Alpha-to-beta cell trans-differentiation for treatment of diabetes

Mohamed Saleh1,2, George K. Gittes1 and ©Krishna Prasadan1

1Division of Pediatric Surgery, UPMC Children’s Hospital of Pittsburgh, Pittsburgh, PA 15224, U.S.A.; 2Division of Pediatric Endocrinology, UPMC Children’s Hospital of Pittsburgh, Pittsburgh, PA 15224, U.S.A.

Correspondence: Krishna Prasadan (prasadank@upmc.edu)

Introduction

Type 1 diabetes is a chronic disease characterized by autoimmune-mediated destruction of the insulin-producing β cells in the pancreas [1]. Destruction of β cells leads to insulin deficiency, hyperglycemia, and eventually the development of clinical diabetes. The treatment options for type 1 diabetes are limited to insulin replacement therapy. While, type 2 diabetes results from long-standing insulin resistance [2]. In type 2 diabetes, β cell mass is reduced by 40–60% compared with weight-matched controls [3,4]. The underlying etiology of β cell mass loss in type 2 diabetes is thought to be due to an increase in β-cell apoptosis rate [5], as chronic exposure to insulin resistance imposes a high workload on β cells in order to meet the higher demand for insulin secretion, which makes β cells vulnerable to endoplasmic reticulum (ER) stress [6,7]. Besides the decreased β cells in patients with type 2 diabetes, β-cell dysfunction also occurs early in the natural course of type 2 diabetes, with a decline in β cell function of 75–80% in subjects in the upper third of impaired glucose tolerance (2 h plasma glucose = 180–199 mg/dl) [6,8,9]. Additionally, reduced first-phase insulin secretion was found to be the earliest and most detrimental defect in β cells in humans with impaired glucose tolerance (prediabetes) and type 2 diabetes [10,11].

There is no cure for type 1 and type 2 diabetes. The prevailing therapeutic approaches to type 2 diabetes have focused on drugs that either improve insulin resistance or increase insulin secretion and decrease glucagon secretion [12]. However, these medications increase the risk of side effects such as dysregulated insulin secretion, weight gain, hypoglycemia, and gastrointestinal, renal, and cardiovascular side effects [13,14]. Although the pathophysiology of type 1 and type 2 diabetes are different, loss of β cell mass with subsequent insulin deficiency represents the end result that directly causes diabetes. Therefore, a better therapeutic strategy would be to enhance β cell mass and restore insulin secretory capacity, which might cure different types of diabetes.
Key transcription factors involved in the development of β cells

In the developing pancreas, cellular differentiation and lineage selection are regulated by a cascade of transcription factors and signaling molecules that coordinate the timing and development of the exocrine and endocrine cells from progenitor cells (Figure 1). The differentiation of endocrine cell types in the pancreas changes throughout embryogenesis. Specifically, α cells are the first to form, with glucagon-positive cells appearing early.

Figure 1. Mouse pancreas development originates from the foregut endoderm under the control of several transcription and growth factors, specifically pdx1.

Loss of pdx1 prevents the formation of the pancreas, while overexpression of endocrine-specific transcription factor, ngn3, in foregut endoderm will result in an immature pancreas containing only α cells. Loss of ngn3 expression in these cells prevents endocrine development. pdx1 positive progenitors develop both trunk and tip progenitors, ngn3 expression in trunk progenitor then leads those into an endocrine lineage and generates all four endocrine cell types. pdx1 and MafA expression in select endocrine progenitors gave way to β cells, while MafB expression is required for α cell formation. Forced expression on pdx1, MafA, and ngn3 in acinar cells reprograms them into β cells, while forced expression of pdx1 and MafA in α cells converts them to β cells.
in the developing mouse pancreas at E9.5, with a subset of these cells co-express insulin [15,16]. This finding suggests that pancreatic endocrine progenitor cells co-express a set of islet hormones whose expression is selectively up or down-regulated as the endocrine lineage selection occurs. Beyond α cells, other types of endocrine cells, including β cells, are not generated in significant numbers in mouse until E13.5 or later [17].

