pancreatic β cell, followed by injection of constructed DNA into the pronuclei of fertilized oocytes from CD-1 mice (Park et al., 2005). The three founder animals obtained were housed under a specific pathogen-free environment on a 12 h light/dark cycle conditions with free access to food and water. The mice used in the studies described here were heterozygous for the transgene.

3.3 Bioluminescent Signal in the Pancreas of MIP-luc Transgenic Mice

Luciferase expression in the pancreas in MIP-luc transgenic mice injected with D-luciferin was monitored by measuring the intensity of the bioluminescent signal (photons/second/region of interest), using the Xenogen IVIS Imaging System. As expected, a strong signal in the upper abdomen was confirmed to be derived from pancreatic β cells (Park et al., 2005; 2009). These studies were the first to explore the possibility of utilizing the MIP-luc mouse to monitor longitudinal changes in β mass in vivo. The MIP-luc transgene represents an indirect imaging agent, with the luciferase activity reflecting the number of cells expressing the transgene as well as the transcriptional modulation of the Ins1 promoter in expressing cells. However, initially it was still unclear whether the signal is correlated with β cell mass or metabolic status of the animal.

To investigate whether the bioluminescent signal intensity in MIP-luc transgenic mice is correlated with β cell mass and thus whether the signal can be used for non-invasive monitoring of changes in β cell mass in individual animals, BLI was performed with MIP-luc transgenic mice fed a high-fat diet, resulted in increased luminescence in β cell mass with age as well as the increase in β cell mass (Park and Bell, 2009). As expected, MIP-luc mice revealed a highly significant correlation between body weight and β cell mass. The mice also showed a strong correlation between bioluminescent signal and β cell mass.

Studies of β cell regeneration in the MIP-luc transgenic mouse model may provide additional insights into the process of β cell regeneration by facilitating sequential real-time measurements of β cell mass in living animals (Virostko et al., 2009; Grossman et al., 2010).
4 Bioluminescent Imaging in p7kb-Bmp4Luc Transgenic Mice

We established transgenic mice that allowed us to monitor Bmp4 expression by BLI for toxicological and nutritional screening of drugs and foods in vivo. Bmp4-Bmp receptor 1A (BmpR1A) signaling in β cells has recently been reported to be required for insulin production and secretion (Goulley, et al., 2007). In addition, Bmp4 blocks the differentiation and promotes the expansion of endocrine progenitor cells (Hua, et al., 2006). Bmp4 therefore regulates the maintenance of homeostasis in the pancreas by mediating two processes, enhancement of secretion of insulin and induction of expansion of progenitor cells (Figure 2).

Figure 2: Bmp4 functions in the pancreas. In pancreatic epithelial progenitor cells, Id2 induced by Bmp4 signaling binds to NeuroD, consequently represses cell differentiation and promotes cell expansion. In pancreatic β cells, autocrine Bmp4 activates insulin gene expression and insulin secretion mediated by Pdx1 expression.

In our transgenic mice harboring the mouse Bmp4 enhancer/promoter-firefly luciferase expression units, bioluminescent signal was detected mainly in the pancreas in three independent lines of transgenic mice. Furthermore, the bioluminescent signal was enhanced in association with the autophagy response to 24-h fasting. These results suggest that pancreatic expression of Bmp4 is involved in responding to the physiological environment, including through autophagy (Yasunaga et al., 2011). These mouse models represent useful tools for toxicological screening, and for investigating the mechanisms responsible for pancreatic Bmp4 function in vivo, with relevance to improving our understanding of pancreatic diseases including diabetes.

