

How to make a functional β -cell

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Summary

Insulin-secreting pancreatic β -cells are essential regulators of mammalian metabolism. The absence of functional β -cells leads to hyperglycemia and diabetes, making patients dependent on exogenously supplied insulin. Recent insights into β -cell development, combined with the discovery of pluripotent stem cells, have led to an unprecedented opportunity to generate new β -cells for transplantation therapy and drug screening. Progress has also been made in converting terminally differentiated cell types into β -cells using transcriptional regulators identified as key players in normal development, and in identifying conditions that induce β -cell replication *in vivo* and *in vitro*. Here, we summarize what is currently known about how these strategies could be utilized to generate new β -cells and highlight how further study into the mechanisms governing later stages of differentiation and the acquisition of functional capabilities could inform this effort.

Key words: β cell, Diabetes mellitus, Mammalian metabolism

Introduction

Diabetes mellitus is a metabolic disease that results from a failure in glucose regulation, causing severe hyperglycemia, tissue/organ damage and increased morbidity and mortality. Pancreatic β -cells respond to high blood glucose levels by secreting the peptide hormone insulin, which acts on other tissues to promote glucose uptake from the blood, for example in the liver where it promotes energy storage by glycogen synthesis (Powers and D'Alessio, 2011). The Centers of Disease Control (CDC) estimated that 25.8 million Americans had diabetes in 2010, and more than 300 million people are affected worldwide according to the International Diabetes Federation, thus making diabetes a major worldwide healthcare challenge.

Diabetes is classified into two related but distinct diseases with different causes. Type 1 diabetes results from autoimmune destruction of insulin-producing β -cells in the pancreas. Type 2 diabetes, which is commonly associated with obesity, occurs when insulin demand due to persistently high blood sugar overwhelms the capacity of β -cells to produce sufficient insulin to prevent hyperglycemia. In Type 2 diabetes, peripheral tissues, such as fat and muscle, also become resistant to the effects of insulin. This high demand on β -cells frequently leads to β -cell malfunction, de-differentiation and death (Ashcroft and Rorsman, 2012; Talchai et al., 2012). The number of β -cells lost in Type 2 diabetes is unclear but can approach 60% (Butler et al., 2003; Rahier et al., 2008), and the remaining β -cells are likely to be in some way dysfunctional.

In diabetes, the persistent misregulation of glucose homeostasis also leads to a variety of secondary complications including cardiovascular disease, retinopathy and associated blindness,

neuropathy that can result in amputations, and kidney disease leading to renal failure (Powers and D'Alessio, 2011). According to the CDC, diabetes is the leading cause of kidney failure, blindness and amputations in American adults. Cardiovascular complications, which are even more common, lead to greatly increased healthcare costs and reduced life expectancy (Caro et al., 2002). Improving glycemic control could thus prevent these complications and result in improved patient health (Fonseca, 2003).

A number of drugs exist to improve glycemic control and treat diabetes, including administration of insulin itself. Treatment of Type 1 diabetes requires continuous administration of exogenous insulin, whereas Type 2 diabetes can often be controlled by other oral or injected therapeutics that act on the β -cells or peripheral tissues. However, none of these therapies matches the precision of endogenous β -cells, and all have side effects including risk of ketosis and coma (Nathan et al., 2009). For Type 1 diabetes, insulin is the only option, and multiple blood glucose tests and insulin doses per day, every day for the patient's entire life, are required.

Alternative therapeutic options are crucial in order to address these healthcare challenges and the field of regenerative medicine is poised to contribute. Strategies that induce replication and regeneration of existing β -cells could enhance the number of β -cells available to control blood glucose, and studies of β -cell replication in a variety of genetic models have identified candidate pathways. In addition, the discovery of pluripotent embryonic stem cells (ESCs) capable of developing into any cell type has inspired a more radical strategy in which faulty or missing tissues are completely replaced. Precedents for this approach have been set by studies for other tissues, including ESC-derived cardiomyocytes that engraft into injured heart muscle and prevent arrhythmias (Shiba et al., 2012) and ESC-derived oligodendrocyte progenitor cells that restore mobility in rats that have suffered spinal cord injuries (Keirstead et al., 2005). β -cells make an especially attractive case for cell replacement strategies because only a single cell type is missing and replacement can occur in non-endogenous sites, a surgical advantage as cells can be placed subcutaneously in minimally invasive surgeries. Transplantation into a non-endogenous site also offers the opportunity to protect the replacement cells from autoimmune attack because the replacement cells can be transplanted inside immunoprotective devices, enabling allogeneic replacement strategies as well as protected autologous transplant for Type 1 diabetics. The transplantation of pancreatic islets into the hepatic portal vein has already been demonstrated as a very effective treatment for diabetes (Lacy and Scharp, 1986; Mullen et al., 1977; Bellin et al., 2012; Shapiro et al., 2000; Shapiro et al., 2006). However, the demand for human cadaveric pancreata, from which the islets are isolated, far outstrips the supply, especially as single patients often require more than one donor. These results firmly establish the clinical value of generating β -cells from alternative sources. In addition to their clinical value, a reliable and reproducible source of human β -cells would be of great benefit for *in vitro* studies of metabolism and β -cell function. The development of new sources of human β -cells would also

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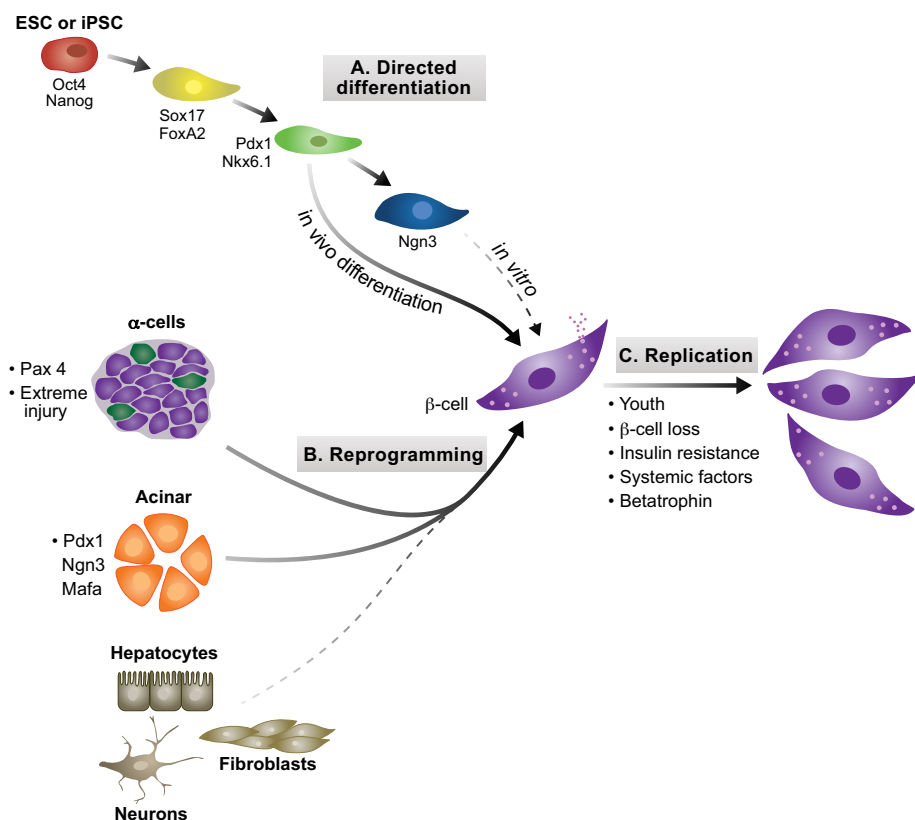


Fig. 1. Strategies to generate new β -cells.

(A) Directed differentiation using growth factors and small molecules can direct a pluripotent stem cell (red) through the stages of pancreatic differentiation in a manner that mimics normal development. Currently, functional β -cells can only be differentiated through an *in vivo* transplantation step, but deriving a bona fide β -cell fully *in vitro* (dashed line) is a major goal. A subset of important genes expressed at each stage is listed. (B) Reprogramming of terminally differentiated cell types, such as acinar or α -cells, can be used to generate β -cells *in vivo*, using the overexpression or injury strategies listed. Reprogramming other mature cell types, such as hepatocytes, fibroblasts or neurons, *in vitro* into β -cells (dashed line) remains to be achieved. (C) Inducing the replication of existing β -cells is the primary strategy for generating new endogenous β -cells. Replication may be recapitulated *in vitro* or induced *in vivo* with new small molecules or proteins based on the strategies listed.

provide a potentially unlimited source of cells for the development of a novel drug-screening platform for diabetes.

Here, we present an update on the progress in generating new β -cells from three complementary strategies – pluripotent stem cell differentiation, reprogramming from other cell types, and induction of replication in existing β -cells (Fig. 1) – based on work in both murine and human systems.

Generating β -cells from pluripotent stem cells

Type 1 diabetics lack a sufficient number of β -cells and many patients appear to have none. In Type 2 patients, β -cell mass is also insufficient to maintain glycemic control. Therefore, strategies to create new β -cells for therapeutic replacement have garnered significant excitement in the last two decades. A major advance toward this goal was the identification of pluripotent human ESCs (hESCs) that are capable of generating tissues from all three developmental germ layers (Thomson et al., 1998). In the decade following this discovery, an additional source of pluripotent stem cells was identified – induced pluripotent stem cells (iPSCs) reprogrammed from murine fibroblasts (Takahashi and Yamanaka, 2006; Wernig et al., 2007; Yu et al., 2007). Soon thereafter, iPSCs were also engineered from human cells (Lowry et al., 2008; Nakagawa et al., 2008; Takahashi et al., 2007; Yu et al., 2007). One of the remarkable features of iPSCs is that, like ESCs, they have the capacity to generate all cell types (Okita et al., 2007; Wernig et al., 2007). Thus, these cells present an unprecedented opportunity to generate replacement tissues *in vitro*, including autologous cells from patient-specific cells. To this aim, autologous mouse iPSCs were differentiated into hematopoietic progenitors that were shown to be effective in treating sickle cell defects in an anemia mouse model (Hanna et al., 2007).

Similar disease-curing strategies through the directed differentiation of pluripotent stem cells (Fig. 1A) should be possible for diabetes, although one major hurdle exists: discovering strategies that can recapitulate the development of functional β -cells (summarized in Fig. 2) *in vitro*. During development, cells of the fertilized embryo first select which germ layer fate to acquire (ectoderm, mesoderm or endoderm); β -cells derive from the endodermal layer. After endodermal specification, signals from adjacent developing tissues induce specification of pancreatic progenitors that have the potential to generate all three pancreatic cell types: ductal, acinar and endocrine (Fig. 2). The exocrine tissue of the pancreas is composed of ductal and acinar cells, whereas islets provide the endocrine function of the pancreas. After selection of endocrine fate, those endocrine progenitors must then be specified to become one of the five endocrine cell types of the islet: insulin-producing β -cells, glucagon-producing α -cells, somatostatin-producing δ -cells, pancreatic polypeptide-producing (PP) cells, or ghrelin-producing ϵ -cells (Fig. 2). A decade ago, even the first step of this process – the controlled induction of endoderm – had not been achieved from ESCs. In the intervening years, however, remarkable progress has been made toward the ultimate aim of creating fully functional β -cells from pluripotent cells *in vitro*.