In mice, early embryonic pdx1 positive cells represent progenitors of all of the mature endocrine and exocrine pancreatic cells [18]. Pdx1 expression becomes limited to β cells late in the development of the murine pancreas as β cells mature [19]. Also, pdx1 is known to regulate the insulin genes in rodents [20]. Besides, PDX1 is required for normal β cell function, and loss of its expression from one allele in adult humans causes diabetes [21,22]. Conditional deletion of pdx1 in the developing β cells in rodents results in hyperglycemia, reduced number of β cells and an increased number of α cells [23,24]. In the developing mouse pancreas, all endocrine cells develop from neurogenin-3 (ngn3) positive endocrine progenitor cells [18,25]. Ngn3 [a class A Basic Helix-Loop-Helix Protein (bHLH)] is expressed in duct-like epithelial cells that are centrally located within the developing mouse pancreas; as these cells differentiate, they down-regulate ngn3 and aggregate into proto-islet structures [26]. Loss of function mutation of ngn3 prevents endocrine development and leads to death in mice postnatally [25,27–29]. Although it is critical for all pancreatic endocrine cell identity, forced expression of ngn3 early during the mouse pancreas development under the pdx1 promoter led to the formation of a premature cluster of endocrine cells containing only α cells [26], suggesting a role for other factors besides ngn3 later in development for proper endocrine development and specification.

The Maf family proteins, MafA, and MafB have a central role in the late development and maturation of endocrine cells in rodents [30–32]. In the embryonic mouse pancreas, a significant portion of insulin-positive cells express MafB, and as part of the β cell maturation process, these cells transitioned through a MafB and MafA double-positive phase (insulin intermediate cells) followed by full maturation to a MafB negative and MafA positive β cells. This transition of β cells to become MafA positive only coincides with increased pdx1 expression in these mature cells [33]. MafB (−/−) null mutant embryonic pancreas had reduced numbers of insulin and glucagon-positive cells, yet, the total number of endocrine cells appeared to remain the same [34,35]. Unlike mice, adult human islet β cells express MAFB [36]. Human progenitor stem cells lacking MAFB expression failed to differentiate into α or β cells, but formed delta and pancreatic polypeptide cells [36].

The MafA null mutant showed that MafA is necessary for maturation but not for the specification of pancreatic β cells. [33]. Losing MafA during the development of mouse pancreas did not alter the proportion of insulin-positive cells at birth, suggesting normal development and lineage selection [33]. However, the MafA deficient mice developed diabetes postnatally, suggesting that MafA regulates maturation and is required for glucose-responsive expression of insulin in adult β cells [33]. To this point, MafA interacts with NeuroD1 and Pdx1 [29] to activate the insulin gene in mice [30]. Besides Pdx-1 and MafA, in rodents, the mature β-cell expresses Pax4, Nkx 2.2, and Nkx 6.1, which are also required to maintain normal function [37].

Non-endocrine cells as a source of new β cells

There have been several attempts in the past to convert non-endocrine cells into insulin-producing cells [38,39], including viral-mediated expression of transcription factors in human hepatocytes [38], mouse gastrointestinal cells [40], and acinar cells [41,42]. Ectopic PDX1 expression in adult human liver cells induced the development of functional insulin-producing cells; these cells when transplanted under the renal capsule of diabetic, immunodeficient mice ameliorated hyperglycemia [43]. Also, In vivo recombinant-adenovirus-mediated gene transfer of pdx1 into liver cells ameliorated hyperglycemia in diabetic mice treated with streptozotocin [44]. Furthermore, treatment with exendin-4 (Glucagon-like peptide agonist) enhanced the proliferation and maturation of PDX1-expressing human liver cells toward a β cell phenotype [45]. In addition, plasmid-based pdx1, MafA, and ngn3 (PMN) gene delivery into the inferior vena cava transiently induced insulin transcripts in rat livers [46]. Also, systemic administration of a single adenoviral vector encoding pdx1, MafA, and ngn3 factors reprogrammed duct-like SOX9-positive cells in the liver into insulin-producing cells and improved hyperglycemia in non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice treated with streptozotocin [47]. Notably, those insulin-producing duct-like cells showed some degree of glucose responsiveness ex vivo [47]. In immunocompetent mice, adenoviral vector-mediated PMN delivery transiently induced insulin-producing SOX9-positive duct-like cells in the liver [48].

