

Current status of regeneration of pancreatic β -cells

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ABSTRACT

Newly generated insulin-secreting cells for use in cell therapy for insulin-deficient diabetes mellitus require properties similar to those of native pancreatic β -cells. Pancreatic β -cells are highly specialized cells that produce a large amount of insulin, and secrete insulin in a regulated manner in response to glucose and other stimuli. It is not yet explained how the β -cells acquire this complex function during normal differentiation. So far, *in vitro* generation of insulin-secreting cells from embryonic stem cells, induced-pluripotent stem cells and adult stem/progenitor-like cells has been reported. However, most of these cells are functionally immature and show poor glucose-responsive insulin secretion compared to that of native pancreatic β -cells (or islets). Strategies to generate functional β -cells or a whole organ *in vivo* have also recently been proposed. Establishing a protocol to generate fully functional insulin-secreting cells that closely resemble native β -cells is a critical matter in regenerative medicine for diabetes. Understanding the physiological processes of differentiation, proliferation and regeneration of pancreatic β -cells might open the path to cell therapy to cure patients with absolute insulin deficiency. (*J Diabetes Invest*, doi: 10.1111/jdi.12062, 2013)

KEY WORDS: Diabetes, Insulin, Regenerative medicine

INTRODUCTION

Pancreatic β -cells are highly differentiated and specialized cells with complex biological functions. The most outstanding feature of β -cells is that they produce a large amount of insulin, the only hormone that lowers blood glucose, and secrete it continuously in response to changes in the extracellular glucose concentration. Insulin secretion from pancreatic β -cells also is modified by a variety of nutrients, hormones and neuronal signals in the maintenance of systemic glucose homeostasis¹.

For treatment of diabetes with absolute insulin deficiency, insulin is generally used. However, normal pancreatic β -cells adjust insulin secretion continually in response to varying blood glucose levels; exogenous insulin administration cannot maintain blood glucose levels within the narrow physiological range that protects from development of various diabetic complications. Although transplantation of the pancreas or pancreatic islets has been an efficient therapeutic option for cure of patients with insulin-deficient diabetes, the chronic shortage of donor organs limits widespread application of such transplantation. Thus, regenerative medicine, including generation of pancreatic β -cells, pancreatic islets or whole pancreas, is an

intriguing approach to the development of future therapy for diabetes². In the present review, we discuss functional differentiation of native pancreatic β -cells as the scientific basis of regenerative medicine in the field of diabetes, and introduce approaches to regeneration of β -cells (Figure 1).

ACQUISITION OF INSULIN SECRETORY FUNCTION IN THE PANCREATIC β -CELL

Pancreatic β -cells secrete adequate amounts of insulin in response to extracellular glucose concentration so that blood glucose levels are controlled within a narrow physiological range; severe hypoglycemia or hyperglycemia seldom occurs in healthy subjects. This is because the β -cell has properties of: (i) insulin biosynthesis; (ii) glucose sensing; (iii) metabolism-secretion coupling; and (iv) regulated exocytosis (Figure 2).

Although our knowledge of the development of well-regulated insulin secretion is limited, analyses of developing and differentiating pancreatic β -cells provide clues. It is known that fetal and neonatal pancreatic β -cells lack glucose responsiveness³. By using rat models, it was found that fetal pancreatic β -cells show lower facilitation of glucose oxidation than adult β -cells⁴. As forced depolarization can stimulate insulin secretion similarly in both fetal and adult β -cells^{4,5}, fetal β -cells seem to show insulin biosynthesis, granule formation and expression of soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins. Thus, immaturity in glucose metabolism might account for the lack of glucose responsiveness in fetal pancreatic β -cells. In fact, expression levels of key enzymes [mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH) and mitochondrial malate dehydrogenase] in NADH (the

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Received 9 January 2013; accepted 21 January 2013

