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Pancreatic β cell regeneration: to β or not to β

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Diabetes is a major worldwide health problem which results from the loss and/or dysfunction of pancreatic insulin-producing β cells in the pancreas. Therefore, there is great interest in understanding the endogenous capacity of β cells to regenerate under normal or pathological conditions, with the goal of restoring functional β cell mass in patients with diabetes. Here, we summarize the current status of β cell regeneration research, which has been broadly divided into three *in vivo* mechanisms: 1. proliferation of existing β cells; 2. neogenesis of β cells from adult ductal progenitors; and 3. transdifferentiation of other cell types into β cells. We discuss the evidence and controversies for each mechanism in mice and humans, as well as the prospect of using these approaches for the treatment of diabetes.

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Introduction

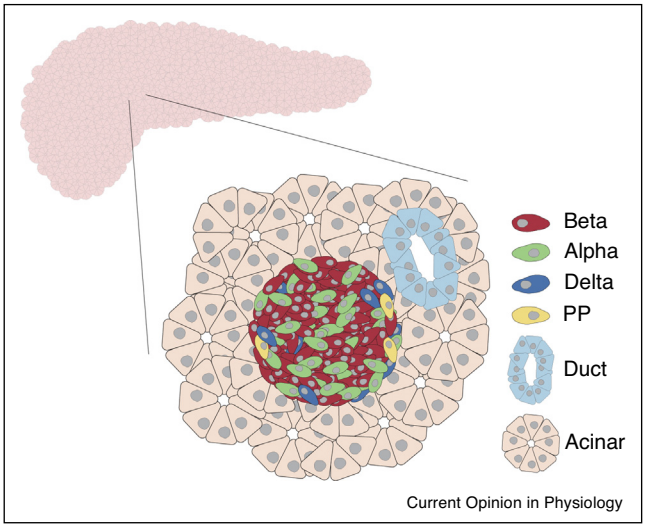
The pancreas is an endodermally derived organ consisting of exocrine tissue that secretes digestive enzymes into the stomach and duodenum, and endocrine tissue responsible for producing hormones to maintain glucose homeostasis. The endocrine cells are organized into distinct micro-organs known as Islets of Langerhans and are embedded within the exocrine tissue (Figure 1). The four main endocrine cell types of the adult pancreas are α, β, δ, and PP cells, which produce glucagon, insulin, somatostatin, and pancreatic polypeptide, respectively. Of particular importance are the insulin-producing β cells; the condition of insufficient functional β mass cells results in diabetes mellitus, a disease which affects over 400 million people worldwide and is currently increasing in incidence [1]. There are two main types of diabetes: type 1 diabetes (T1D) is caused by an autoimmune attack on the β cells, whereas type 2 diabetes (T2D) results from β cell dysfunction and/or peripheral insulin resistance and subsequent insulin insufficiency. Complications associated with long-term T1D and T2D include cardiovascular disease, kidney disease, neuropathy, stroke and premature death [1]. Together, they pose a major public health challenge and will continue to be an increasing burden on the healthcare system and society in the future.

Current treatment options for diabetes include exogenous insulin administration and transplantation using pancreas tissue or islets isolated from cadaveric donors; however, there are several caveats associated with these approaches. Patients receiving exogenous insulin are prone to wide-ranging fluctuations in blood glucose levels and potentially life-threatening bouts of hypoglycemia, although in recent years the development of continuous glucose monitoring technology and closed loop insulin pumps have greatly improved more consistent glucose control [2]. Alternatively, patients can receive islet transplantation; however, each recipient requires islets from at least two donor pancreata and requires continuous treatment with immunosuppressive drugs [3]. Furthermore, islet transplantation only confers insulin-independence for approximately five years, necessitating a life-long supply of donor tissue [3]. Because of the challenges associated with these current diabetes treatments, there is immense interest in understanding whether pancreatic β cells have the ability to regenerate under both normal and pathogenic conditions. This knowledge could facilitate the development of unlimited sources of replacement β cells, either through β cell regeneration *in vivo* or by generating new β cells using *in vitro* systems. In this review, we will predominantly focus on research efforts associated with *in vivo* β cell regeneration, which can be broadly divided into three categories (1) proliferation of existing β cells, (2) neogenesis: differentiation of new β cells from a progenitor population and (3) transdifferentiation of non-β cells into β cells (Figure 2). Researchers have long debated whether these regenerative processes normally occur in mice and humans, and whether they can be activated under certain pathogenic conditions or in response to exogenous stimuli (reviewed in [4,5]). Here, we will review the recent advances, caveats and controversies surrounding each of these mechanisms.