Making definitive endoderm

Studies in mice, including important genetic models described below, identified transcription factors that are key regulators of pancreatic development. Sox17 and FoxA2 (Hnf3beta) are required to generate endodermal tissue and the gut tube that derives from it (Ang and Rossant, 1994; Kanai-Azuma et al., 2002; Weinstein et al., 1994). FoxA2 acts in part through mediating nucleosome depletion and subsequent gene activation (Li et al., 2012). Molecular signals, in particular the TGF β superfamily member

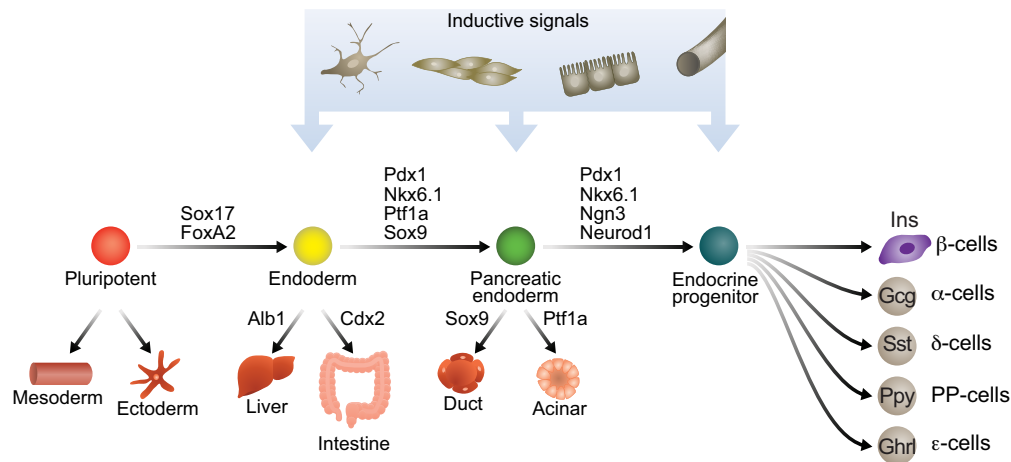


Fig. 2. An overview of fate choices during normal β -cell development. Pluripotent cells first acquire the identity of one of three germ layers; pancreatic cells arise from the endodermal layer. A subset of endoderm is specified by Pdx1 expression to become pancreatic endoderm, which will subsequently differentiate to a pancreatic ductal, acinar or endocrine fate. Endocrine progenitors express Ngn3 and differentiate further into the five hormone-expressing cell types of the islet according, at least in part, to which other transcription factors are expressed. A subset of relevant transcription factors is listed. For a more extensive review, see Pan and Wright (Pan and Wright, 2011). Ins, insulin; Gcg, glucagon; Sst, somatostatin; Ppy, pancreatic polypeptide; Ghrl, ghrelin.

Nodal, that induce embryonic differentiation into endoderm have also been identified, using first frog and fish and later murine models (Brennan et al., 2001; Tremblay, 2010). Another TGF β family member, activin, has similar receptor binding patterns as Nodal, is easier to produce as a recombinant protein, and can signal via similar downstream pathways (Chen et al., 2013). These features of activin A enabled researchers to develop the first efficient protocol for generating definitive endoderm *in vitro* from pluripotent stem cells (D'Amour et al., 2005).

The transition to pancreatic endoderm

Definitive endoderm can subsequently differentiate into pancreatic endoderm, provided that the appropriate cues are present. The key pancreatic regulator Pdx1 (Ipf1) is expressed early in embryogenesis downstream of FoxA1 and FoxA2, and marks the region of endoderm committed to a pancreatic fate (Gao et al., 2008; Ohlsson et al., 1993). In fact, deletion of this transcription factor prevents pancreatic acinar and endocrine development (Jonsson et al., 1994; Offield et al., 1996) and, based on lineage-tracing experiments, all pancreatic cell types arise from Pdx1-positive cells (Gannon et al., 2000; Gu et al., 2002). Additionally, genetic analysis of patients suffering from a rare monogenic form of diabetes called MODY (mature onset diabetes of the young) confirms the importance of PDX1 and other transcription factors in pancreatic differentiation, as causative mutations of these genes have been identified in these patients (Ashcroft and Rorsman, 2012). For example, pancreatic agenesis is seen in patients harboring certain homozygous point mutations in the *PDX1* coding sequence (Stoffers et al., 1997). The transcription factors Hnf1b (Tcf2), Hnf6 (Onecut1) and Prox1 (Wandzioch and Zaret, 2009) are also expressed in tissues from which the pancreas, as well as the liver, is derived. In line with this, *HNF1B*, *HNF4A* and *HNF1A* have all been identified as MODY genes and a cause of human diabetes (Ashcroft and Rorsman, 2012). Thus, analysis of these factors serves to guide whether stem cell differentiation *in vitro* is recapitulating the essential gene expression patterns observed during normal β -cell development (Fig. 2).

The pancreatic epithelium marked by Pdx1 expression can be further subdivided into regions that will have different cell fates. Of particular importance, pancreatic multipotent progenitors have been identified in the tip and trunk regions of early branching structures of the developing mouse pancreas (Schaffer et al., 2010; Kopp et al., 2011a; Zhou et al., 2007). Based on lineage tracing of carboxypeptidase A1 (Cpa1)-positive cells marked at E12.5 or earlier, tip multipotent progenitors that co-express Cpa1, the transcription factors Pdx1 and Ptf1a and high levels of cMyc generate the three major cell types of the pancreas: endocrine, acinar and ductal cells. Lineage tracing of Ptf1a-expressing cells revealed similar results (Kawaguchi et al., 2002). Ptf1a itself is required for the development of the exocrine pancreas, in particular the acinar cells. In the absence of acinar cells, endocrine cells in *Ptf1a* null mice develop but mis-migrate to the splenic mesenchyme (Krapp et al., 1998). After E12.5, the pancreatic epithelium differentiates into at least two progenitor regions – the *Ptf1a*⁺/*Cpa1*⁺ tip and the *Sox9*⁺/*Hnf1b*⁺/*Nkx6.1*⁺ trunk – that later differentiate into acinar or ductal and endocrine cells, respectively (Kopp et al., 2011b; Schaffer et al., 2010).

The development of pancreatic endoderm expressing these key transcription factors is inhibited by sonic hedgehog (Shh) signaling (Apelqvist et al., 1997). *In vivo* signals from the developing notochord repress this signaling in the adjacent endoderm, allowing it to differentiate into the pancreatic lineage (Hebrok et al., 1998; Kim and Melton, 1998; Kim et al., 1997). Specification of both the pancreatic and liver endoderm from the endodermal germ layer also requires retinoic acid signaling (Chen et al., 2004; Martín et al., 2005; Molotkov et al., 2005; Stafford and Prince, 2002). Expansion and branching of this Pdx1-positive pancreatic epithelium is promoted by Fgf10, which is presumably released by the surrounding mesenchyme (Bhushan et al., 2001). Importantly, the combination of these signals – retinoic acid, the Shh inhibitor cyclopamine, and Fgf10 – is sufficient to induce pancreatic epithelium *in vitro* from definitive endoderm derived from pluripotent stem cells (D'Amour et al., 2006). For an extended review of signaling during pancreatic differentiation see

McCracken and Wells (McCracken and Wells, 2012). Similar protocols have been developed to enhance pancreatic progenitor development and induce the expression of factors such as Nkx6.1 and Ptf1a, and these include the addition of bone morphogenetic protein (BMP) inhibitors and protein kinase C (PKC) activators (Chen et al., 2009; Kroon et al., 2008; Nostro et al., 2011; Rezania et al., 2012).

Differentiation to endocrine cells

One of the more important advances in this field was the demonstration that, when transplanted, the pancreatic progenitors generated from two of these protocols can further differentiate into functional, glucose-responsive β -cells *in vivo* after 4 months (Kroon et al., 2008; Rezania et al., 2012). These *in vivo* differentiated cells co-express insulin and key transcription factors, including Pdx1, Nkx6.1 and Mafa, and are sufficient to rescue diabetes in murine models, suggesting that they closely resemble true β -cells. Although a remarkable advance, the generation of true β -cells *in vitro* has not yet been achieved, and the mechanisms through which differentiation from ESC-derived cells occurs *in vivo* remain unknown. Furthermore, the number and identity of the cells within the pancreatic progenitor population that are capable of *in vivo* differentiation have not yet been established.

Past research has established that progenitors of the endocrine lineage are pancreatic endoderm cells that transiently express the transcription factor Ngn3 (Neurog3) (Gradwohl et al., 2000; Gu et al., 2002). Lineage tracing has demonstrated that all islet endocrine cell types derive from Ngn3-positive cells (Gu et al., 2002). Therefore, the induction of NGN3 expression in hESC-derived pancreatic progenitors is likely to be required to generate β -cells, whether *in vivo* during transplantation or *in vitro*. Ngn3 expression levels are regulated by Notch signaling, which represses Ngn3 expression and maintains the progenitor phenotype (Apelqvist et al., 1999; Jensen et al., 2000). Directed differentiation protocols using different combinations of Notch inhibitors, exendin-4, BMP inhibitors, PKC activators, keratinocyte growth factor (KGF, or Fgf7) or epidermal growth factor (EGF) have generated Ngn3-positive cells *in vitro* after pancreatic epithelium induction (D'Amour et al., 2006; Kroon et al., 2008; Rezania et al., 2012; Schulz et al., 2012). Several days after the appearance of Ngn3-positive cells, endocrine cells expressing insulin or glucagon (or both) appear in these cultures. ESC-derived pancreatic progenitors can also generate some Ngn3-positive cells (and subsequent endocrine cells) without addition of any factors to the medium in the last stage (Kroon et al., 2008). The most efficient current protocols generate greater than 90% Pdx1-positive and greater than 60% Nkx6.1-positive pancreatic endoderm (Rezania et al., 2012; Schulz et al., 2012). Nonetheless, despite variations in the protocol, growth factors, small molecules and cell lines used, none of the endocrine cells generated *in vitro* function as true β -cells.

Most importantly, ESC-derived insulin-expressing cells fail to secrete insulin appropriately in response to the addition of various concentrations of glucose. Normal islets or dispersed β -cells release high levels of insulin in response to high levels of glucose in the glucose-stimulated insulin secretion (GSIS) assay and can do so repeatedly (Ashcroft and Rorsman, 2012) (Fig. 3). However, ESC-derived β -cells fail to increase the amount of insulin secreted in response to high versus low glucose (D'Amour et al., 2006). This function is the key aspect of β -cell identity and the mechanism through which β -cells control glucose metabolism *in vivo*. Generating β -cells that can perform GSIS *in vitro*, as cadaveric human islets can, is an important challenge for the field. Of note,

Gadue and colleagues recently generated insulin-positive cells from endodermal progenitor cell lines that secrete insulin, but it is not yet clear how this insulin secretion compares to that obtained by existing protocols or whether these cells co-express β -cell transcription factors or function *in vivo* after transplantation (Cheng et al., 2012). An analysis of young mouse islets of different ages revealed the upregulation of some genes, including urocortin 3 (*Ucn3*), that correlates with increasing insulin secretion and might be relevant to the acquisition of function in ESC-derived β -cells (Blum et al., 2012; van der Meulen et al., 2012). In addition, the insulin-expressing cells that have been generated from ESCs thus far fail to express key β -cell-specific transcription factors, including Pdx1, Nkx6.1 and Mafa, as well as other metabolic enzymes and cell surface transporters related to normal β -cell function (Xie et al., 2013). The reasons for this anomaly are not yet clear.