4.1 The Function of Bmp4 in the Pancreatic Cell

Bmp4 is a multifunctional growth and differentiation factor that belongs to the transforming growth factor β superfamily. Bmp4 is essential for mouse development, and most Bmp4-null mouse embryos die at
the onset of gastrulation, as a result of failure of mesodermal development (Winnier et al., 1995). Bmp4 has been reported to perform two functions in the pancreas; autocrine control of Bmp4, and production and secretion of insulin stimulated by Bmp4 signaling (Gannon et al., 2007). Bmp4 and BmpR1A are expressed in pancreatic β cells, and autocrine Bmp4 signaling induces Smad signaling through the receptor complex (at least one BmpR1A and BmpR2). Mice with attenuated BmpR1A signaling in β cells show decreased expression of key genes involved in insulin gene expression, proinsulin processing, glucose sensing, secretion-stimulus coupling, incretin signaling, and insulin exocytosis; these mice consequently develop diabetes as a result of impaired insulin secretion (Figure 2) (Goulley et al., 2007; Scott et al., 2009). Moreover, heterozygous knock-out BmpR1A mice demonstrate abnormal glucose metabolism (Goulley et al., 2007; Scott et al., 2009).

However, a recent report showed that Bmp4 stimulation blocked the differentiation and promoted the expansion of endocrine progenitor cells, thereby revealing a novel paradigm of signaling explaining the balance between expansion and differentiation through regulation of Id2 in pancreatic duct epithelial progenitors (Figure 2) (Hua et al., 2006). Likewise, Bmp4 has been reported to enhance mouse embryonic stem cell self-renewal (Ying et al., 2003), and is necessary for the production of hematopoietic progenitors (Park et al., 2004). Bmp4 thus exhibits divergent and complex functions by interacting with many cells and organs in vivo, including regulating the maintenance of homeostasis in the pancreas.

4.2 Generation of p7kb-Bmp4Luc Transgenic Mice

We constructed a reporter plasmid carrying a 7-kb enhancer and promoter region of the Bmp4 gene upstream of the firefly luciferase gene (Figure 3, upper panel) (Yasunaga et al., 2011). Bmp4-Luc transgenic mice were generated by pronuclear microinjection of linearized p7kb-Bmp4Luc DNA into BDF1xBDF1 fertilized eggs; three founders were obtained using this method. One of these p7kb-Bmp4Luc transgenic mice showed a strong bioluminescent signal in the upper abdomen (Figure 3, left panel).

For in vivo imaging or BLI, transgene-positive mice were backcrossed with Hos: HR-1 hairless mice for more than four generations. The mice used in the studies described here were age matched (8–15-week-old) male mice heterozygous for the transgene. All mice were bred and housed under a 12 h light-dark cycle with free access to food and water.

We also analyzed single-view 3D tomography for bioluminescent reporters that can be analyzed in an anatomical context using a digital mouse atlas with IVIS Spectrum (Caliper Life Sciences). As shown in Figure 3 right panel, the rainbow spot (red arrow) indicates the origin of highest luminescence intensity in the body. This rainbow spot is located in the pancreas (shown as a brown structure), not in liver (shown as a red structure). 3D diffuse bioluminescence tomography (coronal section; red surface, sagittal; blue surface, subject height; green surface) can be performed to determine the source of localization in pancreas.

4.3 Bioluminescent Signal in Pancreas of p7kb-Bmp4Luc Transgenic Mice

Luciferase expression in the pancreas in these transgenic mice was monitored by measuring the intensity of the bioluminescent signal (photons/second/region of interest), using the Xenogen IVIS Imaging System. A strong signal observed in the upper abdomen was confirmed by dissection to be derived from the pancreas (Figure 4) (Yasunaga et al., 2011). To identify which tissues produced bioluminescent signals,
In vivo bioluminescent signals in p7kb-Bmp4Luc transgenic mice. The left panel shows BLI of the mice, and the right panels show three dimensional analysis of BLI (Xenogen IVIS Lumina; 2D and Spectrum; 3D). The scale is in p/sec/cm²/sr. (Yasunaga unpublished data).