β cell proliferation

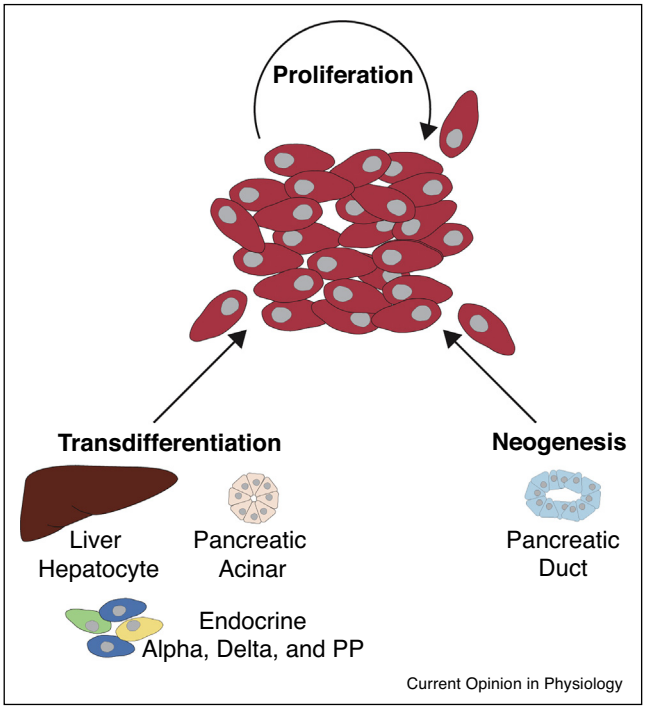
Self-renewal of existing β cells is an attractive approach for generating new β cells for therapeutic purposes. β cells normally proliferate in the developing (embryonic and neonatal) mouse and human pancreas and can be stimulated to replicate by a number of metabolic stressors including pregnancy and obesity [6–8]. During the early

Figure 1



Overview of pancreatic cell types.
The pancreas is composed of acinar, ductal and endocrine cells. The endocrine cells represent 1–5% of the total pancreas area and form clusters of hormone-producing cells known as islets, which are composed of α, β, δ and PP cells. Loss of functional β cell mass leads to diabetes.

Figure 2



Three mechanisms of beta cell regeneration.
1. Existing beta cells can be stimulated to proliferate either *in vivo* or *in vitro*. 2. Pancreatic duct cells can be induced to create a new source of beta cells, potentially by re-activating developmental programs initially used during embryogenesis for β cell specification. 3. Additional cell types, such as pancreatic acinar tissue, non-β endocrine cells, and hepatocytes from the liver can be genetically reprogrammed into functional β cells.

postnatal period, proliferation is the primary mechanism of β cell expansion to generate sufficient β cell mass in an organism [9,10]. However, β cell proliferation rapidly declines early in life, and in adults the rate of β cell division is very low [11,12]. To date, the identification of molecules that can activate replication in adult β cells has proven challenging, partly due to species-dependent molecular differences between mouse and human β cells; factors that can stimulate replication of mouse β cells do not necessarily induce expansion of human cells [13,14]. Another impediment is due to the fact that adult β cells are refractory to mitogens which are able to stimulate proliferation in juvenile β cells from younger donors [15•]. Unlike juvenile β cells, adult β cells have increased expression of cell cycle inhibitors such as p16INK4a and a reduction in cell cycle activators including FoxM1, cyclins, and cyclin-dependent kinases that render them resistant to proliferation [16–19]. Furthermore, it is hypothesized that adult β cells are resistant to rapid turnover to prevent hyperinsulinemia and thus, one valid concern is that inducing unconstrained β cell growth in people could lead to the formation of insulinomas and potential lethality due to hypoglycemia.