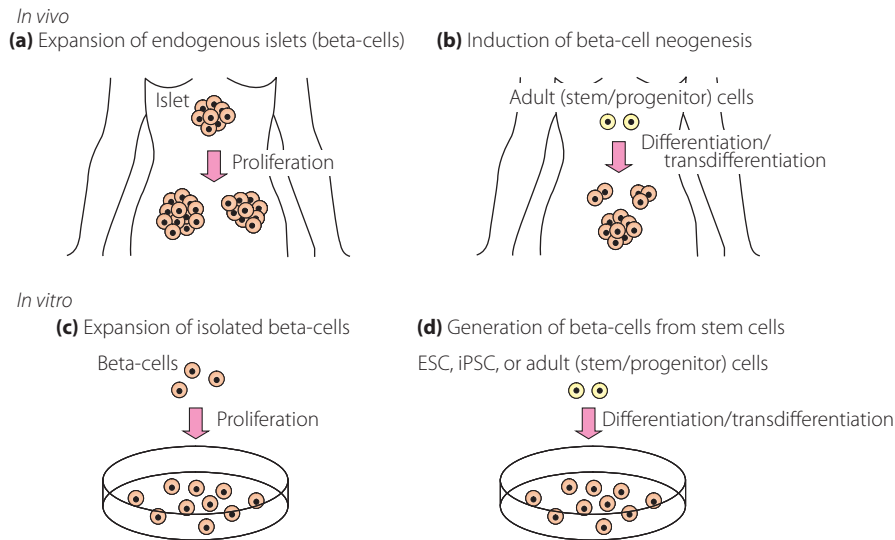


Figure 1 | Possible approaches to regeneration of pancreatic β -cells. New pancreatic β -cells could be generated by induction of self-replication (proliferation) of pre-existing islet β -cells or differentiation/transdifferentiation of adult stem/progenitor cells (neogenesis) *in vivo*. In addition, the new β -cells could also be obtained by forced proliferation of isolated pre-existing β -cells or differentiation/transdifferentiation of embryonic stem cells (ESCs)/induced-pluripotent stem cells (iPSCs) or adult stem/progenitor cells *in vitro*.

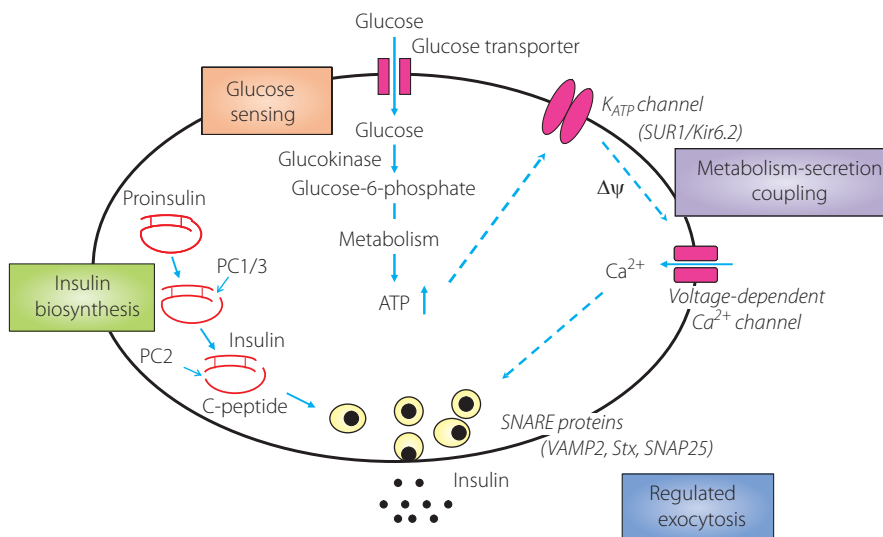


Figure 2 | Generalized mechanism of glucose-induced insulin secretion in pancreatic β -cells. Glucose entering β -cells through glucose transporters is metabolized, leading to an increase in the adenosine triphosphate (ATP) concentration, closure of the ATP-sensitive K^+ (K_{ATP}) channels, depolarization of the β -cell membrane and opening of the voltage-dependent Ca^{2+} channels, which allows Ca^{2+} influx. The resultant rise in intracellular Ca^{2+} concentration triggers soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)-dependent exocytosis of insulin granules. β -Cells thus must possess functions of: (i) insulin biosynthesis; (ii) glucose sensing; (iii) metabolism-secretion coupling; and (iv) regulated exocytosis. PC1/3, prohormone convertase 1/3; PC2, prohormone convertase 2; SNAP25, synaptosomal-associated protein 25; Stx, syntaxin; VAMP2, vesicle-associated membrane protein 2.

reduced form of nicotinamide adenine dinucleotide) shuttles, which have an important role in adenosine triphosphate (ATP) production, are significantly lower in fetal than in adult β -cells, and forced expression of mGPDH improves glucose-induced insulin secretion in fetal pancreatic islets⁶. Immaturity of glucose

metabolism in fetal pancreatic β -cells is also supported by a study using human and porcine fetuses⁷, and by a recent DNA microarray study in fetal rat β -cells⁸. However, the mechanism of β -cell acquisition of glucose-responsiveness during growth is still largely unknown.

