

# Imaging of insulin factory: Is it just imagination or approaching reality?

The determination of clinical staging and the severity of disease are essential to the management and prediction of the prognosis of a patient. Insulin secretory capacity and  $\beta$ -cell mass are both considered to be indicators of severity and staging of either in type 1 or type 2 diabetes. Rapid destruction of  $\beta$ -cells by immune processes is a key pathogenetic process in type 1 diabetes. At onset of the disease, nearly all pancreatic  $\beta$ -cells are destroyed in type 1 diabetes. More recently, also in type 2 diabetes, slow but progressive decline of  $\beta$ -cell mass often accompanied by amyloid deposition was found to be a pathological hallmark. Thus, we have to make a decision about which stage of diabetes the patient stands in when the diagnosis is made. To this end, we need to know to what extent the islets are damaged in a given diabetic patient to determine the best choice of treatment. We are also curious to know whether the treatment can stop the destruction of  $\beta$ -cells or if it promotes their replication. Furthermore, in type 1 diabetic patients, although islet transplantation is a final choice of treatment modalities, it is crucial to follow the survival of the implanted cells to know whether islet transplantation has been successful or not.

As such, quantitation of  $\beta$ -cell mass in diabetic patients seems to be a prime subject of research and investigators have made efforts to explore the blood biomarkers to predict the approximate value of  $\beta$ -cell mass; fasting hyperglycemia, glycated hemoglobin concentrations, C-peptide, insulinogenic index or homeostasis model assessment of  $\beta$ -cell function (HOMA- $\beta$ ) have been explored as well. These indices are found to be incom-

plete, however, for the reflection of actual  $\beta$ -cell mass. Alternatively, the ultimate goal might be direct visualization of  $\beta$ -cells by body imaging.

The major problem of the imaging of the islet is that its volume is too small. The total volume might reach only 1–2 g (cc) in approximately 100 g pancreas (only 1–2% of the pancreas). The size of each islet (50–500  $\mu$ m) to make an image is also too small. We frequently encounter numerous small islets composed of just a few endocrine cells. In addition, they distribute widely in the whole pancreas with low contrast difference from the surrounding tissues. The islet composed of heterogeneous cells yields an inhomogeneous image, not much is discriminated from the intensities of the exocrine pancreas, which makes it difficult to identify the specificity of the tissues. Such anatomical characteristics inevitably require augmentation of the islet image in contrast to background parenchyma. Although optical methods using laser scanning microscopy showed some promise for the visualization of live  $\beta$ -cells in animal models, it was not feasible to apply to humans because of its invasiveness and limited tissue penetration of light<sup>1</sup>. For the detection of 3-D architecture of islets, application of the imaging to positron emission tomography (PET) scans or magnetic resonance imaging (MRI) is more realistic because of non-invasiveness. High-resolution PET and MRI scanners have been developed in the past decade, and the background pathology of the pancreas in diabetic patients has also been established. Because of the high sensitivity of clinical imaging modalities, PET scan was preliminarily applied to humans, but the results were disappointing. As the spatial resolution of PET scan is fairly limited, application of MRI is proposed to be more suitable for the imaging of native islets of the

pancreas located in deep positions of the body<sup>1</sup>.