Acinar cells were another cell type targeted as a potential source of new insulin-producing cells. Forced expression of ngn3 alone in mouse acinar cells induced conversion into delta cells, while forced ngn3 and MafA expression converted acinar cells into α-like cells [41]. However, a combination of the three transcription
factors, mgn3, MafA and pdx1, converted the acinar cells into beta-like cells and improved hyperglycemia in toxin-induced-diabetic mice [41,42].

Besides pancreas acinar cells and liver cells, several studies have targeted gastrointestinal cells as a potential source to form insulin-producing cells. The gastrointestinal tissue is abundant with adult stem/progenitor cells that are continuously forming epithelial cells, including enteroendocrine cells [49,50]. In fetal and adult mice, specific deletion of FoxO1 in mgn3-positive enteroendocrine progenitors converted them into insulin-positive cells [51]. This ablation of the FoxO1 in the enteroendocrine cells increased the expression of beta-cell transcription factors pdx1, mgn3, MafA and Nkx6.1 [51]. In human pluripotent stem (iPS) cells, FOXO1 inhibition induced the generation of insulin-producing cells that express all markers of mature pancreatic beta cells [52]. Similar to acinar cells and liver cells, forced expression of mgn3, MafA and pdx1 in mice intestinal crypt cells and human intestinal organoids converted them to beta-like cells [53]. In this study, expression of mgn3, MafA and pdx1 in intestinal cells lead to modest but significant improvement in glucose tolerance in Streptozotocin-treated mice [53]. Similarly, forced expression of mgn3, MafA and pdx1 reprogrammed gastrointestinal enteroendocrine cells to insulin-producing cells with the highest efficiency being observed in the stomach antrum [54].

One major problem with targeting non-endocrine cells as a source of new insulin-producing cells is that these attempts only resulted in a partial improvement in glycemia, specifically fasting glucose, in diabetic mouse models. The overall glucose tolerance, however, despite the improvement in fasting glucose levels, remained quite abnormal, indicating that the newly formed beta-like cells can secrete some basal insulin, but they cannot respond adequately to a glucose challenge, which obviously raises concerns about the potential translatability of this approach.

A second drawback with reprogramming a non-endocrine cell such as an acinar cell to become an insulin-producing cell (or any islet endocrine cell for that matter), mgn3 is necessary to initiate an endocrine lineage identity and to suppress the acinar cell phenotype [41]. Subsequently, pdx1 and MafA further convert mgn3 positive cells into insulin-producing cells. Here, the continued expression of mgn3 in differentiated islet cells is a significant drawback of this approach when trying to generate beta cells from non-endocrine cells because mgn3 expression is normally low or absent from differentiated islet endocrine cells [55]. Thus, the use of a triple transcription factor vector encoding pdx1, MafA and mgn3 would lead to constitutive expression of relatively high levels of mgn3 in the trans-differentiated beta-like cells, which may have negative consequences. Thus, targeting endocrine cells seems a preferable approach to regenerating beta-like cells.