It has been reported that addition of glucagon-like peptide-1 (GLP-1), an incretin hormone, induces both first and second phase glucose-induced insulin secretion in human fetal islets-like cell clusters (immature islets)⁹. Although this finding reflects an acute effect of GLP-1 on the function of immature β -cells, GLP-1 also has roles in differentiation of pancreatic β -cells. A study has shown by using fetal pig islets that GLP-1 facilitates differentiation of the immature precursor of pancreatic β -cells to mature β -cells¹⁰. Because GLP-1 begins to secret from L-cells on the gut tube by intake of foods after delivery, it is possible that GLP-1 has physiological roles in functional differentiation of pancreatic β -cells.

In contrast, it is known that terminal differentiation of pancreatic β -cells requires expression of the transcription factor v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MafA), which regulates expression of genes participating in glucose-induced insulin secretion including *GLUT2*, glucokinase, *SUR1* and *Kir6.2*¹¹. A recent report showed the possibility that MafA is involved in the acquisition of glucose responsiveness in neonatal rat pancreatic β -cells¹², an important role in functional maturation of the β -cells. Although the relationship between MafA and incretins is unknown, as MafA increases the expression of the GLP-1 receptor¹³, their interaction or cooperation in differentiation of pancreatic β -cells seems likely. However, further studies are required to understand the whole picture.

REGENERATION OF PANCREATIC β -CELLS *IN VIVO*

Most studies on regeneration of pancreatic β -cells *in vivo* have been carried out in rodents using pancreatic injury models. Nicotinamide, an inhibitor of poly(adenosine diphosphate-ribose) synthetase/polymerase, prevents the development of diabetes in experimental animals after administration of the β -cell toxins, streptozotocin and alloxan¹⁴. *In vitro* studies have shown that the agent has beneficial effects on proliferation and differentiation of pancreatic endocrine cells^{15,16}, but the mechanism is not known. Exendin-4, an analog of GLP-1, has been reported to enhance both proliferation and neogenesis of pancreatic β -cells in rats with 90% pancreatectomy¹⁷. Betacellulin, a growth factor belonging to the epidermal growth factor (EGF) family, has been shown to promote neogenesis of β -cells and ameliorate glucose intolerance in mice with selective alloxan perfusion¹⁸, and is also reported to enhance proliferation of β -cells in 90% pancreatectomized rats¹⁹. The *Reg* gene, which is induced in regenerating pancreatic islets, has been identified²⁰.

There are several lines of studies suggesting the cell origin of regenerated pancreatic β -cells. In transgenic mice expressing interferon-gamma specifically in pancreatic β -cells, a dramatic proliferation of pancreatic ductal cells, and the appearance of primitive endocrine cells and their subsequent differentiation into endocrine cells has been reported²¹. During regeneration, transitional intermediate cells expressing both carbonic anhydrase II and amylase²², and bearing both endocrine and exocrine

granules²³ appear. The authors speculate from these findings that pancreatic duct cells represent facultative progenitors in adult pancreas. However, their results also suggest that pancreatic acinar cells give rise to intermediate cells that have characteristics of pancreatic duct cells, and then differentiate into endocrine cells. It has been reported that overexpression of transforming growth factor- α induces expansion of pancreatic and duodenal homeobox 1 (Pdx1)-expressing ductal epithelium in the pancreas, and that focal areas of islet neogenesis can be observed²⁴. As pancreatic acinar cells isolated from transforming growth factor- α transgenic mice convert into ductal cells^{25,26}, the expanded pancreatic ductal cells expressing Pdx1 in these mice might well be derived from pancreatic acinar cells. In addition, some pancreatic injury models have been shown to exhibit pancreas regeneration. After ligation of the pancreatic duct in rats, replacement of exocrine acini by duct-like structures is observed²⁷. This acinoductal metaplasia has been thought to be at least in part the result of transdifferentiation of amylase-positive pancreatic acinar cells into amylase-negative and cytokeratin-positive duct-like cells²⁸. By treating the rats with dexamethasone to inhibit loss of amylase expression, transitional cells co-expressing amylase and cytokeratin 20 were detected²⁸, supporting the notion of acinar-to-ductal transdifferentiation. Furthermore, insulin-positive cells that also express amylase have been found, indicating acinar-to-endocrine transdifferentiation.