The search for factors that can activate replication in adult β cells is further complicated by recent insight into β cell heterogeneity. While it is clear that different subpopulations of β cells exist within an islet, it is not known whether all β cells have the capacity to proliferate [20–22]. Flattop (Fltp), an effector of Wnt/planar cell polarity signaling, was recently shown to mark a population of mature β cells with greater functionality, but lower proliferative potential, suggesting that there may be a subset of β cells that have a greater ability to proliferate than others [23••]. Therefore, islets may contain heterogeneous populations of islets β cells representing a continuum of functionality versus self-replication. The identification of markers delineating cells with a higher proliferative capacity would potentially allow researchers to specifically target this population. However, β cell proliferation often appears to occur at the expense of insulin secretion, and replicating β cells tend to more closely resemble immature β cells. For example, when replication in adult mouse β cells was induced by exogenously expressing c-Myc, these β cells displayed reduced expression of genes important for glucose sensing and insulin secretion (Glut2, PC1/3), as well as transcriptional markers of mature β cells (Pdx1, MafA, Nkx2.2) [24]. Thus, the balance between proliferation and functionality must also be considered when identifying new molecules to expand β cell mass.

One promising target which has been recently shown to reproducibly affect human β cell proliferation is the dual-specificity tyrosine phosphorylation–regulated kinase 1A (DYRK1A). Inhibitors of DYRK1A, including harmine, 5-iodo-tubericidin (5-IT), INDY and GNF4877, have been

shown to increase proliferation of sorted human β cells *in vitro* as well as inducing proliferation of β cells transplanted into mice, apparently without inducing de-differentiation [25•,26•,27,28]. Mechanistic studies suggest DYRK1A inhibitors promote cell cycle progression in part by stimulating activation of the nuclear factor activated in T cells (NFaT) signaling pathway [25•,26•]. Furthermore, DYRK1A inhibitors appear to synergize with inhibitors of the transforming growth factor-β superfamily (TGFβSF), which has by itself been shown to regulate β cell proliferation [29••,30]. The practical use of DYRK1A inhibitors in humans, however, is hindered by the fact that they are not β cell specific and can enhance proliferation of many other cell types, including pancreatic α and ductal cells [29••,30]. Therefore, for therapeutic purposes, it will be necessary to develop methods to target these inhibitors specifically to β cells. A more immediate use for DYRK1a inhibitors, may be in *in vitro* β cell culture systems to expand exogenous or stem cell-derived β cells for transplantation purposes.