**Why alpha cells may be an optimal source for new beta cell formation**

Recently, researchers have shifted their focus toward alpha cells as a source for the replacement of beta cells. Several reasons favor alpha cells as a proper source for beta cell replacement compared with non-endocrine cells, including: (1) alpha and beta cell lineages appear to arise from a common precursor [15,56], which may facilitate reprogramming. (2) evidence already exists for the potential interconversion between alpha and beta cells; postnatal deletion of pdx1 in mouse beta cells led to loss of beta cells with an increase in alpha cells, accompanied by a change in islet morphology, with glucagon-positive cells in the periphery and center of the islet, rather than the usual periphery only in mice. In addition, some cells were double-positive for both glucagon and insulin [24]. Also, it is reported that a massive loss of beta cells in the adult mouse pancreas led to the conversion of alpha cells into beta cells, again with the appearance of bi-hormonal cells expressing both insulin and glucagon [57], additionally, monoclonal antibodies to glucagon receptor were found to induce alpha cell hyperplasia and subsequently alpha cells were converted to beta-like cells [58], thus supporting the ability to reprogram alpha cells into insulin-producing cells therapeutically since it can happen spontaneously. (3) the similarities in the function of alpha cells and beta cells, as both cells have Slc2a2 transporter that allows glucose sensing within a physiologic range [59]. Also, alpha cells and beta cells have similar machinery to metabolize glucose and secrete hormones [60]. (4) alpha cells are located anatomically in the islet, receiving the same blood supply [15], additionally, in humans, alpha and beta cells being located in islets, they receive sympathetic nerve supply through the splanchnic nerve with the neural cell bodies originate from the superior mesenteric and celiac ganglia, while the parasympathetic innervation comes from the vagus nerve [61], which is ideal for the optimal function of newly formed beta-like cells [62]. (5) alpha cells represent ~35% of the islet cells in humans [63], which makes them an abundant source for beta cell replacement. (6) Reduction in alpha cell mass in mice does not have a negative impact on glucose metabolism [64]. In view of these reasons, alpha cell appears to be an ideal therapeutic target for replacement of beta cells to treat diabetes.
Therapeutic attempts to reprogram α cells into insulin-producing cells

Several attempts were made to reprogram α cells into insulin-producing cells ex vivo and in vivo.

For example, in vivo forced expression of pax4 in mouse islet progenitors induced production of α-like cells that then converted into β-like cells, forming large islets with β cell predominance; ngn3 reactivation was crucial in this process [65]. In this model, the pax4 forced expression in islets not only increased β cell mass, but led to improved glucose tolerance. Furthermore, the ectopic expression of pax4 in adult α cells continuously converted them into β cells and reversed hyperglycemia in streptozotocin-treated animals; interestingly, this effect was only seen in mice younger than four weeks [65].

Sangan et al. [66] reprogrammed an α cell line, αTC1.9, into insulin-producing cells by ectopic expression of HNF4α, which resulted in glucagon suppression and induced a β-like cell phenotype; however, that reprogramming was incomplete because certain β cell-specific transcription factors such as pdx1 were not induced.

Similarly, Zhang et al. delivered pax4 via a viral vector (adenovirus 5) into αTC1.9 cells leading to an induction of insulin synthesis and suppression of glucagon. Here, pax4 expression led to an up-regulation of the β cell transcription factors pdx1, MafA, ngn3, and nkx 6.1 in the αTC1.9 cells. Also, direct infusion of adenovirus 5 carrying a pax4 expression cassette into the pancreas via the pancreatic duct resulted in a small improvement in glucose tolerance in toxin-induced diabetic mice, though not a biologically significant improvement [67].

Furthermore, inactivation of arx and dnmt1 in adult mouse pancreatic α cells led to conversion of a subset (50–80% over three months) of these α cells into β-like cells with the capacity to secrete insulin in response to glucose stimulation, yet this insulin secretion capacity was significantly lower than true β cells [68]. Based on the results of that study, a follow up study used the anti-malaria drug artemether to suppress the α cell transcription factor arx in mice to promote trans-differentiation into β-like cells [69]. However, the key initial experiments in this study were carried out in islet cell lines, but subsequent validation experiments in vivo showed some degree of trans-differentiation, but without a clear demonstration of α to β cell conversion; moreover, artemether was found to abrogate β cell calcium signaling and insulin secretion in response to glucose [70].

In the same context, another study reported that the prolonged exposure of wild-type mice to GABA resulted in the conversion of α cells into β-like cells through the down-regulation of Arx expression [71]. In this study GABA treatment successfully reversed hyperglycemia in Streptozotocin-treated mice [71]. Also, Young-sun et al. have shown that glucagon-like peptide 1 promoted the formation of new β-like cells from α cells in mice via FGF21 after chemical ablation of β cells and normalization of blood glucose in streptozotocin-treated animals; interestingly, this effect was only seen in mice younger than four weeks [65].