Although histological analysis has shown that neogenesis or regeneration of pancreatic β -cells occurs in certain conditions, the cellular origin of the new β -cells has not been shown. Recent studies using genetic cell lineage tracing or other cell labeling methods suggest that adult pancreatic β -cells are not derived from non- β -cells²⁹⁻³¹. Using genetic cell lineage tracing, Dor *et al.*²⁹ showed that adult pancreatic β -cells in mice are maintained predominantly by self-replication of pre-existing β -cells. They labeled pancreatic β -cells selectively with human alkaline phosphatase by Cre-loxP-based conditional recombination in adult pancreas, and chased the fate of pre-existing β -cells. In this system, when new β -cells in an adult pancreas are generated from non- β -cells, such as stem/progenitor cells, the frequency of the labeled β -cells should decrease after a chase period. The results showed that the labeling frequency of the β -cells remained unchanged, indicating that the new β -cells were generated primarily from pre-existing β -cells. They concluded that terminally differentiated β -cells retain proliferative capacity, and cast doubt on a significant role for adult stem cells in β -cell replenishment²⁹. Georgia and Bhushan³⁰ reported that during neonatal development, cyclin D2 expression in the endocrine pancreas coincided with the replication of endocrine cells and a massive increase in islet mass. Cyclin D2 is not required for exocrine and ductal cell proliferation, but is required for replication of endocrine cells. In cyclin D2^{-/-} mice, pancreatic islets are much smaller and β -cell mass is reduced to 25% in comparison with wild-type mice. Thus, cyclin D2 plays a key role in β -cell replication, which might be the primary

mechanism for maintaining postnatal β -cell mass³⁰. Furthermore, Teta *et al.*³¹ showed by using a DNA analog-based lineage-tracing technique that unlike gastrointestinal and skin epithelia, specialized progenitors do not contribute to adult β -cell mass, not even during acute β -cell regeneration. Instead, adult β -cells exhibit equal proliferation potential, and expand from within a vast and uniform pool of mature β -cells³². Thus, it is likely that pancreatic β -cell mass is maintained primarily by self-replication of pre-existing β -cells in adult mice, although the existence of pancreatic tissue-specific stem/progenitor cells cannot be excluded.

The turnover rate of pancreatic β -cells in adult is generally low, expansion of the β -cells being detected only in several conditions including neonate, pregnancy and obesity. The mass of pancreatic β -cells increases in the neonatal period as the body and organs grow. However, the increases in β -cell number and the weight of the whole pancreas are not a coincidence^{33,34}, suggesting different mechanisms in the regulation of the mass of pancreatic endocrine and exocrine cells. We have recently shown that non- β -cells contribute to an increase in mass expansion of pancreatic β -cells from neonate through to youth³⁴. Although the signals leading to proliferation of pancreatic β -cells are not yet well characterized, a recent study has shown that EGF-receptor signaling is required for expansion of β -cells in mice under high-fat diet conditions and pregnancy³⁵. In addition, during pregnancy, an increase in insulin resistance in pregnant mothers maintains the nutrient flow to the growing fetus. Prolactin and placental lactogen counterbalance this resistance, and prevent maternal hyperglycemia by driving expansion of the maternal population of insulin-producing β -cells. It should be noted that hepatic activation of extracellular regulated kinase signaling induces proliferation of pancreatic β -cells through a neuronal-mediated relay of metabolic signals³⁶. Such interorgan interaction might serve as an intriguing target for regenerative medicine for diabetes.

In addition, there are several reports showing identification of functional pancreatic adult stem/progenitor cells *in vivo*. It has been shown that progenitors for β -cells are activated in injured adult mouse pancreas, and are located in the ductal lining. Differentiation of the adult progenitors is Ngn3 dependent, and gives rise to all islet cell types both *in situ* and cultured in embryonic pancreas explants³⁷. That study strongly suggests that adult β -cells can be generated not only from pre-existing β -cells, but also from non- β -cells. However, because such progenitors can be detected only when the cells begin to express Ngn3, their precise origin and properties are not ascertained. Although Inada *et al.*³⁸ reported that by using lineage tracing utilizing human carbonic anhydrase II (CAII) promoter a subset of adult β -cells are generated from pancreatic duct cells, Solar *et al.*³⁹ showed that the duct cells (HNF 1 β as the marker) lose potential for differentiation into β -cells after birth. Thus, the origin of newly formed β -cells in the adult pancreas remains to be identified.