Neogenesis

Pancreatic β cells are initially formed during embryonic development from an endocrine progenitor population that lies within the pancreatic ductal epithelium and is marked by the transcription factor Neurogenin3 (Ngn3). In mice and humans, Ngn3+ endocrine progenitor cells differentiate into all four adult endocrine cell types during embryogenesis but decline in numbers upon birth [31–34]. Ngn3 null mice lack all islet endocrine cells indicating Ngn3 is absolutely required for endocrine neogenesis during development [32]; whereas in humans, the known NGN3 mutations variably contribute to diabetes [35,36]. Because endocrine cells originate from the ductal epithelium during development, many researchers have examined whether the embryonic endocrine differentiation program can be re-activated in adult pancreatic ducts to serve as a potential source of new β cells. However, whether this occurs endogenously or under certain pathological conditions remains controversial. Several studies using pancreatic injury models, such as pancreatic duct ligation or partial pancreatectomy have shown the reappearance of Ngn3 positive progenitor cells within the adult ductal epithelium and the presence of small clusters of endocrine cells close to these ducts, suggesting neogenesis can occur [37–41]. However, studies using similar approaches provide evidence that neogenesis does not occur, suggesting this mechanism is difficult to activate or is relatively rare [42–45]. Genetic lineage tracing experiments in mice using a Cre-lox system to genetically label specific populations of putative ductal progenitor cells with β-galactosidase or fluorescent reporter proteins also demonstrated contradictory results. Lineage tracing of the ductal tree using an inducible Cre recombinase (CreER) driven by a fragment of the human carbonic anhydrase promoter provided evidence that mature ducts can give rise to endocrine cells, whereas experiments using Hnf1CreER and Sox9CreER showed evidence to the contrary [46–48]. Recent studies in cultured pancreas and organoid systems also suggest that mouse ductal cells can be induced to differentiate into β cells under specific culture conditions indicating that although the occurrence of *in vivo* β cell neogenesis remains controversial, ductal cells could potentially serve as a source of *in vitro* derived β cells [49].

In humans, obtaining proof of β cell neogenesis has also been challenging. Potential evidence of ductal derived β cells has been proposed based on the observation of islet cell clusters that are adjacent or closely opposed to ducts in donor pancreata [50,51]. Ductal cells positive for immature β cell markers have also been detected in samples from pregnant humans and individuals with T2D, and appear to increase in numbers in obese individuals. Furthermore, human ductal cells can be induced to express pancreatic markers and insulin in *ex vivo* culture systems [52,53]. Valdez *et al.*, were also able to demonstrate the induction of endocrine differentiation in the human PANC1 pancreatic ductal cell line downstream of NGN3 activation by proinflammatory cytokines [54]. However, without the ability to perform genetic lineage tracing of human ductal cells, it is difficult to confirm that human β cell neogenesis appreciably occurs *in vivo*.

Transdifferentiation from other cell types

While it remains unclear whether and under which conditions ductal cells can be re-activated to differentiate into β cells, there is mounting evidence that other differentiated tissue types can be reprogrammed into β cells in a process broadly referred to as transdifferentiation. During embryonic development, the pancreas forms from a region of foregut endoderm marked by pancreatic and duodenal homeobox factor 1 (Pdx1) that is posterior to the antral stomach, adjacent to the budding liver, and anterior to the duodenum [55]. Because of their common developmental lineage, it is attractive to speculate that cells from these closely related endodermal organs could be reprogrammed into pancreatic endocrine cells. Indeed, a number of studies have demonstrated that insulin positive cells can be induced *in vivo* in the livers of mice by the adenoviral transduction of one or a combination of key pancreatic transcription factors, including Pdx1, NeuroD1, or a combination of Ngn3, Pdx1 and MafA (known as the PNM factors) [56–59]. Furthermore, the ectopic β cells that are generated in the liver are capable of secreting insulin and these mice are resistant to chemically induced diabetes [58,59]. More recently, the novel factor TGIF2, a modulator of BMP/TGF-β signaling that is expressed in common hepatic and pancreatic endoderm progenitors, but becomes restricted to the pancreas during development, was shown to induce pancreatic progenitor gene expression in the adult mouse liver [60•]. Currently, it is not known whether terminally

differentiated hepatocytes or a more "stem cell-like" population within the liver are capable of transdifferentiating to insulin-expressing cells, and this may also depend on the type of viral vector used and the method of delivery.

Two groups also used genetically modified mice to express the PNM factors within specific tissue compartments of the gut, and identified ectopic insulin-expressing cells in the intestinal crypts and antral stomach, suggesting that several foregut endoderm tissues are intrinsically competent to be reprogramed into insulin-producing cells [61,62]. Downregulation of the transcription factor Foxo1 in Ngn3-expressing gastrointestinal enteroendocrine cells in mice or cultured organoids was also able to induce insulin expression, providing yet another potential source of insulin producing cells [63,64]. However, whether these mechanisms can be induced in human cells requires further exploration.