Following the fate of pancreatic progenitors

Lineage-tracing experiments that address how many of the pancreatic progenitors go on to produce the functional graft have not been performed, in part due to the challenges of hESC genomic engineering. For example, only a subset of Pdx1- and Nkx6.1-expressing cells may be competent to generate the graft of insulin-expressing cells (Fig. 4A), indicating the existence of an additional, heterogeneously expressed factor. If only a few cells are competent, this could explain why it takes 4 months for the transplanted graft to become functional and able to control diabetes in the host (Kroon et al., 2008; Rezania et al., 2012).

The analysis of an equivalent pancreatic progenitor stage during human fetal development has also not been performed, and therefore comparisons between endogenous human pancreatic progenitors and those derived from stem cells have not been possible. This type of analysis could determine the identity of additional regulators that are normally present in this cell type but are either not expressed in stem cell-derived progenitors or are expressed in only a subset of cells.

Identifying signals for endocrine differentiation *in vitro*

If pancreatic progenitors are correctly programmed, a second hypothesis to explain the lack of proper subsequent differentiation *in vitro* is the presence of incorrect or incomplete signals to specify β -cell fate. One strategy would be to identify small molecules or culture conditions that promote the induction of endocrine fate at the same time as promoting the expression of key transcription factors such as Pdx1 and Nkx6.1. In addition, much focus has been placed on screening for small molecules that modulate transcription factor expression, but it might be equally relevant to screen for factors that modulate the expression of *Ucn3*, metabolic enzymes or membrane transporters such as glucokinase or glucose transporters that might be misexpressed in ESC-derived endocrine cells (Xie et al., 2013) (Fig. 4B).

Alternatively, functional β -cells could be generated from ESC-derived pancreatic progenitors via viral-, plasmid- or RNA-based expression of missing genes, in much the same way that functional iPSCs have been generated (Okita et al., 2010; Takahashi and Yamanaka, 2006; Warren et al., 2010). One prerequisite to this approach is to know precisely which genes are misexpressed in *in vitro* derived cells. To address this, a recent study by Sander and colleagues analyzed gene expression and chromatin status along the steps of directed differentiation *in vitro* as well as in functional endocrine cells generated from *in vivo* engraftment (Xie et al., 2013). Although a number of gene expression differences were identified between *in vitro* and *in vivo* differentiated endocrine

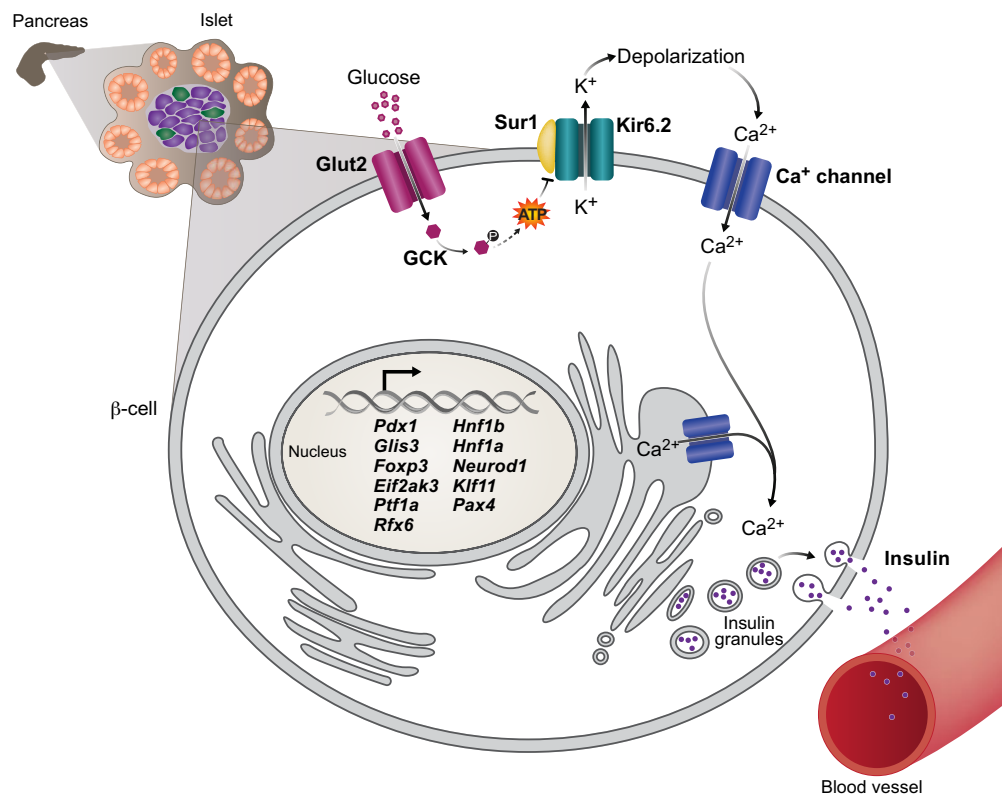


Fig. 3. Functional β -cells respond to increasing glucose levels by increasing insulin secretion. In glucose-stimulated insulin secretion (GSIS), glucose is transported into the cell via glucose transporters [e.g. *Glut1* (*Slc2a1*) or *Glut2* (*Slc2a2*), pink], where it is phosphorylated by glucokinase (*GSK*) and converted into ATP by subsequent metabolic reactions. Rising ATP levels (e.g. rising ATP:ADP ratios) trigger the closure of potassium channels [*Sur1* (*Abcc8*) and *Kir6.2* (*Kcnj11*) subunits], membrane depolarization, and the opening of calcium channels (blue). The resultant rise in intracellular calcium levels triggers the exocytosis of insulin-containing granules and hence leads to increased insulin levels in adjacent blood vessels. Human genetic studies of maturity onset diabetes of the young (MODY) patients have identified a number of mutations that trigger diabetes, including those in genes encoding transcription factors (depicted in the nucleus) and components of the GSIS pathway indicated in this figure.

cells, this analysis was performed on mixed populations of cells of which only a minority were actually insulin-expressing endocrine cells.

The exogenous expression of genes required for β -cell identity, especially when combined with siRNA-mediated knockdown of those that are overexpressed, might reveal the combination of factors required for β -cell differentiation and function. In addition to protein-coding genes, true β -cells also express non-coding RNAs including microRNAs and long non-coding RNAs (lncRNAs) (Lynn et al., 2007; Morán et al., 2012), which may also modulate β -cell identity. For example, miR-375 has been shown to inhibit GSIS, and deletion of the microRNA-processing enzyme *Dicer* prevents β -cell differentiation in mouse knockout models (Lynn et al., 2007; Melkman-Zehavi et al., 2011; Poy et al., 2004). Thus, in addition to identifying the proteins that need to be induced or repressed to confer true β -cell identity, microRNAs might also need to be induced or exogenously expressed before β -cell fate can be engineered from pancreatic progenitors *in vitro*.

Maintaining β -cell identity and function during *in vitro* culture

Finally, a third hypothesis to explain the lack of functional β -cells found in ESC-derived cultures is that existing protocols are capable of generating true β -cells but we simply lack the culture conditions

to maintain these cells *in vitro* or to train these new young β -cells to respond to glucose appropriately. Human β -cells are generally agreed to be a difficult cell type to keep alive and functional *in vitro*. Even 48–72 hours of culture of human islets results in the loss of half the islet cells, as well as dramatically decreased GSIS *in vitro* and a reduced ability to restore normoglycemia after transplantation into diabetic mice (Noguchi et al., 2012). These data suggest that, even if true β -cells could be generated through directed differentiation strategies, they might not survive and function very well using current culture protocols. However, several groups have reported the ability to maintain β -cells *in vitro* for several weeks in certain culture conditions so that cells maintain their ability to be functionally engrafted after transplantation (Gaber et al., 2001; Keymeulen et al., 2006).

Unlike cell fate choices in which an inductive cue need only be transitory, the maintenance of the terminal function of a cell *in vitro* requires the maintenance of an environment that is sufficiently similar to the *in vivo* context. When fully differentiated and functional human islets are cultured, parts of the islet niche are already present, including the extracellular matrix (ECM) and adjacent endothelial and mesenchymal cells carried over from the islet isolation. The addition of appropriate ECM proteins or other cell types to ESC-derived insulin-positive cells may improve their terminal differentiation and function *in vitro*. Human islets are surrounded by a milieu of ECM and mesenchymal, endothelial,