Introduction of MRI imaging of the transplanted islets *in vivo* in animals showed promising images, providing us with an expectation for the detection of native islets. It has not been successful, however, to detect native islets *in situ*, mostly because of an insufficient spatial resolution, which requires a high magnetic field for imaging. Under such circumstances, the paper written by Lamprianou *et al.*<sup>2</sup> at the University of Geneva has shown a great advance in visualization of  $\beta$ -cell images. They introduced a combination of glucose infusion and manganese-enhanced high-field magnetic resonance imaging (MEHFMRI) to amplify the islet image without application of a specific probe to bind  $\beta$ -cells. The manganese was expected to enhance the image difference between islet and non-islet tissues to which glucose infusion further amplified the difference. Although they had to exteriorize the pancreas for the minimization of body movement, native individual islets were clearly visualized by MEHFMRI in living anesthetized mice. Diabetic mice induced by streptozotocin (STZ) showed a drastic decrease in the islet image to approximately 50%, which was confirmed by immunostained sections of the same animals. Indeed, the distribution and contour of the native islets in a living mouse corresponded well with those shown on the histological sections. Thus, the data show that MEHFMRI can quantitatively visualize individual islets in the intact mouse pancreas, both *ex vivo* and *in vivo*. The findings they presented appear to give us great promise for non-invasive monitoring of  $\beta$ -cell mass. At the same time, however, the study showed that there are many barriers that should be overcome before the methods can be applied to humans; specificity of

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$\beta$ -cells, precision, reproducibility and cost performance.

To make an image of the islet in live individuals is not an easy task because of insufficient information of the islet structure, and  $\beta$ -cell mass in normal and pathological conditions. Translation from mouse studies to humans requires caution, because rodent islets are very different from that of humans; the size distribution, composition of endocrine cells and vascular flow as well. In laboratory animals, the imaging of islets was challenged by the use of T1 contrast media to trace the materials incorporated into or attached to  $\beta$ -cells. Iron oxide, fluorine-19(<sup>19</sup>F) nanoparticles or gadolinium-diethylenetriamine pentaacetic acid (GdDTPA) were tested, but the images are yet to be satisfactory<sup>3</sup>. Infusion of manganese and glucose used by Lamprianou *et al.* to augment the islet image might be the first step for the separation of islets from the surrounding tissues. Manganese, T1 contrast agent, is known to be incorporated through calcium channels into  $\beta$ -cells, and is therefore

used for characterization of isolated islet potency and functionality of both grafted and endogenous islets. However, the uptake of manganese might also be incorporated into non- $\beta$ -cells and be potentially cytotoxic. To obtain specific images of the islets or  $\beta$ -cells, a strong and specific affinity of the probe that binds to  $\beta$ -cells might be essential. The lack of specificity for manganese might have yielded a considerable discrepancy of the  $\beta$ -cell mass between the MRI images and data from the histological sections in their study.

The use of tracers bound with  $\beta$ -cells might further promote studies on islet imaging by PET. For identification of  $\beta$ -cells, numerous probes were tested for their utility: labeled antibodies targeting  $\beta$ -cell surface molecules, such as IC2 or R2D6; labeled probes for sulfonylurea receptor-1, such as glibenclamide, tolbutamide and repaglinide; for glucose transporter 2 (GLUT2), such as alloxan or D-mannoheptulose; and for secretory granules, such as zinc, serotonin, L-Dopa or vesicular monoamine transporter

type 2 (VMAT2; Table 1)<sup>4</sup>. Among these agents, the probe that might not affect the  $\beta$ -cell survival or glycemia would be preferable. Although these pioneering works have shown some progress in better identification of the islets *in vivo*, there appears to be a great deal of room for the improvement. More recently, labeled glucagon-like peptide-1 (GLP-1) analog, exenatide or GLP-1 antagonist have also been introduced and preliminary studies have shown promise to warrant further examination<sup>5</sup>.

Caution might be required for the experimental conditions used by Lamprianou *et al.*<sup>2</sup>, as they had to exteriorize the pancreas to minimize the body movement to capture a detailed image. The image of the unexteriorized pancreas would be of considerable interest for comparison with the exteriorized pancreas, as we cannot take an image of an exteriorized pancreas in humans. It should also be pointed out that the loss of  $\beta$ -cells in their STZ-model was undervalued compared with the actual loss on the sections. Size frequency histograms of the detected islets were