More recently, the focus has shifted to overexpression of MafA and pdx1 in α cells to convert them into β-like cells. In adult mice, induced expression of MafA and pdx1 in ngn3 positive endocrine progenitor cells led to the development of a β-like cell phenotype; similarly, pdx1 and MafA overexpression in α cells led to its trans-differentiation into β-like cells [73]. This latter study was followed by a study that used an in vivo infusion of adeno-associated virus (AAV) carrying pdx1 and MafA expression cassettes into the pancreatic duct, leading to reprogramming of α cells into functional β-like cells with normalization of blood glucose in both β cell-toxin-induced diabetic mice and in autoimmune NOD mice (Figure 2). In that study, the euglycemia persisted in the autoimmune NOD mice for four months before the recurrence of hyperglycemia, perhaps because the immune system began to recognize and destroy the newly formed β-like cells. This gene therapy strategy also induced α to β cell conversion in toxin-treated human islets, which restored blood glucose levels in NOD/SCID mice upon transplantation [74].

A subsequent study sought to better study human islet cell plasticity, specifically the ability of human α cells to transform into β cells. In vitro infection of human α-cell-only pseudoislets with adenovirus expressing PDX1 and MAFα led to conversion of ~35% of these α cells into insulin-positive cells [75]. Moreover, transplantation of pseudoislets, made of α cells infected with this same pdx1 and MafA adenovirus, into diabetic immunodeficient NOD/SCID/Il2rg<sup>−/−</sup> (NSG) mice led to improved insulin secretion and glucose tolerance. The improvement fell short of full normalization, likely due to an inadequate mass of transplanted reprogrammed α cells [75].

A cell to β cell conversion; challenges and future directions

Translation of this therapeutic gene strategy to treat diabetes in humans seems technically applicable via the noninvasive procedure endoscopic retrograde cholangiopancreatography (ERCP). However, finding a proper viral vector that could carry the genes and targets human α cells with high affinity in vivo remains a challenge.
Xiao et al. and Furuyama et al. used AAV serotype 8 virus to deliver \textit{pdx1} and \textit{MafA} to human $\alpha$ cells \textit{ex vivo} \cite{74,75}. However, directly infecting islets \textit{ex vivo} with the virus differs significantly from using an \textit{in vivo} pancreatic duct infusion. In cell culture, the virus is placed in direct contact with the islets, making the pathway to viral infection of the islets very different. \textit{In vivo}, the virus must pass out of the pancreatic ductal system, crossing the pancreatic duct epithelium and ductal basement membrane, or crossing the acinar cells and the acinar basement membrane, across the interstitial space before finally reaching the islets, which also \textit{in vivo} are surrounded by a basement membrane ‘capsule’ \cite{76}. This capsule is degraded during the islet isolation process and therefore not a barrier to \textit{in vitro} islet infection by virus. During this journey, undesirable trapping of the virus in exocrine cells may occur before they can reach the islets and potentially preventing adequate numbers of virus from reaching the $\alpha$ cells. Finding the ideal vector (virus type and serotype) for infecting $\alpha$ cells in humans will likely require further studies in non-human primates. This primate optimization will be necessary before pursuing clinical trials in humans to ensure the safety of the viral therapy, minimizing the risk of adverse extrapancreatic side effects, and optimizing efficacy. Efficacy will entail infecting and reprogramming an adequate number of $\alpha$ cells into insulin-producing cells enough to reverse hyperglycemia.

The immunogenicity of the newly formed $\beta$ cells from $\alpha$ cells is also an important aspect that needs to be addressed before applying this therapeutic strategy in type 1 diabetes. Xiao et al. have shown that \textit{pdx1} and \textit{MafA} gene therapy maintained euglycemia in NOD mice for four months, suggesting that the newly formed $\beta$ cells are not quickly recognized by the autoimmune response \cite{74}. Thus, this gene therapy could be combined with immunomodulation or immunosuppression to prolong the lifespan of the newly formed $\beta$-like cells.