these signals were detected in tissues dissected from transgenic mouse incubated with fresh medium containing D-luciferin substrate. Bioluminescent signals were strongly detected in the pancreas, weakly detected in lung and kidney, but not detected at all in the liver and spleen. To determine if the expression pattern of the luciferase proteins was consistent with that of Bmp4, endogenous Bmp4 and transduced luciferase protein expression were examined by western blotting analysis in various tissues from transgenic mice. Bmp4 was abundantly expressed in the pancreas compared with other tissues, while luciferase expression was also notably higher in the pancreas. It is noteworthy that this reporter is strongly expressed in pancreas. Since the pancreas tissue contains strong RNase and protease activities, any biochemical assay of gene expression requires particular care and attention. However, due to the non-invasive nature of BLI, consideration of the potential disadvantages due to RNase and protease activities is not necessary. Moreover, relatively high level of BMP4 and low level of Luc in the liver may be influenced by their stability (Figure 4).

We undertook further studies to establish whether the expression of Bmp4 in pancreas responds to the physiological environment, including factors such as nutrient starvation, pancreatic acute disease, and psychosomatic stress.

4.4 Increase of Bioluminescent Signal in Pancreas after 24 h Fasting

The pancreas stringently maintains blood sugar concentrations during feeding and fasting by homeostatic regulation. To test whether p7kb-Bmp4Luc transgenic mice were able to respond to feeding or fasting, they were fasted for 24 h prior to BLI. The intensity of the bioluminescent signal in the pancreas of fasted mice was increased by approximately 3-fold, compared with that in fed mice. Furthermore, western blot analysis showed that Bmp4 protein levels in the pancreas of mice fasted for 24 h were also increased approximately 3-fold compared with fed mice (Figure 5) (Yasunaga et al., 2011). These results suggest that
Figure 4: Analysis of Bmp4 and luciferase proteins in pancreas. The left panel shows BLI of a mouse which has undergone a laparotomy. The right panel indicates ex vivo imaging of dissected tissues. The scale is in p/sec/cm²/sr. A standard “ROI” template was used for each experiment to quantify the luminescence. Images were processed using Living Image software (Caliper Life Sciences, Hopkinton, MA). The bottom panel shows western blot analysis of various tissues from the mouse. Expression levels are normalized by α-tubulin expression using Image Gauge software (Fuji Film, Tokyo Japan) (Yasunaga et al., 2011).

Bmp4 expression in the pancreas responds to feeding or fasting, and that pancreatic function responding to the physiological environment is able to be determined quantitatively in transgenic mice without sacrifice.

Autophagy induced by deprivation of nutrients is an evolutionarily conserved lysosomal degradation pathway in which the cell self-digests its own proteins and organelles, and thus maintains macromolecular synthesis and ATP production (Hailey et al., 2010). Beta-cell-specific autophagy-deficient mice show hypoinsulinemia and hyperglycemia, both of which are diabetic phenotypes (Hur et al., 2010; Jung et al., 2008). Autophagy is required to maintain the structure, mass and function of pancreatic β cells (Ebato et al., 2008; Fujimoto et al., 2009). In our experiments, expression of LC3 (autophagy marker)-positive cells was increased in the pancreas of 24-h-fasted transgenic mice (Yasunaga et al. 2011). Our observations suggest that fasting or starvation stress in pancreatic cells either independently induces both an autophagy response and Bmp4 expression, or autophagy signals induce Bmp4 expression. Further studies are needed to clarify this issue.
Figure 5: The effect of 24-h fasting on bioluminescent signals in the pancreas of p7kb-Bmp4Luc transgenic mice (BLI; in vivo, ex vivo imaging and western blot analysis). The scale is in p/sec/cm2/sr. (Yasunaga et al., 2011)