IN VITRO EXPANSION OF β -CELLS

In vitro expansion of pancreatic β -cells represents an attractive strategy for obtaining a large amount of β -cells for transplantation. Indeed, human β -cells possess proliferation capacity when cultured on extracellular matrices with growth factors and hormones^{40–42}. However, the capacity is very limited while preserving the β -cell phenotype⁴³, expansion of β -cells often occurs along with loss of the β -cell phenotype (i.e., expression and secretion of insulin)⁴⁴. Such phenotypic changes of β -cells sometimes appear to resemble epithelial-to-mesenchymal transition (EMT). EMT was originally defined in the context of developmental stages: a biological process that allows a polarized epithelial cell to undergo multiple biochemical changes that enable it to assume a mesenchymal cell phenotype⁴⁵. The first report that referred phenotypic changes of pancreatic β -cells to the EMT was carried out by Gershengorn *et al.*⁴⁶, and a similar phenomenon was observed by others⁴⁷. However, other studies using lineage tracing showed that murine β -cells do not undergo EMT^{48–50}. They found that most proliferative mesenchymal cells migrating out from pancreatic islets *in vitro* were not derived from β -cells, and suggested that these cells do not represent a useful source for the generation of physiologically competent β -cells for treatment of diabetes^{48,49}. At that time, the journal, *Diabetes*, stated that ‘EMT participation in mechanism of β -cell expansion has been preempted’⁵¹. Nevertheless, we and others have shown direct evidence of EMT-like phenotypic changes in mouse and human pancreatic β -cells^{52,53}. We have established a culture system using fetal pancreatic cells as feeder cells that induce dedifferentiation of adult β -cells, and we have developed a method whereby pre-existing pancreatic β -cells can be traced throughout the culture process, even when the cells lose insulin expression⁵³. We showed that pancreatic β -cells of adult mice undergo dedifferentiation into mesenchyme-like fibroblastoid cells (i.e., EMT), and that the process is associated with the progression of the cell cycle. The dedifferentiated cells can redifferentiate into insulin-positive cells⁵³. Human pancreatic β -cells were also reported to have the potential to proliferate on EMT and redifferentiate into islet progenitor-like cells⁵⁴. These studies suggest the feasibility of the generation of a large amount of insulin-secreting cells by *in vitro* expansion of pancreatic β -cells.

EXPERIMENTAL GENERATION OF FUNCTIONAL β -CELLS IN VITRO

Table 1 shows a list of experimental generations of insulin-producing cells *in vitro*. The first report of the *in vitro* generation of β -like cells from mouse ES cells was by Lumelsky *et al.*⁵⁵ in 2001. Their differentiation protocol was based on that for neuronal cells. The generation of insulin-positive cells from human ES cells has also been reported⁵⁶. However, as these insulin-positive cells showed low insulin production capacity and poor insulin secretory response, the cells could not be identified as β -cells. In fact, later studies showed that ES cell-derived insulin-positive cells can be generated as a result of uptake of exogenous insulin^{57,58}. In 2006, a new protocol was

Table 1 | Experimental regeneration of insulin-producing cells *in vitro*

Original cell type	Features of differentiation protocol	Efficiency of generation of insulin+ cells	Other β -cell markers	Insulin production		Glucose-induced insulin secretion	Reversal of hyperglycemia in transplanted animals	Remarks	Reference
				Content	Ratio to normal islet ^a				
Mouse ES cell	Selection of nestin-positive cells	15%	GLUT2, Pdx1, IAPP (mRNA)	145 ng/mg protein ^b	1/250	Yes	No		55
Human ES cell	Spontaneous differentiation	60–70% (1–3% strongly stained)	Gck, GLUT2, Pdx1 (mRNA)	NA	NA	No	NA		56
Human ES cell	Induction of definitive endoderm	3–12%	Pdx1, Nkx2.2, IAPP	20–200 pmol/ μ g DNA	1/25–1/2.5	No	NA		59
Human iPS cell	Induction of definitive endoderm	NA	Pdx1	NA	NA	Yes	NA	iPS cells generated from foreskin fibroblast	61
Human iPS cell	Induction of definitive endoderm	NA	Pdx1, Nkx2.2 (mRNA)	NA	NA	Yes	NA	iPS cells generated from skin fibroblast of patient with type 1 diabetes	62
Mouse iPS cell	Three-stage differentiation	50%	Pax4, IAPP (mRNA)	NA	NA	Yes	Yes	iPS cells generated from skin fibroblast	63
Putative progenitor cell (mouse)	Culture onto Matrigel TM matrix	4.7%	Pdx1, GLUT2	40.2 ng/ μ g DNA ^b	1/3.5	Yes	NA	Clonally identified neurosphere-like putative progenitor cells	66
Putative progenitor cell (mouse)	Culture with GLP-1	NA	Pdx1, IAPP, GLUT2 (mRNA)	NA	NA	NA	NA	FACS purification of c-Met ⁺ c-Kit ⁻ CD45 ⁻ TER119 ⁻ cells	67
Putative progenitor cell (rat/human)	Nestin-positive	NA	Pdx1, GLUT2, synaptophysin (mRNA)	NA	NA	NA	NA		16
Mouse pancreatic duct cell	Culture with high concentration of glucose	NA	NeuroD	0.5 pg/IPC-derived islet	1/150,000	NA	Yes	Cells isolated from pre-diabetic NOD mouse	68, 69
Human pancreatic duct cell	Culture onto Matrigel TM matrix with nicotinamide and KGF	NA	NA	57–440 ng/ μ g DNA	1/53–1/7	Yes ^c	NA		70