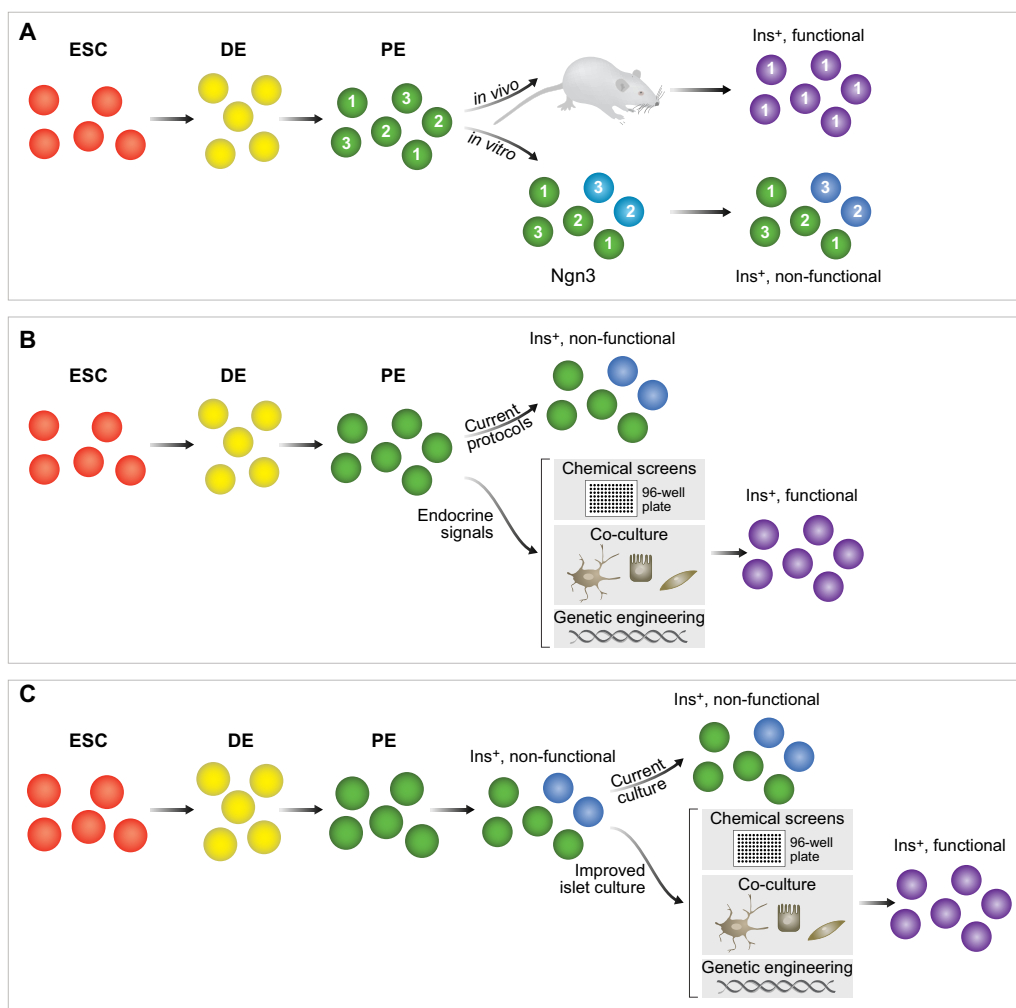


Fig. 4. Identifying how to improve directed differentiation into β -cells. (A) Lineage tracing of ESC-derived pancreatic endoderm could reveal how heterogeneous this population is and which cells are competent to generate functional β -cells *in vivo*. Depicted is a hypothetical outcome whereby pancreatic progenitor cell type 1, but not types 2 or 3, generates functional β -cells in the graft. Types 2 and 3 may turn on insulin expression *in vitro* but may not be functional, and *in vitro* cues may be insufficient to direct further differentiation of the type 1 cells. (B) Chemical screening for novel small molecules or growth factors, co-culture with instructive cell types or genetic engineering strategies might be necessary to trigger the *in vitro* differentiation of pancreatic endoderm cells into functional endocrine cells that express the normal set of transcription factors. (C) Current islet culture conditions are not optimal for inducing the function of the insulin-positive cells made in the dish. Thus, *in vitro* derived cells might not be instructed to function in the absence of appropriate culture conditions. The identification of novel culture conditions will improve the chances of generating β -cells *in vitro*. DE, definitive endoderm; PE, pancreatic endoderm.

neuronal and exocrine cells, many of which act to support β -cell identity and function. Mesodermally derived aortic endothelial cells, for example, help maintain Pdx1 expression (Lammert et al., 2001) and induce Ptf1a expression (Yoshitomi and Zaret, 2004), and mature islets secrete Vegfa to attract endothelial cells (Nikolova et al., 2006). Organ-specific mesenchymal cell lines can also induce replication in hESC-derived definitive endoderm (Sneddon et al., 2012). Finally, it should be noted that bona fide β -cells differentiate and function in a three-dimensional environment, whereas the majority of research efforts are performed in two-dimensional tissue culture.

Taken together, these observations suggest that modifications to the culture conditions might improve functional β -cell generation (Fig. 4C). Alternatively, the co-differentiation of whole islets or islet organoids, rather than β -cells in isolation, might ultimately prove a more successful approach to differentiating true β -cells.

Reprogramming other cell types into β -cells

An alternative strategy to differentiating β -cells in a stepwise fashion from pluripotent stem cells is the reprogramming of terminally differentiated cell types into β -cells. This type of direct reprogramming has made it possible to generate iPSCs, cardiomyocytes, hepatocytes and neurons from fibroblasts (Huang et al., 2011; Ieda et al., 2010; Takahashi and Yamanaka, 2006; Vierbuchen et al., 2010). Remarkably, each of these cases required the overexpression of only two to four genes. In the last decade, similar strategies have proven successful for reprogramming other cell types into β -cells.

Acinar to β -cell reprogramming

One example of this reprogramming-based approach was the demonstration of the direct conversion of mouse acinar cells to β -cells *in vivo* via viral expression of particular genes (Zhou and

Melton, 2008). This example showed that, from an initial test of nine transcription factors expressed in β -cells and their immediate precursors, three factors – Ngn3, Pdx1 and Mafa – were sufficient, once transduced into acinar cells, to reprogram those cells to a β -cell fate (Zhou and Melton, 2008). Lineage tracing *in vivo* confirmed that Cpa1-positive mature acinar cells could convert into insulin-expressing cells post-transduction. Importantly, these insulin-expressing cells also co-expressed key markers of β -cell function including Nkx6.1, Glut2 (Slc2a2) and glucokinase, but no longer expressed key regulators of acinar function including Ptf1a or amylase and did not express any of the other islet hormones. Finally, these induced cells were sufficient to improve glycemic control in diabetic mice, a key characteristic of bona fide β -cells (Zhou and Melton, 2008).

Nonetheless, these factors are not universally sufficient to generate β -cells; they are insufficient to reprogram skeletal muscle or fibroblasts into β -cells. Although acinar cells have been reprogrammed *in vivo*, no mouse or human cell type has been fully reprogrammed into a β -cell *in vitro*. This might relate to the poorly defined culture conditions required for the maintenance of β -cell identity and function *in vitro*, as discussed above. Furthermore, the reprogrammed acinar cells do not aggregate into islets, perhaps owing to the lack of other reprogrammed islet cell types such as α -cells, and hence these factors appear to be sufficient only to generate β -cells. Most importantly in the context of potential therapeutic application, this achievement will need to be recapitulated either *in vivo* without viruses or, perhaps as a safer option, *in vitro* for subsequent cell transplantation. The latter goal would be particularly important to achieve from human cells, as it is not clear whether the same factors used for the transdifferentiation of mouse acinar cells will be sufficient in another species. Reprogramming from other more easily accessible and expandable cell types would also be advantageous. However, directly reprogramming such cell types, which include fibroblasts, hepatocytes and pluripotent stem cells, into functional β -cells might require a different, or expanded, set of transcription factors together with optimized culture conditions to provide the necessary niche.

α -cell to β -cell reprogramming

More recently, an additional example of adult cell reprogramming to β -cells has been described from adult mouse α -cells. Mansouri and colleagues discovered that the ectopic expression of Pax4 was sufficient to convert α -cells into β -cells *in vivo* (Collombat et al., 2009). Conversely, loss of Pax4 leads to loss of β -cells and a concomitant increase in the number of α -cells (Sosa-Pineda et al., 1997). These data reflect the shared developmental trajectory of these two cell types and their similar gene expression programs.

Interestingly, conditions of near complete β -cell ablation may trigger a similar sort of reprogramming in α -cells. Herrera and colleagues created a transgenic mouse model that permits α -cell lineage tracing and near total β -cell ablation using the diphtheria toxin receptor system (Thorel et al., 2010). Nearly complete β -cell destruction led to the eventual regeneration of β -cells but, surprisingly, many of these cells derived from former α -cells. Transgenically marked α -cells began producing insulin and to co-express the adult β -cell markers Pdx1 and Nkx6.1.

The functional similarity and shared ancestry of these two cell types might make their interconversion possible in extreme circumstances, which in this case might be an extreme niche that entirely lacks local insulin signaling. Recent studies revealed that α -cells harbor bivalent chromatin signatures at genes that are active in β -cells, such as *Pdx1* and *Mafa* (Bramswig et al., 2013). A

bivalent chromatin signature contains both active and repressive histone marks, suggesting that part of the reason that α -cells may have the plasticity to be reprogrammed into β -cells is that β -cell-specific genes are already poised to be active. Treatment of islets with a histone methyltransferase inhibitor resulted in Pdx1 and insulin expression in glucagon-positive cells, suggesting the potential for partial transcriptional reprogramming, a finding that warrants further investigation (Bramswig et al., 2013).

Although it is not yet clear whether complete α -cell to β -cell transdifferentiation can occur in humans, if appropriate immunoregulatory conditions could be established in severe cases of Type 1 diabetes it might be possible to mimic ‘extreme β -cell loss’ and observe α -cell transdifferentiation. Alternatively, one may hypothesize that the transplantation of hESC-derived α -cells (in the absence of β -cells) should also lead to transdifferentiation of some of those α -cells into β -cells *in vivo*. Finally, a clear demonstration of this α -cell transdifferentiation *in vitro*, either by Pax4 overexpression or by some modulation of glucagon or insulin levels in the culture niche, would provide stronger support for the utility of this strategy. Given the newly established ability to genetically manipulate human pluripotent stem cells (Ding et al., 2013), one could lineage trace *in vitro* derived α -cells using the glucagon promoter and follow their differentiation in culture or after transplantation into mice.

Reprogramming from other cell types

A particular form of injury to the pancreas, partial duct ligation (PDL), has been argued to increase islet mass, potentially via transdifferentiation of adjacent tissues (Wang et al., 1995). In this injury model, the pancreatic ductal cells proliferate while acinar tissue is simultaneously lost. Based on these and other observations, Xu and colleagues suggested that PDL can induce Ngn3 re-expression and that the new adult endocrine progenitors are able to differentiate into new β -cells (Xu et al., 2008). The molecular triggers for this conversion and whether it can take place in humans or *in vitro* remain unknown. This study also suggested that it was ductal cells that turned on Ngn3 expression to transdifferentiate into endocrine cells; however, ductal-specific lineage tracing to test this hypothesis indicated that Sox9-positive ductal cells are not a source of new β -cells after PDL or after β -cell ablation (Kopp et al., 2011b; Solar et al., 2009). In addition, several recent studies have demonstrated that total β -cell number may not actually change after PDL and that the β -cell mass increase might be an artifact of the estimation methodology (Chintinne et al., 2012; Rankin et al., 2013). However, a new study using Ptf1a lineage tracing demonstrated that Ptf1a-positive acinar cells can transdifferentiate into Ngn3-positive progenitors after PDL and that these cells subsequently differentiate into new endocrine cells at very low frequency (Pan et al., 2013). The insulin-expressing cells derived from the former Ptf1a-positive acinar cells in this model express mature β -cell markers, including Pdx1, Nkx6.1 and Mafa, similar to the β -cells derived from virally reprogrammed acinar cells (Pan et al., 2013; Zhou and Melton, 2008).

These lineage-tracing studies performed after injury also provide evidence for the limited role that β -cell neogenesis plays postnatally in maintaining β -cell mass. The bulk of evidence to date suggests that the pancreas has a limited capacity to regenerate, and the reader is referred to other reviews for a more extensive discussion of the contradictory studies on the potential of β -cell neogenesis to contribute new β -cells (Collombat et al., 2010; Desgraz et al., 2011).

The role that reprogramming will play in regenerative medicine for the treatment of diabetes remains to be seen. Clearly, terminally differentiated cells can be reprogrammed into other cell types by a variety of overexpression strategies. These strategies for β -cells have been performed exclusively *in vivo* thus far, and so a demonstration of successful reprogramming *in vitro* would be a significant advance. Successful reprogramming would need to be validated by more than the simple detection of insulin-expressing cells, as many of the reprogramming factors, such as Pdx1, can bind and activate the insulin promoter directly (Ohlsson et al., 1993). The induction of other canonical β -cell genes, such as *Glut2* and *Nkx6.1*, in the 'reprogrammed' cell would provide stronger evidence of cell fate change than the expression of insulin alone. In addition, whole-genome expression profiling of reprogrammed β -cells and comparison with endogenous β -cells can help to address how close the reprogrammed cell is to a bona fide β -cell. Establishing function by *in vitro* GSIS assays or transplantation and regulation of host blood glucose levels can provide valuable additional evidence as to how closely a reprogrammed cell resembles a β -cell.