4.5 Increase of Bioluminescent Signal in Transgenic Mice with Pancreatic Duct Ligation

Recent studies indicate that adult pancreatic cells are more “plastic” than previously assumed, and evidence indicating islet neogenesis is emerging in several animal models. One intriguing hypothesis has explained islet neogenesis in the context of pancreatic injury-induced activation of facultative stem/progenitor cells, which results in expansion of β cell mass (Dor et al., 2008). Robust injury using partial pancreatic duct ligation (PDL) creates an appropriate microenvironment to unambiguously demonstrate the existence of multipotent endocrine progenitors in the adult mouse pancreas (Xu et al., 2008). To determine whether p7kb-Bmp4Luc transgenic mice were able to respond to PDL, BLI was performed on these mice for 7 days after the PDL treatment. The intensity of the bioluminescent signal in the pancreas of PDL mice was increased by approximately 2-fold, compared with that in sham-operated mice (Figure 6) (Yasunaga et al. unpublished data). In a previous report, maximal transcript levels of Ngn3 (a marker of endocrine progenitor cells) were observed within 7 days of treatment, with a subsequent slow decrease (Xu et al., 2008). These results suggest that changes in Bmp4 expression in the pancreas in response to PDL may correlate with the process of pancreatic regeneration, and that bioluminescence from our transgenic mice may enable monitoring of the regeneration process through expression of Bmp4.
5 Conclusion

Although the functions of Bmp4 in the pancreas are not fully understood, BLI of p7kb-Bmp4Luc transgenic mice achieved non-invasive monitoring of a number of pancreatic functions. Pancreatic disease models such as acute pancreatitis, diabetes mellitus and pancreatic tumors based on p7kb-Bmp4Luc transgenic mice may be suitable for evaluating disease onset, and these transgenic mice may prove to be a useful tool as an “In Vivo Screening System” for future toxicological screening studies, and for further studies to reveal the functions of Bmp4 in the pancreas (Figure 7).

Taken together, non-invasive and real-time imaging of β cell mass and function using multi imaging techniques, combination of MRI, CT/PET, FLI and BLI will provide us many information and unexpected hypothesis to process of developmental diabetes, such as interaction between pancreas and the other organs. The paradigm shift of pancreatic diseases results might lead us to find a novel target of prevention and therapy. In addition, developments of reliable imaging systems in human will contribute to a personalized medicine with continuous monitoring of therapeutic value and side effects of drugs. We expected that non-invasive in vivo imaging technology will bring benefits to diabetes research and therapy as well as basic biological research.
In the Future

Figure 7: In the future, BLI of p7kb-Bmp4Luc transgenic mice may prove to be a useful tool as an “In Vivo Screening System” for a range of studies on pancreatic diseases.

6 Summary

Type-2 diabetes accounts for almost 90% of all cases of diabetes in adults worldwide, and results from the development of insulin resistance in some tissues and a concomitant impairment of insulin secretion in the pancreatic β cell. A progressive loss of β cell mass and function triggers the transition from glucose intolerance to overt diabetes during the course of the disease. Thus, monitoring β cell mass and function will be instrumental in defining the approaches that best facilitate β cell recovery and regeneration in pancreatic diseases such as type-2 diabetes and acute pancreatitis.

Beta cell mass and function are most commonly estimated from measurements of insulin secretion or morphometric analysis of histological sections. Currently, molecular imaging in the whole body is advancing in the fields of positron emission tomography-computed tomography (PET/CT), magnetic resonance imaging (MRI) Fluorescence imaging (FLI) and bioluminescence imaging (BLI).

In this article, we have summarized recent advances made in molecular imaging techniques such as BLI, in particular the use of transgenic mice to monitor expression of Ins1 and Bmp4 and thus non-invasively image the pancreatic β cell for research on diabetes. Our model, Bmp4-Luc transgenic mice, has revealed that pancreatic gene expression is altered in response to environmental changes such as food
uptake, stresses and certain pancreatic diseases. Our mice model will assist in the prescription of appropriate drug therapies, in terms of dose, and of the timing of drug administration.

References