**Table 1** | Potential  $\beta$ -cell imaging probes

Target	Agent	Application	Results of specificity
Islet cell antigen	[ <sup>111</sup> In]-IC2 antibody	Immunohistology, nuclear imaging	High
	[ <sup>125</sup> I]R2D6 antibody	<i>In vivo</i> binding assay/distribution	Insufficient
	[ <sup>125</sup> I]K14D10-antibody and its Fab fragment	<i>In vivo</i> binding assay/distribution	Insufficient
Glucose metabolism	D-[U- <sup>14</sup> C]-glucose	<i>In vivo</i> binding assay/distribution	Insufficient
	2-Deoxy-2-[ <sup>18</sup> F]fluorodeoxyglucose	<i>In vivo</i> distribution/ <i>in vitro</i> binding	Insufficient
GLUT2	[2-( <sup>14</sup> C)]-alloxan	<i>In vitro</i> binding/ <i>in vivo</i> distribution	No $\beta$ -cell tropism/toxic
	D-( <sup>3</sup> H)-mannoheptulose	<i>In vitro</i> binding/ <i>in vivo</i> distribution	No $\beta$ -cell tropism
Secretory granules	<sup>65</sup> Zinc	<i>In vitro</i> binding/ <i>in vivo</i> distribution	Insufficient
	Serotonin, L-Dopa, Dopamine	<i>In vitro</i> specificity	Insufficient
(VMAT2)	{( <sup>11</sup> C)}-dihydrotetrabenzene	Longitudinal PET study (rat/human)	Good in rat, not in human
Sulfonylurea receptor 1	2-[ <sup>18</sup> F]fluoroethoxybenzenesulfonyl) 3-butyl urea	<i>In vitro</i> binding assay	Insufficient
	2-[ <sup>18</sup> F]fluoroethoxy-5-bromoglibenclamide	<i>In vitro</i> binding/ <i>in vivo</i> distribution/human-PET	Insufficient
	<sup>99</sup> Tc-glibenclamide	<i>In vitro</i> binding/ <i>in vivo</i> distribution	Insufficient
	Glibenclamide -glucose-conjugate	<i>In vitro</i> binding/ <i>in vivo</i> distribution	Low plasma binding
	[ <sup>18</sup> F]-repaglinide, [ <sup>11</sup> C]-repaglinide	<i>In vitro</i> binding/ <i>in vivo</i> distribution/human-PET	Insufficient
GLP-1 receptor	[Lys40(Ahx-DTPA)N-H2] exendin	<i>In vivo</i> distribution/bioimaging	Good for insulinoma
	GLP-analog (exendin 4)	Binding/ <i>in vivo</i> bioimaging	Good
	GLP-1 antagonist (exendin9-39)	Binding assay/ <i>in vivo</i> distribution	Good
Unknown	RIP11-phage clone	<i>In vitro</i> binding/ <i>in vivo</i> distribution	Fair
	$\beta$ -cell specific single-chain antibody	<i>In vitro</i> assay/immunohistology	Good

Modified from reference 4. GLP-1, glucagon-like peptide-1; GLUT, glucose transporter; PET, positron emission tomography; VMAT2, vesicular monoamine transporter 2.

shifted to the right in MRI images in contrast to those on immunostained sections, suggesting negligence of small islets. As the human pancreas is much replete with small islets, much more precision will be required for the application to humans. In particular, the difference in the  $\beta$ -cell mass between type 2 diabetes and their controls might not be so large that detection of small islets would be of paramount importance.

With the recent progress in dynamic changes of islet pathology, we begin to consider that the natural history of either type 1 or type 2 diabetes might follow their distinct courses. Treatment for diabetes has long been used to prevent or not to worsen its vascular complications by tight blood glucose control. With the background basis of islet pathology, we know that the new treatment should pre-

vent the loss of  $\beta$ -cells or restore them. Indeed, we hope that incretin therapies for type 2 diabetes will serve this purpose. A number of agents to prevent  $\beta$ -cell loss and to promote its replication are also being developed. To this end, future investigation to develop the methods for accurate measurement of  $\beta$ -cell mass by body imaging without invasive procedures is extremely important.

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