In addition, reprogramming from accessible tissues, including direct reprogramming from ESCs or fibroblasts and particularly human tissues, should be a near-term goal. Finally, the misregulated insulin-expressing cells that are currently being generated from pluripotent stem cells represent a potentially ideal target for reprogramming, as much of the pancreatic program should already be in place.

Regenerating β -cells by induced replication

Whereas β -cell neogenesis may be achieved from pluripotent stem cells or via reprogramming, the strategy for generating more β -cells endogenously is to induce replication from existing β -cells (Fig. 1C). Whereas tissues such as the blood or skin are regenerated via the differentiation of tissue-specific stem cells, new pancreatic β -cells normally derive from the replication of existing β -cells (Dor and Melton, 2004). The replication of endogenous β -cells would provide an autologous source of new β -cells and potentially decrease the burden on existing β -cells that are overworked in Type 2 diabetes. In addition, it should be noted that some Type 1 diabetes patients retain some β -cells; in one study, 16% of patients had residual β -cell function as measured by detectable C-peptide levels (Schölin et al., 2004). C-peptide, or connecting peptide, is released from proinsulin processing and secreted in an equimolar ratio with insulin. The persistence of functional β -cells in this patient subpopulation suggests that they might benefit from therapies that induce replication of these residual cells, if this strategy can be combined with therapies to control the autoimmune attack.

Despite these potential advantages, a particular risk of such a replication induction strategy is the inadvertent promotion of tumorigenesis. The risk of cancer would be particularly important to consider if acinar or ductal tissue was induced to proliferate alongside β -cells. Conversely, this risk would be ameliorated if the replication-inducing agent of choice was developed to have a high specificity for β -cells relative to other cell types.

Young β -cells have higher replication rates

The rate of β -cell replication drops precipitously during human aging, from 3% replicating cells among fetal β -cells to less than 0.5% by 6 months of age, and even lower thereafter (Kassem et al., 2000; Perl et al., 2010; Teta et al., 2005). In another study, 2.5% of β -cells in young mice (5 weeks old) replicated, whereas the cells of old mice replicated at a rate of only 0.2% (Stolovich-Rain et al.,

2012). The rate of β -cell replication in a diphtheria toxin-based β -cell-ablation model reaches 7.5% in young mice, but still reaches as high as 1% in old mice (Stolovich-Rain et al., 2012), suggesting that an intrinsic capacity to replicate is maintained in old age but also that youth itself is one variable that promotes β -cell replication. Whether this change in replication capacity is autonomous or due to systemic 'aging' factors remains to be determined.

The replication of β -cells also increases during pregnancy, potentially via the activities of prolactin and placental lactogen, although the increase in β -cell mass may be less in humans than in rodents (Butler et al., 2010; Parsons et al., 1992; Van Assche et al., 1978). Prolactin may act in part via repressing the transcriptional regulator menin, a protein that when overexpressed can prevent pregnancy-associated replication (Karnik et al., 2007). Alternatively, pregnancy may primarily influence the amount of insulin secretion rather than the number of β -cells (Brelje et al., 2004).

Screening for small-molecule effectors that modulate replication

In mice, treatment with exendin-4, a glucagon-like peptide 1 receptor (Glp1r) agonist, led to a threefold increase in BrdU-positive replicating β -cells (Xu et al., 1999; Stoffers et al., 2000). Exendin-4 has a longer half-life than glucagon-like peptide 1 itself, making it more clinically useful (Eng et al., 1992). The replication-inducing effects of exendin-4 have also been observed in young (<22 years) human islets transplanted into mice but not in old (>35 years) human islets (Caballero et al., 2013; Tian et al., 2011). Additional molecular regulators of β -cell replication in rodents have been identified through high-throughput screening of reversibly immortalized β -cell lines or primary rodent islets. These regulators include phorbol esters, thiophene-pyrimidines, dihydropyridine derivatives and adenosine kinase inhibitors (Annes et al., 2012; Wang et al., 2009). Glucose or Glp1r agonists have an additive effect on the replication induced by most of these recently identified factors (Annes et al., 2012; Wang et al., 2009); however, it remains to be seen whether these small molecules will have an inductive effect on human islets. More recently, Schultz and colleagues identified a novel small molecule, WS6, that increased both rat and human β -cell replication *in vitro* by sixfold in dispersed islets and by more than tenfold in intact islets (Shen et al., 2013).

Identifying mechanisms of replication induction using the LIRKO model

Given the role of insulin signaling in the development of diabetes, liver-specific insulin receptor knockout (LIRKO) mice were generated to investigate the role of insulin signaling in hepatocytes. Surprisingly, mutation of the insulin receptor in hepatocytes resulted not only in insulin resistance and glucose intolerance but also led to a dramatic sixfold increase in islet mass (Michael et al., 2000). These studies prompted the idea that blocking the insulin receptor with a small molecule or peptide antagonist could recapitulate these effects, including the induction of β -cell replication. In fact, treatment with one such insulin receptor antagonist, a novel peptide named S961, results in hyperinsulinemia in rats (Schäffer et al., 2003; Schäffer et al., 2008; Vikram and Jena, 2010).

Furthermore, an additional study of the LIRKO model revealed that β -cells were specifically induced to replicate in this context with no effect on α -cells or other non-pancreatic tissues (El

Ouaamari et al., 2013). This study also demonstrated that the induced replication could occur in a normal parabiotic partner (with normal blood glucose and insulin levels) if that animal was joined with a LIRKO mouse partner, suggesting both that a systemic factor was at work and that it could work independently of blood glucose levels. Furthermore, LIRKO-derived serum or a LIRKO liver explant system was sufficient to recapitulate replication induction in isolated mouse islets *in vitro*, although the number of replicating cells analyzed was very small and the specific factor(s) responsible were not identified.

Roles for insulin resistance and glucose levels in β -cell replication

Glucose itself induces β -cell replication in both rodents and humans, although clearly in the case of diabetes this replication is insufficient to keep up with demand or with the autoimmune attack (Bonner-Weir et al., 1989; Porat et al., 2011). Human islets transplanted into immunodeficient mice can also be induced to replicate following infusion of glucose (Levitt et al., 2011). Furthermore, humans with mutations (V91L) in glucokinase that increase its affinity for glucose similarly show increased β -cell replication and, as a consequence, larger islets (Kassem et al., 2010).

Similarly, a high-fat diet induces insulin resistance, which results in β -cell replication (Terauchi et al., 2007); the total number of β -cells in mice on such a diet more than doubled in 20 weeks. The process of replication under these conditions also requires glucokinase, as mice haploinsufficient for glucokinase fail to increase the number of β -cells. By contrast, treatment with glucokinase activators doubles β -cell replication in both young and old mice (Stolovich-Rain et al., 2012). Anti-diabetic therapeutics based on the strategy of glucokinase activation are currently being investigated, and one beneficial side effect beyond their effects on hepatic glucose metabolism may be induced β -cell replication (Matschinsky, 2009).

Recent work in our laboratory extended these studies by examining the organ-specific transcriptional changes induced by insulin resistance in order to identify the downstream factors that enhance β -cell replication. Analysis of hepatocyte transcriptional changes downstream of S961 revealed upregulation of a novel hormone, betatrophin (Yi et al., 2013). Remarkably, induction of betatrophin expression independently of insulin receptor antagonism increased β -cell replication in mice by more than tenfold. These data suggest that a novel therapeutic strategy might be possible for generating more β -cells through increasing the levels of this newly discovered hormone.

Conclusions and perspectives

In summary, unprecedented progress toward the goal of making more β -cells has been realized in the last decade. At the same time, however, additional challenges beyond those discussed above remain for this field. In particular, we have limited the scope of this review to the β -cell but acknowledge that addressing the immune system problem will be essential for treating Type 1 diabetes, whether through systemic immunomodulation or transplantation of new β -cells inside an immunoprotective capsule. If new stem cell-derived allogeneic β -cells are to be transplanted into patients with Type 2 diabetes to improve glycemic control, the issue of immune system rejection will also have to be addressed.

In this regard, a promising strategy to address the immune issue is the encapsulation of new β -cells in an immunoprotective device that permits nutrient diffusion but inhibits immune cell infiltration.

One such device has been successfully used for the protected transplantation of human islets and hESC-derived pancreatic progenitors into mice (Lee et al., 2009; Xie et al., 2013). An alternative strategy to avoid allogeneic immune rejection might be to develop protocols for the differentiation or reprogramming of patient-specific iPSCs into β -cells, particularly iPSCs that have been generated by non-viral methods. Early reports suggested that transplantation of undifferentiated iPSCs into syngeneic mice resulted in immune attack (Zhao et al., 2011). However, no patient-specific iPSC therapeutic strategy is likely to involve transplantation of undifferentiated pluripotent stem cells. Two more recent studies demonstrated that the transplantation of differentiated cells from syngeneic iPSCs generated no immune reaction or rejection and that the results of the previous study might be an artifact of using retrovirally derived iPSCs (Araki et al., 2013; Guha et al., 2013). Thus, iPSC-derived β -cells have particular potential to contribute to therapies in the future if scientific strategies can be developed that overcome the heterogeneity of differentiation propensities in individual cell lines.

The clinical utility of new sources of β -cells will require methods to generate homogenous cell populations that lack residual multipotent cells that could form cysts or tumors. Given the tremendous progress that has been made towards homogeneity in the early stages of directed differentiation (to the point of 99% definitive endoderm at present), this goal should be achievable for the late stages of differentiation once the appropriate developmental cues are discovered. In addition, these cell preparations will have to be generated on a very large scale compared with that typical in academia, and the first notable progress toward this goal has been reported recently (Schulz et al., 2012).

In summary, tremendous progress has been made toward both understanding how β -cells arise and proliferate during normal development and how functional β -cells might be generated through novel methods. The challenge of reconstructing a bona fide β -cell *in vitro*, whether through stem cell differentiation or reprogramming, remains open.

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Competing interests statement

The authors declare no competing financial interests.

References

- Ang, S. L. and Rossant, J. (1994). HNF-3 beta is essential for node and notochord formation in mouse development. *Cell* **78**, 561-574.
- Annes, J. P., Ryu, J. H., Lam, K., Carolan, P. J., Utz, K., Hollister-Lock, J., Arvanites, A. C., Rubin, L. L., Weir, G. and Melton, D. A. (2012). Adenosine kinase inhibition selectively promotes rodent and porcine islet β -cell replication. *Proc. Natl. Acad. Sci. USA* **109**, 3915-3920.
- Apelqvist, A., Ahlgren, U. and Edlund, H. (1997). Sonic hedgehog directs specialised mesoderm differentiation in the intestine and pancreas. *Curr. Biol.* **7**, 801-804.
- Apelqvist, A., Li, H., Sommer, L., Beatus, P., Anderson, D. J., Honjo, T., Hrabe de Angelis, M., Lendahl, U. and Edlund, H. (1999). Notch signalling controls pancreatic cell differentiation. *Nature* **400**, 877-881.
- Araki, R., Uda, M., Hoki, Y., Sunayama, M., Nakamura, M., Ando, S., Sugiura, M., Ideno, H., Shimada, A., Nifuji, A. et al. (2013). Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells. *Nature* **494**, 100-104.