Table 1 (Continued)

Original cell type	Features of differentiation protocol	Efficiency of generation of insulin+ cells	Other β -cell markers	Insulin production		Glucose-induced insulin secretion	Reversal of hyperglycemia in transplanted animals	Remarks	Reference
				Content	Ratio to normal islet ^a				
Rat pancreatic exocrine cell	Culture in suspension	5.57%	Pdx1, NeuroD, Gck, GLUT2 (mRNA)	0.4 pmol/ μ g DNA	1/1,250	NA	NA		76
Rat pancreatic exocrine cell	Culture with EGF and LIF, and inhibition of Notch signaling	30%	Pdx1	26.1 pg/cell	1/2	Yes	Yes	Further differentiation after transplantation	74, 75
Mouse pancreatic exocrine cell	Activation of EGF signaling and spheroid formation	5%	Pdx1, NeuroD, Nkx2.2, Gck, GLUT2, Kir6.2, SUR1 (mRNA)	600 ng/mg protein ^b	1/20	Yes	NA	Direct evidence for acinar cell origin	77, 78, 80
Human pancreatic exocrine cell	Activation of EGF signaling and spheroid formation	NA	Pdx1, Gck, Kir6.2, SUR1, NeuroD, MafA (mRNA)	2.8–130 mg/ μ g protein	1/90–1/2	Yes	NA		82

c-Met⁺, c-Met positive; c-Kit⁻, c-Kit negative; CD45⁻, CD45 negative; DNA, deoxyribonucleic acid; EGF, epidermal growth factor; ES, embryonic stem; FACS, fluorescence-activated cell sorting; Gck, glucokinase; GLP-1, glucagon-like peptide-1; GLUT, glucose transporter; IAPP, islet amyloid polypeptide; IPC, islet-producing stem cells; iPS, induced pluripotent; KGF, keratinocyte growth factor; Kir, inward-rectifier potassium ion channel; LIF, leukemia inhibitory factor; MafA, musculoaponeurotic fibrosarcoma oncogene homolog A; mRNA, messenger ribonucleic acid; NA, not applicable; NeuroD, neurogenic differentiation; Nkx2.2, NK2 homeobox 2; NOD, non-obese diabetic; Pdx1, pancreatic and duodenal homeobox 1; SUR1, sulphonylurea receptor 1; TER119⁻, TER119 negative. ^aCalculation based on our own measurement of insulin content of normal islet: 250 μ g/mg protein [75 ng/islet, 3 μ g (500 pmol)/ μ g DNA]. ^bInsulin content was measured in total (including both insulin-positive and -negative cells). ^cInsulin secretion was measured as accumulation for 24 h.

developed to generate insulin-secreting cells, in which differentiation processes mimic pancreatic organogenesis by directing cells through stages resembling definitive endoderm, gut-tube endoderm, pancreatic endoderm and endocrine precursor⁵⁹. However, the insulin-secreting cells made by this method showed poor glucose responsiveness and appeared immature. A later study made by the same group showed that glucose-responsive insulin-secreting cells can be generated by transplantation of the cells at the stage of pancreatic endoderm⁶⁰. Insulin-secreting cells have also been generated from induced-pluripotent stem (iPS) cells^{61–63}, but precise analysis of the insulin secretion has not been carried out.

Several studies have attempted to identify stem/progenitor cells in the adult pancreas. Zulewski *et al.*¹⁶ showed that cells expressing the neural stem cell marker nestin occur in human and rat pancreatic islets, and that these cells can be isolated and cultured for a long time. It has been shown that cultured nestin-positive cells can be differentiated into insulin-producing cells^{16,64}, and that such cells from human fetal pancreas when transplanted can be expanded and differentiated into islet-like cell clusters, which can reverse hyperglycemia in diabetic mice⁶⁵. Clonal identification of multipotent precursors from adult mouse pancreas has also been reported⁶⁶. These candidate progenitor cells proliferate in the serum-free conditions used for neural stem cell (NSC) culture and form spherical cell clusters like NSCs by floating culture. Interestingly, the cells in the spherical clusters show characteristics of both pancreatic and neural precursors. Furthermore, the potential progenitors give rise to multiple types of neural cells, including neurons and glial cells, and also differentiate into insulin-producing (β) cells, glucagon-producing (α) cells, and somatostatin-producing (δ) cells. The insulin-producing cells derived from these progenitors are glucose-competent in terms of Ca^{2+} responsiveness and insulin secretion. Suzuki *et al.*⁶⁷ reported isolation of pancreatic progenitor cells from adult mouse by using fluorescence-activated cell sorting. The isolated cells express c-Met, the receptors for hepatocyte growth factor, but do not express hematopoietic and vascular endothelial antigens, such as CD45, TER119, c-Kit and Flk-1. Thus, hepatocyte growth factor/c-Met signaling might play an important role in the maintenance of these progenitor-like cells. The cells formed clonal colonies *in vitro*, and differentiated into multiple pancreatic lineages from single cells. However, no functional analysis has been carried out for the differentiated pancreatic cells induced from these candidate progenitors. Taken together, these findings suggest that although stem/progenitor-like cells might be obtained from the adult pancreas, it is not yet clear that such isolated stem/progenitor-like cells have the full potential to differentiate into native pancreatic β -cells and function as stem/progenitors in the pancreas *in vivo*.