Within the pancreas itself, terminally differentiated exocrine tissue has also been suggested as source of *de novo* endocrine cells. In 2008, Zhou *et al.*, found that adenoviral delivery of the PMN factors into the pancreas of an adult immune compromised mouse, could convert acinar cells into insulin producing β cells [65]. However, the endogenous capacity of exocrine tissues to generate β cells without adenoviral administration has been called into question. Clayton *et al.*, used an inducible transgenic mouse model system to express the PMN factors in pancreatic acinar cells and found that both the level of PMN factor expression and inflammation influence reprogramming outcomes [66]. There have also been conflicting results using genetic lineage tracing. Experiments labeling acinar cells with Elastase-CreER demonstrated that exocrine cells do not contribute to the endocrine compartment under normal or several different injury conditions, whereas a recent report using a similar Cre line found that acinar cells could differentiate into β cells following EGF and CNTF treatment after alloxan induced-injury [67,68].

Recent attention has been also focused on understanding whether other endocrine cell types within the islet have the regenerative potential to convert into β cells. Although endocrine cells were once thought to be a stable, terminally differentiated population, studies have shown that they are considerably more plastic under stress conditions or upon genetic manipulation [69–72]. In an adult mouse model of extreme β cell killing and hyperglycemia, α cells began to co-express insulin, and some of these bihormonal cells were shown through genetic lineage tracing to become monohormonal insulin positive cells over time [73]. Subsequent studies have shown that reprogramming can occur throughout the mouse lifetime in response to physiological stimuli such as multiple rounds of pregnancy, and that δ cells can also convert to β cells in young mice after β cell injury [74,75]. Reprogramming of α cells to β cells has also recently been suggested to occur normally without stimuli or injury. A population of immature β cells identified by the presence of insulin expression, but absence of the maturity marker urocortin3 (Ucn3) were found at the periphery of the islet, and are thought to be in a transition state between mature α cells and β cells [76••]. Lineage tracing confirmed that these cells once expressed glucagon. In contrast to previous studies, which found no contribution of other endocrine cell types to the β cell pool throughout the lifetime of an islet, this study suggests that there may be a dedicated population of cells, at least in the mouse, which exhibit lineage plasticity.

Because of the inability to perform lineage tracing experiments in humans, the question still remains whether endocrine cells can transdifferentiate into β cells in patients with pancreatic disease. Insulin and glucagon double-positive cells have been detected in tissue sections from patients with T1D and T2D [77,78]; however, polyhormonal cells can also be detected in the human pancreas during normal development [79]. Therefore, it is not known whether these cells are undergoing de-differentiation to a more embryonic-like state or whether they are in the process of transdifferentiatiating to another endocrine cell type. More broadly, the question of whether the process of endocrine transdifferentiation in humans or mice requires de-differentiation before reprogramming has not yet been answered definitively, as both direct reprogramming and transdifferentiation associated with dedifferentiation (with or without re-expression of Ngn3) have been reported [77,80].

The molecular mechanisms underlying the process of transdifferentiation of other endocrine cells to β cells have not been fully elucidated. Ectopic expression of the transcription factor Pax4 and inhibition of the α cell gene Arx in mice appear to allow α cells to convert to β cells [81,82]. More recently, it was reported that the deletion of the DNA methyltransferase, Dnmt1, together with Arx is necessary for conversion of α cells to functional β cells [77]. The expression of Pdx1 and MafA specifically in α cells using a genetic approach or throughout the pancreas using a viral approach can also induce insulin expression in α cells and, in the latter study could rescue blood glucose levels in the non-obese diabetic (NOD) model of autoimmune diabetes [83,84]. Interestingly, α and PP cells sorted from human donor islets were also able to be reprogramed into insulin secreting cells when transduced with Pdx1 and MafA, indicating that these factors are key regulators of the β cell fate [85]. Insight into the plasticity of α cells comes from a study that profiled epigenetic histone marks of sorted human endocrine cells. Compared to exocrine and β cells, the pattern of histone marks in the genome of α cells more closely resembles the pattern in pluripotent cells,

suggesting α cells may be transcriptionally poised to undergo lineage reprogramming [86].