- Ashcroft, F. M. and Rorsman, P. (2012). Diabetes mellitus and the β cell: the last ten years. *Cell* **148**, 1160-1171.
- Bellin, M. D., Barton, F. B., Heitman, A., Harmon, J. V., Kandaswamy, R., Balamurugan, A. N., Sutherland, D. E., Alejandro, R. and Hering, B. J. (2012). Potent induction immunotherapy promotes long-term insulin independence after islet transplantation in type 1 diabetes. *Am. J. Transplant.* **12**, 1576-1583.
- Bhushan, A., Itoh, N., Kato, S., Thiery, J. P., Czernichow, P., Bellusci, S. and Scharfmann, R. (2001). Fgf10 is essential for maintaining the proliferative capacity of epithelial progenitor cells during early pancreatic organogenesis. *Development* **128**, 5109-5117.
- Blum, B., Hrvatin, S., Schuetz, C., Bonal, C., Rezanian, A., and Melton, D. A. (2012). Functional beta-cell maturation is marked by an increased glucose threshold and by expression of urocortin 3. *Nat. Biotechnol.* **30**, 261-264.
- Bonner-Weir, S., Deery, D., Leahy, J. L. and Weir, G. C. (1989). Compensatory growth of pancreatic β -cells in adult rats after short-term glucose infusion. *Diabetes* **38**, 49-53.
- Bramswig, N. C., Everett, L. J., Schug, J., Dorrell, C., Liu, C., Luo, Y., Streeter, P. R., Naji, A., Grompe, M. and Kaestner, K. H. (2013). Epigenomic plasticity enables human pancreatic α to β cell reprogramming. *J. Clin. Invest.* **123**, 1275-1284.
- Brelje, T. C., Stout, L. E., Bhargroo, N. V. and Sorenson, R. L. (2004). Distinctive roles for prolactin and growth hormone in the activation of signal transducer and activator of transcription 5 in pancreatic islets of langerhans. *Endocrinology* **145**, 4162-4175.
- Brennan, J., Lu, C. C., Norris, D. P., Rodriguez, T. A., Beddington, R. S. and Robertson, E. J. (2001). Nodal signalling in the epiblast patterns the early mouse embryo. *Nature* **411**, 965-969.
- Butler, A. E., Janson, J., Bonner-Weir, S., Ritzel, R., Rizza, R. A. and Butler, P. C. (2003). β -cell deficit and increased β -cell apoptosis in humans with type 2 diabetes. *Diabetes* **52**, 102-110.
- Butler, A. E., Cao-Minh, L., Galasso, R., Rizza, R. A., Corradin, A., Cobelli, C. and Butler, P. C. (2010). Adaptive changes in pancreatic beta cell fractional area and beta cell turnover in human pregnancy. *Diabetologia* **53**, 2167-2176.
- Caballero, F., Siniakowicz, K., Hollister-Lock, J., Duran, L., Katsuta, H., Yamada, T., Lei, J., Deng, S., Westermarck, G. T., Markmann, J. et al. (2013). Birth and death of human β -cells in pancreas from cadaver donors, autopsies, surgical specimens, and islets transplanted into mice. *Cell Transplant* doi:10.3727/096368912X659916.
- Caro, J. J., Ward, A. J. and O'Brien, J. A. (2002). Lifetime costs of complications resulting from type 2 diabetes in the U.S. *Diabetes Care* **25**, 476-481.
- Chen, C. Z., Li, L., Lodish, H. F. and Bartel, D. P. (2004). MicroRNAs modulate hematopoietic lineage differentiation. *Science* **303**, 83-86.
- Chen, S., Borowiak, M., Fox, J. L., Maehr, R., Osafune, K., Davidow, L., Lam, K., Peng, L. F., Schreiber, S. L., Rubin, L. L. et al. (2009). A small molecule that directs differentiation of human ESCs into the pancreatic lineage. *Nat. Chem. Biol.* **5**, 258-265.
- Chen, A. E., Borowiak, M., Sherwood, R. I., Kweudjeu, A. and Melton, D. A. (2013). Functional evaluation of ES cell-derived endodermal populations reveals differences between Nodal and Activin A-guided differentiation. *Development* **140**, 675-686.
- Cheng, X., Ying, L., Lu, L., Galvão, A. M., Mills, J. A., Lin, H. C., Kotton, D. N., Shen, S. S., Nostro, M. C., Choi, J. K. et al. (2012). Self-renewing endodermal progenitor lines generated from human pluripotent stem cells. *Cell Stem Cell* **10**, 371-384.
- Chintinne, M., Stangé, G., Denys, B., Ling, Z., In 't Veld, P. and Pipeleers, D. (2012). Beta cell count instead of beta cell mass to assess and localize growth in beta cell population following pancreatic duct ligation in mice. *PLoS ONE* **7**, e43959.
- Collombat, P., Xu, X., Ravassard, P., Sosa-Pineda, B., Dussaud, S., Billestrup, N., Madsen, O. D., Serup, P., Heimberg, H. and Mansouri, A. (2009). The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into alpha and subsequently beta cells. *Cell* **138**, 449-462.
- Collombat, P., Xu, X., Heimberg, H. and Mansouri, A. (2010). Pancreatic beta-cells: from generation to regeneration. *Semin. Cell Dev. Biol.* **21**, 838-844.
- D'Amour, K. A., Agulnick, A. D., Eliazar, S., Kelly, O. G., Kroon, E. and Baetge, E. E. (2005). Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nat. Biotechnol.* **23**, 1534-1541.
- D'Amour, K. A., Bang, A. G., Eliazar, S., Kelly, O. G., Agulnick, A. D., Smart, N. G., Moorman, M. A., Kroon, E., Carpenter, M. K. and Baetge, E. E. (2006). Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat. Biotechnol.* **24**, 1392-1401.
- Desgraz, R., Bonal, C. and Herrera, P. L. (2011). β -cell regeneration: the pancreatic intrinsic faculty. *Trends Endocrinol. Metab.* **22**, 34-43.
- Ding, Q., Lee, Y.-K., Schaefer, E. A., Peters, D. T., Veres, A., Kim, K., Kuperwasser, N., Motola, D. L., Meissner, T. B., Hendriks, W. T. et al. (2013). A TALEN genome-editing system for generating human stem cell-based disease models. *Cell Stem Cell* **12**, 238-251.
- Dor, Y. and Melton, D. A. (2004). How important are adult stem cells for tissue maintenance? *Cell Cycle* **3**, 1104-1106.
- El Ouaamari, A., Kawamori, D., Dirice, E., Liew, C. W., Shadrach, J. L., Hu, J. et al. (2013). Liver-derived systemic factors drive β cell hyperplasia in insulin-resistant states. *Cell Reports* **3**, 401-410.
- Eng, J., Kleinman, W. A., Singh, L., Singh, G. and Raufman, J. P. (1992). Isolation and characterization of exendin-4, an exendin-3 analogue, from *Heloderma suspectum* venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. *J. Biol. Chem.* **267**, 7402-7405.
- Fonseca, V. (2003). Clinical significance of targeting postprandial and fasting hyperglycemia in managing type 2 diabetes mellitus. *Curr. Med. Res. Opin.* **19**, 635-641.
- Gaber, A. O., Fraga, D. W., Callicutt, C. S., Gerling, I. C., Sabek, O. M. and Kotb, M. Y. (2001). Improved in vivo pancreatic islet function after prolonged in vitro islet culture. *Transplantation* **72**, 1730-1736.
- Gannon, M., Herrera, P. L. and Wright, C. V. (2000). Mosaic Cre-mediated recombination in pancreas using the pdx-1 enhancer/promoter. *Genesis* **26**, 143-144.
- Gao, N., LeLay, J., Vatamaniuk, M. Z., Rieck, S., Friedman, J. R. and Kaestner, K. H. (2008). Dynamic regulation of Pdx1 enhancers by Foxa1 and Foxa2 is essential for pancreas development. *Genes Dev.* **22**, 3435-3448.
- Gradwohl, G., Dierich, A., LeMeur, M. and Guillemot, F. (2000). neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. *Proc. Natl. Acad. Sci. USA* **97**, 1607-1611.
- Gu, G., Dubauskaite, J. and Melton, D. A. (2002). Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. *Development* **129**, 2447-2457.
- Guha, P., Morgan, J. W., Mostoslavsky, G., Rodrigues, N. P. and Boyd, A. S. (2013). Lack of immune response to differentiated cells derived from syngeneic induced pluripotent stem cells. *Cell Stem Cell* **12**, 407-412.
- Hanna, J., Wernig, M., Markoulaki, S., Sun, C.-W., Meissner, A., Cassady, J. P., Beard, C., Brambrink, T., Wu, L. C., Townes, T. M. et al. (2007). Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science* **318**, 1920-1923.
- Hebrok, M., Kim, S. K. and Melton, D. A. (1998). Notochord repression of endodermal Sonic hedgehog permits pancreas development. *Genes Dev.* **12**, 1705-1713.
- Huang, P., He, Z., Ji, S., Sun, H., Xiang, D., Liu, C., Hu, Y., Wang, X. and Hui, L. (2011). Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. *Nature* **475**, 386-389.
- Ieda, M., Fu, J. D., Delgado-Olguin, P., Vedantham, V., Hayashi, Y., Bruneau, B. G. and Srivastava, D. (2010). Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* **142**, 375-386.
- Jensen, J., Pedersen, E. E., Galante, P., Hald, J., Heller, R. S., Ishibashi, M., Kageyama, R., Guillemot, F., Serup, P. and Madsen, O. D. (2000). Control of endodermal endocrine development by Hes-1. *Nat. Genet.* **24**, 36-44.
- Jonsson, J., Carlsson, L., Edlund, T. and Edlund, H. (1994). Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature* **371**, 606-609.
- Kanai-Azuma, M., Kanai, Y., Gad, J. M., Tajima, Y., Taya, C., Kurohmaru, M., Sanai, Y., Yonekawa, H., Yazaki, K., Tam, P. P. et al. (2002). Depletion of definitive gut endoderm in Sox17-null mutant mice. *Development* **129**, 2367-2379.
- Karnik, S. K., Chen, H., McLean, G. W., Heit, J. J., Gu, X., Zhang, A. Y., Fontaine, M., Yen, M. H. and Kim, S. K. (2007). Menin controls growth of pancreatic beta-cells in pregnant mice and promotes gestational diabetes mellitus. *Science* **318**, 806-809.
- Kassem, S. A., Ariel, I., Thornton, P. S., Scheimberg, I. and Glaser, B. (2000). Beta-cell proliferation and apoptosis in the developing normal human pancreas and in hyperinsulinism of infancy. *Diabetes* **49**, 1325-1333.
- Kassem, S., Bhandari, S., Rodriguez-Bada, P., Motaghedi, R., Heyman, M., García-Gimeno, M. A., Cobo-Vuilleumier, N., Sanz, P., Maclaren, N. K., Rahier, J. et al. (2010). Large islets, beta-cell proliferation, and a glucokinase mutation. *N. Engl. J. Med.* **362**, 1348-1350.
- Kawaguchi, Y., Cooper, B., Gannon, M., Ray, M., MacDonald, R. J. and Wright, C. V. (2002). The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. *Nat. Genet.* **32**, 128-134.
- Keirstead, H. S., Nistor, G., Bernal, G., Totoiu, M., Cloutier, F., Sharp, K. and Steward, O. (2005). Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. *J. Neurosci.* **25**, 4694-4705.
- Keymeulen, B., Gillard, P., Mathieu, C., Movahedi, B., Maleux, G., Delvaux, G., Ysebaert, D., Roep, B., Vandemeulebroucke, E., Marichal, M. et al. (2006). Correlation between beta cell mass and glycemic control in type 1 diabetic recipients of islet cell graft. *Proc. Natl. Acad. Sci. USA* **103**, 17444-17449.
- Kim, S. K. and Melton, D. A. (1998). Pancreas development is promoted by cyclopamine, a hedgehog signaling inhibitor. *Proc. Natl. Acad. Sci. USA* **95**, 13036-13041.
- Kim, S. K., Hebrok, M. and Melton, D. A. (1997). Notochord to endoderm signaling is required for pancreas development. *Development* **124**, 4243-4252.
- Kopp, J. L., Dubois, C. L., Hao, E., Thorel, F., Herrera, P. L. and Sander, M. (2011a). Progenitor cell domains in the developing and adult pancreas. *Cell Cycle* **10**, 1921-1927.