Studies that examine the efficacy of this therapeutic strategy in treating type 2 diabetes in animal models are still lacking. In both impaired glucose tolerance (prediabetes) and T2D, insulin resistance at the hepatic level and peripheral tissues occurs due to impaired insulin signaling \cite{2,77} and inappropriate hyperglucagonemia \cite{78,79}. With long-standing insulin resistance, there is an eventual decline in $\beta$ cell mass and function \cite{8}. Considering that the pathophysiology of type 2 diabetes involves a decrease in insulin secretion secondary to decreased $\beta$ cell mass and function, and inappropriate hyperglucagonemia, the reprogramming of $\alpha$ cells into
insulin-secreting cells seems to be an appealing treatment for this disorder by restoring β cell mass, leading to increased insulin secretion, and by decreasing α cell mass with a potential reduction in the hyperglucagonemia and insulin resistance. Ideally, such a treatment for type 2 diabetes, with the reprogramming of α cells into insulin-producing cells, would be accompanied by lifestyle modification. Otherwise, the persistent chronic exposure of the newly formed β cells to insulin resistance, glucotoxicity and lipotoxicity would likely cause failure of the new β-like cells, with recurrence of hyperglycemia [80]. Thus, more studies are needed to address the potential benefit of using α cell to β cell conversion in treating type 2 diabetes.

Overall, further studies are required to develop and optimize this promising therapeutic strategy given the desparate need to find novel treatments and perhaps a cure for diabetes, a major health and economic problem.

**Perspectives**

- **Importance in the field:** Diabetes is a major health and economic problem in the United States and around the world. There is currently no cure for diabetes. Converting α cells into insulin-producing cells could provide a promising therapeutic strategy to cure diabetes.

- **Current thinking:** The use of viral gene therapy to drive the expression of *pdx1* and *MafA* in α cells, transforming them into functional β cells, has recently become the main direction in this research field. This technique will replace the lost β cell mass and restore insulin secretion capacity in individuals with diabetes.

- **Future directions:** In this field, future directions include: (1) Developing a proper viral vector that could carry the genes and targets human α cells with high affinity *in vivo*. (2) Immunomodulation to prevent the immune-mediated destruction of the newly formed β cells in type 1 diabetes. (3) Investigate the potential efficacy of this approach as a therapeutic stagey for type 2 diabetes.

**Competing Interests**

George Gittes has a potential conflict of interest with Genprex company that contributes to supporting his research work about converting alpha cells into beta cells to treat diabetes.

**Author Contributions**

M.S. and K.P. wrote the review. G.G. reviewed and edited the manuscript.

**Funding**

This work was partially supported by NIDDK funding to GG (RO1DK111460).

**Abbreviations**

AAV, adeno-associated virus; NOD, non-obese diabetic; PMN, *pdx1*, *MafA*, and *ngn3*; SCID, severe combined immunodeficient.

**References**

1. Atkinson, M.A., Eisenbarth, G.S. and Michels, A.W. (2014) Type 1 diabetes. *Lancet* **383**, 69–82. https://doi.org/10.1016/S0140-6736(13)60591-7
2. Defronzo, R.A. (2009) Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes* **58**, 773–795. https://doi.org/10.2337/db09-9028
3. Rahier, J., Guiot, Y., Goebbels, R.M., Sempoux, C. and Henquin, J.C. (2008) Pancreatic beta-cell mass in European subjects with type 2 diabetes. *Diabetes Obes. Metab.* **10**, 32–42. https://doi.org/10.1111/j.1463-1326.2008.00969.x
4. Yoon, K.H., Ko, S.H., Cho, J.H., Lee, J.M., Ahn, Y.B., Song, K.H. et al. (2003) Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea. *J. Clin. Endocrinol. Metab.* **88**, 2300–2308. https://doi.org/10.1210/jc.2002-020735
5. Butler, A.E., Janson, J., Bonner-Weir, S., Ritzel, R., Rizza, R.A. and Butler, P.C. (2003) Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* **52**, 102–110. https://doi.org/10.2337/diabetes.52.1.102
2546

6 Weir, G.C., Butler, P.C. and Bonner-Weir, S. (2021) The beta-cell glucose toxicity hypothesis: attractive but difficult to prove. Metabolism 124, 154870 https://doi.org/10.1016/j.metabol.2021.154870