There is also considerable interest in identifying small molecules and drugs that can induce transdifferentiation and enhance β cell mass in patients with diabetes. Recently, the γ-aminobutyric (GABA) signaling pathway, activated either by GABA itself or by the anti-malarial class of drugs artemisinins, including artemether and artesunate, was reported to induce transdifferentiation of α cells to β cells in *in vitro* and *in vivo* models in rodents, human islets and zebrafish [87•,88•]. Long-term GABA administration resulted in significant β cell hyperplasia which involved activation of a neogenic-like program within the pancreatic ducts 88•]. However, several recent studies demonstrated convincingly that neither GABA nor artemether can induce α to β cell reprogramming. Ackermann *et al.*, found that while islets from mice treated with artemether or GABA had reduced expression of Arx, there was no difference in the number of α cells that had transdifferentiated to β cells [89••]. In a similar study, van der Meulen *et al.* showed that culturing mouse islets with artemether also resulted in reduced expression of α cell genes, but that these α cells failed to transdifferentiate into insulin-positive cells [90••]. Furthermore, data from these groups and others suggest that GABA signaling may in fact reduce insulin expression and impair β cell function [89••,90••,91]. The discrepancy between the ability of GABA to induce transdifferentiation in these studies could be partly due to technical differences in the lineage tracing approaches used. Ackermann *et al.* [89••] and van der Meulen *et al.* [90••] used an inducible glucagon-driven Cre line to mark mature α cells before GABA or artesunate administration, whereas the studies by Ben-Othman *et al.* 88•] and Li *et al.* [87•] utilized a constitutively active Cre recombinase driven by the glucagon promoter which is active during development and also labels immature cells or other endocrine cells which have transitioned through a glucagon-expressing intermediate. Thus, the role of GABA in α to β cell reprogramming remains uncertain.

Conclusions

The ultimate goal of pancreatic regeneration research is to expand endogenous β cell mass without compromising function, to prevent or treat diabetes. Although significant advances have been made in each of the mechanisms discussed in this review, many challenges associated with stimulating β cell regeneration *in vivo* remain. These issues are further compounded by our inability to assess endogenous β cell mass or to track changes in β cell mass in response to disease interventions. In light of the challenges associated with *in vivo* islet regeneration, substantial research efforts are now focused on *in vitro* differentiation of pluripotent embryonic stem (ES) cells or induced pluripotent stem (iPS) into insulin-producing cells which could be used for transplantation. Although these protocols have made significant advances in generating glucose-responsive insulin-expressing cells, the process remains inefficient and variable, and long-term stability of a β cell phenotype post-transplantation has not been determined [92]. Furthermore, with the recent identification of functional heterogeneity within the β cell population [20,23••], questions arise regarding whether multiple different types of β cells are necessary for full optimal glucose control. If so, which intrinsic and extrinsic pathways are required to generate and maintain this heterogeneity?

Despite the many recent scientific advances, it remains uncertain whether human pancreatic β cells possess intrinsic regenerative capacity. However, there are extensive data to suggest that — under the right conditions — the potential for regeneration exists. With the advent of novel technologies such as single cell RNA-sequencing, there is renewed hope that a rare β cell subpopulation with increased regenerative capacity will be identified. The existence of such specialized β cells would overcome many of the current challenges associated with β cell expansion and could provide the basis for efficient β cell regeneration on demand. *In vitro* islet generation from human pluripotent stem cells also continues to be a viable treatment option, and although this approach also presents its own unique challenges, the continual advances in this technology has been remarkable. Overall, there is great promise for both approaches, and a greater understanding of the mechanisms of regeneration holds the potential to substantially improve the future of diabetes treatment.

Conflict of interest statement

Nothing declared.

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