- Kopp, J. L., Dubois, C. L., Schaffer, A. E., Hao, E., Shih, H. P., Seymour, P. A., Ma, J. and Sander, M. (2011b). Sox9+ ductal cells are multipotent progenitors throughout development but do not produce new endocrine cells in the normal or injured adult pancreas. *Development* **138**, 653-665.
- Krapp, A., Knöfler, M., Ledermann, B., Bürki, K., Berney, C., Zoerkler, N., Hagenbüchle, O. and Wellauer, P. K. (1998). The bHLH protein PTF1-p48 is essential for the formation of the exocrine and the correct spatial organization of the endocrine pancreas. *Genes Dev.* **12**, 3752-3763.
- Kroon, E., Martinson, L. A., Kadoya, K., Bang, A. G., Kelly, O. G., Eliazar, S., Young, H., Richardson, M., Smart, N. G., Cunningham, J. et al. (2008). Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nat. Biotechnol.* **26**, 443-452.
- Lacy, P. E. L. and Scharp, D. W. S. (1986). Islet transplantation in treating diabetes. *Annu. Rev. Med.* **37**, 33-40.
- Lammert, E., Cleaver, O. and Melton, D. (2001). Induction of pancreatic differentiation by signals from blood vessels. *Science* **294**, 564-567.
- Lee, S. H., Hao, E., Savinov, A. Y., Geron, I., Strongin, A. Y. and Itkin-Ansari, P. (2009). Human β -cell precursors mature into functional insulin-producing cells in an immunoisolation device: implications for diabetes cell therapies. *Transplantation* **87**, 983-991.
- Levitt, H. E., Cyphert, T. J., Pascoe, J. L., Hollern, D. A., Abraham, N., Lundell, R. J., Rosa, T., Romano, L. C., Zou, B., O'Donnell, C. P. et al. (2011). Glucose stimulates human beta cell replication in vivo in islets transplanted into NOD-severe combined immunodeficiency (SCID) mice. *Diabetologia* **54**, 572-582.
- Li, Z., Gadue, P., Chen, K., Jiao, Y., Tuteja, G., Schug, J., Li, W. and Kaestner, K. H. (2012). Foxa2 and H2A.Z mediate nucleosome depletion during embryonic stem cell differentiation. *Cell* **151**, 1608-1616.
- Lowry, W. E., Richter, L., Yachekchko, R., Pyle, A. D., Tchieu, J., Sridharan, R., Clark, A. T. and Plath, K. (2008). Generation of human induced pluripotent stem cells from dermal fibroblasts. *Proc. Natl. Acad. Sci. USA* **105**, 2883-2888.
- Lynn, F. C., Skewes-Cox, P., Kosaka, Y., McManus, M. T., Harfe, B. D. and German, M. S. (2007). MicroRNA expression is required for pancreatic islet cell genesis in the mouse. *Diabetes* **56**, 2938-2945.
- Martín, M., Gallego-Llamas, J., Ribes, V., Keding, M., Niederreither, K., Chambon, P., Dollé, P. and Gradwohl, G. (2005). Dorsal pancreas agenesis in retinoic acid-deficient Raldh2 mutant mice. *Dev. Biol.* **284**, 399-411.
- Matschinsky, F. M. (2009). Assessing the potential of glucokinase activators in diabetes therapy. *Nat. Rev. Drug Discov.* **8**, 399-416.
- McCracken, K. W. and Wells, J. M. (2012). Molecular pathways controlling pancreas induction. *Semin. Cell Dev. Biol.* **23**, 656-662.
- Melkman-Zehavi, T., Oren, R., Kredo-Russo, S., Shapira, T., Mandelbaum, A. D., Rivkin, N., Nir, T., Lennox, K. A., Bello, M. A., Dor, Y. et al. (2011). miRNAs control insulin content in pancreatic β -cells via downregulation of transcriptional repressors. *EMBO J.* **30**, 835-845.
- Michael, M. D., Kulkarni, R. N., Postic, C., Previs, S. F., Shulman, G. I., Magnuson, M. A. and Kahn, C. R. (2000). Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Mol. Cell* **6**, 87-97.
- Molotkov, A., Molotkova, N. and Duyster, G. (2005). Retinoic acid generated by Raldh2 in mesoderm is required for mouse dorsal endodermal pancreas development. *Dev. Dyn.* **232**, 950-957.
- Morán, I., Akerman, I., van de Bunt, M., Xie, R., Benazra, M., Nammo, T., Arnes, L., Nakić, N., García-Hurtado, J., Rodríguez-Seguí, S. et al. (2012). Human β cell transcriptome analysis uncovers lncRNAs that are tissue-specific, dynamically regulated, and abnormally expressed in type 2 diabetes. *Cell Metab.* **16**, 435-448.
- Mullen, Y. S., Clark, W. R., Molnar, I. G. and Brown, J. (1977). Complete reversal of experimental diabetes mellitus in rats by a single fetal pancreas. *Science* **195**, 68-70.
- Nakagawa, M., Koyanagi, M., Tanabe, K., Takahashi, K., Ichisaka, T., Aoi, T., Okita, K., Mochizuki, Y., Takizawa, N. and Yamanaka, S. (2008). Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat. Biotechnol.* **26**, 101-106.
- Nathan, D. M., Zinman, B., Cleary, P. A., Backlund, J. Y., Genuth, S., Miller, R., Orchard, T. J. and Diabetes Control and Complications Trial/ Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Research Group (2009). Modern-day clinical course of type 1 diabetes mellitus after 30 years' duration: the diabetes control and complications trial/epidemiology of diabetes interventions and complications and Pittsburgh epidemiology of diabetes complications experience (1983-2005). *Arch. Intern. Med.* **169**, 1307-1316.
- Nikolova, G., Jabs, N., Konstantinova, I., Domogatskaya, A., Tryggvason, K., Sorokin, L., Fässler, R., Gu, G., Gerber, H. P., Ferrara, N. et al. (2006). The vascular basement membrane: a niche for insulin gene expression and Beta cell proliferation. *Dev. Cell* **10**, 397-405.
- Noguchi, H., Naziruddin, B., Jackson, A., Shimoda, M., Ikemoto, T., Fujita, Y., Chujo, D., Takita, M., Peng, H., Sugimoto, K. et al. (2012). Fresh islets are more effective for islet transplantation than cultured islets. *Cell Transplant.* **21**, 517-523.
- Nostro, M. C., Sarangi, F., Ogawa, S., Holtzinger, A., Corneo, B., Li, X., Micallef, S. J., Park, I. H., Basford, C., Wheeler, M. B. et al. (2011). Stage-specific signaling through TGF β family members and WNT regulates patterning and pancreatic specification of human pluripotent stem cells. *Development* **138**, 861-871.
- Offield, M. F., Jetton, T. L., Labosky, P. A., Ray, M., Stein, R. W., Magnuson, M. A., Hogan, B. L. and Wright, C. V. (1996). PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development* **122**, 983-995.
- Ohlsson, H., Karlsson, K. and Edlund, T. (1993). IPF1, a homeodomain-containing transactivator of the insulin gene. *EMBO J.* **12**, 4251-4259.
- Okita, K., Ichisaka, T. and Yamanaka, S. (2007). Generation of germline-competent induced pluripotent stem cells. *Nature* **448**, 313-317.
- Okita, K., Hong, H., Takahashi, K. and Yamanaka, S. (2010). Generation of mouse-induced pluripotent stem cells with plasmid vectors. *Nat. Protoc.* **5**, 418-428.
- Pan, F. C. and Wright, C. (2011). Pancreas organogenesis: from bud to plexus to gland. *Dev. Dyn.* **240**, 530-565.
- Pan, F. C., Bankaitis, E. D., Boyer, D., Xu, X., Van de Casteele, M., Magnuson, M. A., Heimberg, H. and Wright, C. V. (2013). Spatiotemporal patterns of multipotentiality in Ptf1a-expressing cells during pancreas organogenesis and injury-induced facultative restoration. *Development* **140**, 751-764.
- Parsons, J. A., Brelje, T. C. and Sorenson, R. L. (1992). Adaptation of islets of Langerhans to pregnancy: increased islet cell proliferation and insulin secretion correlates with the onset of placental lactogen secretion. *Endocrinology* **130**, 1459-1466.
- Perl, S., Kushner, J. A., Buchholz, B. A., Meeker, A. K., Stein, G. M., Hsieh, M., Kirby, M., Pechhold, S., Liu, E. H., Harlan, D. M. et al. (2010). Significant human beta-cell turnover is limited to the first three decades of life as determined by in vivo thymidine analog incorporation and radiocarbon dating. *J. Clin. Endocrinol. Metab.* **95**, E234-E239.
- Porat, S., Weinberg-Corem, N., Tornovsky-Babaey, S., Schyr-Ben-Haroush, R., Hija, A., Stolovich-Rain, M., Dadon, D., Granot, Z., Ben-Hur, V., White, P. et al. (2011). Control of pancreatic β cell regeneration by glucose metabolism. *Cell Metab.* **13**, 440-449.
- Powers, A. C. and D'Alessio, D. (2011). Endocrine pancreas and pharmacotherapy of diabetes mellitus and hypoglycemia. In *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 12th edn (ed. B. A. Chabner, L. L. Brunton and B. C. Knollman). New York: McGraw-Hill.
- Poy, M. N., Eliasson, L., Krutzfeldt, J., Kuwajima, S., Ma, X., Macdonald, P. E., Pfeffer, S., Tuschl, T., Rajewsky, N., Rorsman, P. et al. (2004). A pancreatic islet-specific microRNA regulates insulin secretion. *Nature* **432**, 226-230.
- Rahier, J., Guiot, Y., Goebbels, R. M., Sempoux, C. and Henquin, J. C. (2008). Pancreatic β -cell mass in European subjects with type 2 diabetes. *Diabetes Obes. Metab.* **10** Suppl. 4, 32-42.
- Rankin, M. M., Wilbur, C. J., Rak, K., Shields, E. J., Granger, A. and Kushner, J. A. (2013). Beta cells are not generated in pancreatic duct ligation induced injury in adult mice. *Diabetes* **62**, 1634-1645.
- Rezania, A., Bruin, J. E., Riedel, M. J., Mojibian, M., Asadi, A., Xu, J., Gauvin, R., Narayan, K., Karanu, F., O'Neil, J. J. et al. (2012). Maturation of human embryonic stem cell-derived pancreatic progenitors into functional islets capable of treating pre-existing diabetes in mice. *Diabetes* **61**, 2016-2029.
- Schäffer, L., Brissette, R. E., Spetzler, J. C., Pillutla, R. C., Østergaard, S., Lennick, M., Brandt, J., Fletcher, P. W., Danielsen, G. M., Hsiao, K. C. et al. (2003). Assembly of high-affinity insulin receptor agonists and antagonists from peptide building blocks. *Proc. Natl. Acad. Sci. USA* **100**, 4435-4439.
- Schäffer, L., Brand, C. L., Hansen, B. F., Ribel, U., Shaw, A. C., Slaaby, R. and Sturis, J. (2008). A novel high-affinity peptide antagonist to the insulin receptor. *Biochem. Biophys. Res. Commun.* **376**, 380-383.
- Schaffer, A. E., Freude, K. K., Nelson, S. B. and Sander, M. (2010). Nkx6 transcription factors and Ptf1a function as antagonistic lineage determinants in multipotent pancreatic progenitors. *Dev. Cell* **18**, 1022-1029.
- Schölin, A., Björklund, L., Borg, H., Arnqvist, H., Björk, E., Blomhé, G., Bolinder, J., Eriksson, J. W., Gudbjörnsdóttir, S., Nyström, L. et al. (2004). Islet antibodies and remaining β -cell function 8 years after diagnosis of diabetes in young adults: a prospective follow-up of the nationwide Diabetes Incidence Study in Sweden. *J. Intern. Med.* **255**, 384-391.
- Schulz, T. C., Young, H. Y., Agulnick, A. D., Babin, M. J., Baetge, E. E., Bang, A. G., Bhoumik, A., Cepa, I., Cesario, R. M., Haakmeester, C. et al. (2012). A scalable system for production of functional pancreatic progenitors from human embryonic stem cells. *PLoS ONE* **7**, e37004.
- Shapiro, A. M., Lakey, J. R., Ryan, E. A., Korbitt, G. S., Toth, E., Warnock, G. L., Kneteman, N. M. and Rajotte, R. V. (2000). Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N. Engl. J. Med.* **343**, 230-238.
- Shapiro, A. M., Ricordi, C., Hering, B. J., Auchincloss, H., Lindblad, R., Robertson, R. P., Secchi, A., Brendel, M. D., Berney, T., Brennan, D. C. et al. (2006). International trial of the Edmonton protocol for islet transplantation. *N. Engl. J. Med.* **355**, 1318-1330.