Peck *et al.*^{68,69} isolated and cultured pancreatic ductal cells from prediabetic non-obese diabetic mice, and induced islet-like cell clusters containing insulin-producing cells. Although the insulin secretory capacity of the induced cells was extremely low, transplantation of the cells to diabetic mice could lead to

normalization of blood glucose levels. It is possible that these insulin-producing cells further differentiate *in vivo*, but no relevant functional analyses after transplantation have been carried out. Bonner-Weir *et al.*⁷⁰ generated insulin-producing cells from human pancreatic ductal cells. The ductal cells were expanded as a monolayer, and the cells were then overlaid with a thin layer of Matrigel. The cells formed 3-D structures of ductal cysts from which islet-like clusters budded. These cells secreted insulin, although the amount was low. Generation of insulin-producing cells by this method was reproduced by Gao *et al.*⁷¹. However, they reported the possibility in a later study that contaminated β -cells in the starting material underwent dedifferentiation and redifferentiation during the culture⁷². In contrast, Bouwens *et al.*⁷³ have claimed the importance of transdifferentiation of pancreatic exocrine cells in regeneration of pancreatic cells. They reported that pancreatic exocrine cells can be converted into insulin-producing cells by culturing with EGF and leukemia inhibitory factor^{74,75}. Song *et al.*⁷⁶ reported a similar study. They isolated pancreatic acinar cells from adult rats, and cultured the cells in suspension without adding any growth factor or cytokines. Insulin-positive cells were detected at the periphery of the spherical cell clusters derived from the acinar cells⁷⁶. Although these studies suggest transdifferentiation of pancreatic acinar cells into insulin-producing cells, neither direct evidence of the origin of these cells nor their precise insulin secretory properties were identified. At the same time, we also reported transdifferentiation of pancreatic acinar cells, and utilized the method of cell lineage tracing based on the Cre-loxP system to determine the origin of the newly-made insulin-secreting cells⁷⁷. We have shown that disruption of cadherin-mediated cell–cell adhesion in pancreatic acinar cells induces dedifferentiation, and the subsequent activation of the phosphoinositide 3-kinase/Akt pathway recovers the cell–cell adhesion, which induces redifferentiation into endocrine or ductal cells (Figure 3)^{78,79}. This explains the rarity of *in vivo* transdifferentiation of acinar cells into endocrine cells, as even in severe cases of pancreatitis or cancer, cell–cell contacts are not completely destroyed. The acinar cell-derived insulin-secreting cells secreted insulin in response to a variety of stimuli, including glucose and GLP-1. However, insulin production of the cells was lower than that of native islets. As the newly-made cells expressed genes, such as HNF6 and PGP9.5, which are not expressed in native β -cells, but did not express MafA, which regulates the expression of genes that participate in glucose-induced insulin secretion^{77,78,80,81}, the cells were thought to be in an immature state. Insulin-secreting cells can also be generated from human pancreatic exocrine cells⁸².

INTERCONVERSION OF PANCREATIC ENDOCRINE CELLS

Thorel *et al.*⁸³ reported that α -cells transdifferentiate into β -cells under conditions of severe loss of β -cells. They generated mice carrying a transgene containing the diphtheria toxin receptor under the control of the insulin promoter (RIP-DTR). In male

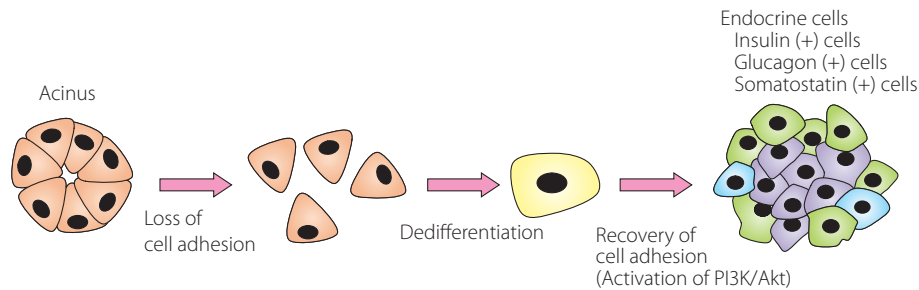


Figure 3 | Model for pancreatic acinar-to- β -cell transdifferentiation. Enzymatic dissociation disrupts epithelial structures of acini, resulting in loss of cadherin-mediated cell–cell adhesion, which causes dedifferentiation of the acinar cells. Meanwhile, epidermal growth factor receptors are activated, which is followed by activation of the phosphoinositide 3-kinase PI3K/Akt pathway. Within a few days of culture, cadherin-mediated cell–cell adhesion is recovered by the enhanced expression of E-cadherin, which is essential for redifferentiation of the dedifferentiated cells into insulin-secreting cells.

RIP-DTR mice, more than 99% of the β -cells in the adult pancreas can be destroyed by injection of diphtheria toxin. When such mice were kept alive for up to 10 months with insulin treatment during the initial 5 months, the β -cell mass increased to 10% of normal animals on average, and glycemic control improved in some of the mice. By using a conditional cell lineage tracing method, the origin of the regenerated β -cells was shown not to be surviving pre-existing β -cells, but rather glucagon-expressing α -cells. Although their findings suggest the usefulness of α -cells as a source of new β -cells, the conversion of α -cells to β -cells decreases the α -cell population, which might cause the loss of glucagon action in the maintenance of glucose homeostasis. However, they later reported that near-total ablation of α -cells did not affect the insulin counter-regulatory response and glucose homeostasis in mice⁸⁴.

Talchai *et al.*⁸⁵ recently showed that pancreatic β -cell dedifferentiation rather than cell death is responsible for diabetic β -cell failure in several different diabetic mouse models. In their experiments, adult mouse β -cells underwent dedifferentiation and reverted to progenitor-like cells expressing Ngn3, Oct4, Nanog and L-Myc under conditions of physiological stress, such as β -cell-specific disruption of FoxO1. The dedifferentiated β -cells can convert to other endocrine cell types, including glucagon-expressing α -cells. It has also been shown that ectopic expression of certain transcription factors can change the fate between α - and β -cells⁸⁶. These studies suggest an attractive future therapeutic strategy for diabetes in which pancreatic β -cells can be generated from α -cells by inducing their transdifferentiation *in vivo*.

PERSPECTIVES

There are many different approaches towards the development of cell replacement therapy for insulin-deficient diabetes mellitus. However, *in vivo* regeneration of β -cells in humans is not realistic at present from both efficacy and safety points of view, and most newly generated insulin-secreting cells *in vitro* are not fully differentiated β -cells, as assessed by both insulin secretory properties and gene expression profile. Another unique

and intriguing strategy of regenerative medicine for diabetes has recently been proposed by Kobayashi *et al.*⁸⁷, who made use of the technique of “blastocyst complementation”, which enables *in vivo* generation of organs derived from pluripotent stem cells (PSCs: ES cells or iPSCs), and generated a rat pancreas in a mouse by injecting wild-type rat PSCs into a *Pdx1*^{-/-} (pancreatogenesis-disabled) mouse blastocyst. Thus, a human pancreas might be generated from human PSCs in a pig for use in organ transplantation, although many issues of concern must be addressed to bring this principle into clinical use.

Human pancreatic α -cells have recently been shown to express GLP-1 and prohormone convertase 1/3 (PC1/3), as well as glucagon and PC2⁸⁸. In addition, a recent study showed ectopic expression of glucose-dependent insulinotropic polypeptide (GIP) in pancreatic β -cells in mice with the complete absence of proglucagon-derived peptide⁸⁹. Thus, it is worth re-evaluating the roles and functions of pancreatic endocrine cells. We must expand our knowledge of the physiological processes of differentiation, proliferation and regeneration of pancreatic β -cells as an essential step on the path to cell therapy to cure patients with absolute insulin deficiency.

ACKNOWLEDGEMENTS

This work was supported by a CREST grant from the Japan Science and Technology Agency, and Grant-in-Aid for Scientific Research and grants for the Global Center of Excellence (COE) Programs ‘Global Center of Excellence for Education and Research on Signal Transduction Medicine in the Coming Generation’, ‘Global Center for Education and Research in Integrative Membrane Biology’, and Kobe Translational Research Cluster, the Knowledge Cluster Initiative from the Ministry of Education, Culture, Sports, Science and Technology, Japan. The authors declare that there is no conflict of interest.

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