- Shen, W., Tremblay, M. S., Deshmukh, V. A., Wang, W., Filippi, C. M., Harb, G., Zhang, Y. Q., Kamireddy, A., Baaten, J. E., Jin, Q. et al. (2013). Small-molecule inducer of β cell proliferation identified by high-throughput screening. *J. Am. Chem. Soc.* **135**, 1669-1672.
- Shiba, Y., Fernandes, S., Zhu, W. Z., Filice, D., Muskheli, V., Kim, J., Palpant, N. J., Gantz, J., Moyes, K. W., Reinecke, H. et al. (2012). Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts. *Nature* **489**, 322-325.
- Sneddon, J. B., Borowiak, M. and Melton, D. A. (2012). Self-renewal of embryonic-stem-cell-derived progenitors by organ-matched mesenchyme. *Nature* **491**, 765-768.
- Solar, M., Cardalda, C., Houbracken, I., Martín, M., Maestro, M. A., De Medts, N., Xu, X., Grau, V., Heimberg, H., Bouwens, L. et al. (2009). Pancreatic exocrine duct cells give rise to insulin-producing beta cells during embryogenesis but not after birth. *Dev. Cell* **17**, 849-860.
- Sosa-Pineda, B., Chowdhury, K., Torres, M., Oliver, G., and Gruss, P. (1997). The pax4 gene is essential for differentiation of insulin-producing β cells in the mammalian pancreas. *Nature* **386**, 399-402.
- Stafford, D. and Prince, V. E. (2002). Retinoic acid signaling is required for a critical early step in zebrafish pancreatic development. *Curr. Biol.* **12**, 1215-1220.
- Stoffers, D. A., Zinkin, N. T., Stanojevic, V., Clarke, W. L. and Habener, J. F. (1997). Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. *Nat. Genet.* **15**, 106-110.
- Stoffers, D. A., Kieffer, T. J., Hussain, M. A., Drucker, D. J., Bonner-Weir, S., Habener, J. F. and Egan, J. M. (2000). Insulinotropic glucagon-like peptide 1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas. *Diabetes* **49**, 741-748.
- Stolovich-Rain, M., Hija, A., Grimsby, J., Glaser, B. and Dor, Y. (2012). Pancreatic beta cells in very old mice retain capacity for compensatory proliferation. *J. Biol. Chem.* **287**, 27407-27414.
- Takahashi, K. and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663-676.
- Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K. and Yamanaka, S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861-872.
- Talchai, C., Xuan, S., Lin, H. V., Sussel, L. and Accili, D. (2012). Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. *Cell* **150**, 1223-1234.
- Terauchi, Y., Takamoto, I., Kubota, N., Matsui, J., Suzuki, R., Komeda, K., Hara, A., Toyoda, Y., Miwa, I., Aizawa, S. et al. (2007). Glucokinase and IRS-2 are required for compensatory beta cell hyperplasia in response to high-fat diet-induced insulin resistance. *J. Clin. Invest.* **117**, 246-257.
- Teta, M., Long, S. Y., Wartschow, L. M., Rankin, M. M. and Kushner, J. A. (2005). Very slow turnover of β -cells in aged adult mice. *Diabetes* **54**, 2557-2567.
- Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S. and Jones, J. M. (1998). Embryonic stem cell lines derived from human blastocysts. *Science* **282**, 1145-1147.
- Thorel, F., Népote, V., Avril, I., Kohno, K., Desgraz, R., Chera, S. and Herrera, P. L. (2010). Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. *Nature* **464**, 1149-1154.
- Tian, L., Gao, J., Weng, G., Yi, H., Tian, B., O'Brien, T. D. and Guo, Z. (2011). Comparison of exendin-4 on beta-cell replication in mouse and human islet grafts. *Transpl. Int.* **24**, 856-864.
- Tremblay, K. D. (2010). Formation of the murine endoderm: lessons from the mouse, frog, fish, and chick. *Prog. Mol. Biol. Transl. Sci.* **96**, 1-34.
- Van Assche, F. A., Aerts, L. and De Prins, F. (1978). A morphological study of the endocrine pancreas in human pregnancy. *Br. J. Obstet. Gynaecol.* **85**, 818-820.
- van der Meulen, T., Xie, R., Kelly, O. G., Vale, W. W., Sander, M. and Huisig, M. O. (2012). Urocortin 3 marks mature human primary and embryonic stem cell-derived pancreatic alpha and beta cells. *PLoS ONE* **7**, e52181.
- Vierbuchen, T., Ostermeier, A., Pang, Z. P., Kokubu, Y., Südhof, T. C. and Wernig, M. (2010). Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* **463**, 1035-1041.
- Vikram, A. and Jena, G. (2010). S961, an insulin receptor antagonist causes hyperinsulinemia, insulin-resistance and depletion of energy stores in rats. *Biochem. Biophys. Res. Commun.* **398**, 260-265.
- Wandzioch, E. and Zaret, K. S. (2009). Dynamic signaling network for the specification of embryonic pancreas and liver progenitors. *Science* **324**, 1707-1710.
- Wang, R. N., Klöppel, G. and Bouwens, L. (1995). Duct- to islet-cell differentiation and islet growth in the pancreas of duct-ligated adult rats. *Diabetologia* **38**, 1405-1411.
- Wang, W., Walker, J. R., Wang, X., Tremblay, M. S., Lee, J. W., Wu, X. and Schultz, P. G. (2009). Identification of small-molecule inducers of pancreatic β -cell expansion. *Proc. Natl. Acad. Sci. USA* **106**, 1427-1432.
- Warren, L., Manos, P. D., Ahfeldt, T., Loh, Y.-H., Li, H., Lau, F., Ebina, W., Mandal, P. K., Smith, Z. D., Meissner, A. et al. (2010). Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell* **7**, 618-630.
- Weinstein, D. C., Ruiz i Altaba, A., Chen, W. S., Hoodless, P., Prezioso, V. R., Jessell, T. M. and Darnell, J. E., Jr (1994). The winged-helix transcription factor HNF-3 beta is required for notochord development in the mouse embryo. *Cell* **78**, 575-588.
- Wernig, M., Meissner, A., Foreman, R., Brambrink, T., Ku, M., Hochedlinger, K., Bernstein, B. E. and Jaenisch, R. (2007). In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* **448**, 318-324.
- Xie, R., Everett, L. J., Lim, H. W., Patel, N. A., Schug, J., Kroon, E. et al. (2013). Dynamic chromatin remodeling mediated by polycomb proteins orchestrates pancreatic differentiation of human embryonic stem cells. *Cell Stem Cell* **12**, 224-237.
- Xu, G., Stoffers, D. A., Habener, J. F. and Bonner-Weir, S. (1999). Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes* **48**, 2270-2276.
- Xu, X., D'Hoker, J., Stangé, G., Bonnè, S., De Leu, N., Xiao, X., Van de Castele, M., Mellitzer, G., Ling, Z., Pipeleers, D. et al. (2008). Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell* **132**, 197-207.
- Yi, P. Y., Park, J. and Melton, D. M. (2013). Betatrophin: a hormone that controls pancreatic beta cell proliferation. *Cell* **153**, 747-758.
- Yoshitomi, H. and Zaret, K. S. (2004). Endothelial cell interactions initiate dorsal pancreas development by selectively inducing the transcription factor Ptf1a. *Development* **131**, 807-817.
- Yu, J., Vodyanik, M. A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J. L., Tian, S., Nie, J., Jonsdottir, G. A., Ruotti, V., Stewart, R. et al. (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science* **318**, 1917-1920.
- Zhao, T., Zhang, Z. N., Rong, Z. and Xu, Y. (2011). Immunogenicity of induced pluripotent stem cells. *Nature* **474**, 212-215.
- Zhou, Q. and Melton, D. A. (2008). Extreme makeover: converting one cell into another. *Cell Stem Cell* **3**, 382-388.
- Zhou, Q., Law, A. C., Rajagopal, J., Anderson, W. J., Gray, P. A. and Melton, D. A. (2007). A multipotent progenitor domain guides pancreatic organogenesis. *Dev. Cell* **13**, 103-114.