7 Rege, N.K., Liu, M., Yang, Y., Dhayalan, B., Wickramasinghe, N.P., Chen, Y.S. et al. (2020) Evolution of insulin at the edge of foldability and its medical implications. Proc. Natl Acad. Sci. U.S.A. 117, 29618–29628 https://doi.org/10.1073/pnas.2010908117

8 Gastaldelli, A., Ferrannini, E., Miyazaki, Y., Matsuda, M. and DeFronzo, R.A. (2004) San Antonio metabolism s. Beta-cell dysfunction and glucose intolerance: results from the San Antonio metabolic (SAM) study. Diabetologia 47, 31–39 https://doi.org/10.1007/s00125-003-1263-9

9 Abdul-Ghani, M.A., Jenkinson, C.P., Richardson, D.K., Tripathy, D. and DeFronzo, R.A. (2006) Insulin secretion and action in subjects with impaired fasting glucose and impaired glucose tolerance: results from the Veterans Administration Genetic Epidemiology Study. Diabetes 55, 1430–1435 https://doi.org/10.2337/db05-1200

10 Weyer, C., Bogardus, C., Mott, D.M. and Pratley, R.E. (1999) The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. J. Clin. Invest. 104, 787–794 https://doi.org/10.1172/JCI7231

11 Del Prato, S. (2003) Loss of early insulin secretion leads to postprandial hyperglycaemia. Diabetologia 46, M2–M8 https://doi.org/10.1007/s00125-002-0930-6

12 Aguilar, R.B. (2011) Evaluating treatment algorithms for patients with type 2 diabetes mellitus: a perspective on the definition of treatment success. Clin. Ther. 33, 408–424 https://doi.org/10.1016/j.clinthera.2011.04.008

13 Godinho, R., Mega, C., Teixeira-de-Lemos, E., Carvalho, E., Teixeira, F., Fernandes, R. et al. (2015) The place of dipeptidyl peptidase-4 inhibitors in type 2 diabetes therapeutics: a “me too” or “the special one” antidiabetic class? J. Diabetes Res. 2015, 806979 https://doi.org/10.1155/2015/806979

14 Marin-Penalver, J.J., Martin-Timon, I., Sevillano-Collantes, C. and Del Canizo-Gomez, F.J. (2016) Update on the treatment of type 2 diabetes mellitus. World J. Diabetes 7, 354–359 https://doi.org/10.4239/wjd.v7.i7.354

15 Herrera, P.L. (2000) Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages. Development 127, 2317–2322 https://doi.org/10.1242/dev.127.11.2317

16 Teitelman, G., Alpert, S., Polak, J.M., Martinez, A. and Hanahan, D. (1993) Precursor cells of mouse endocrine pancreas coexpress insulin, glucagon and the neuronal proteins tyrosine hydroxylase and neuropeptide Y, but not pancreatic polypeptide. Development 118, 1031–1039 https://doi.org/10.1242/.dev.118.4.1031

17 Herrera, P.L., Huarte, J., Sanvito, F., Meda, P., Orci, L. and Vassalli, J.D. (1991) Embryogenesis of the murine endocrine pancreas; early expression of pancreatic polypeptide gene. Development 113, 1257–1265 https://doi.org/10.1242/develop.113.4.1257

18 Gu, G., Dubaukaite, 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 https://doi.org/10.1242/develop.129.10.2447

19 Gu, Y., Montminy, M.R., Stein, R., Leonard, J., Gamer, L.W., Wright, C.V. et al. (1995) Expression of murine STF-1, a putative insulin gene transcription factor, in beta cells of pancreas, duodenal epithelium and pancreatic exocrine and endocrine progenitors during ontogeny. Development 121, 11–18 https://doi.org/10.1242/develop.121.1.11

20 Ohlsson, H., Karlsson, K. and Edlund, T. (1993) IPF1, a homeodomain-containing transactivator of the insulin gene. EMBO J. 12, 4251–4259 https://doi.org/10.1002/j.1460-2075.1993.tb06109.x

21 Hani, E.H., Stoffers, D.A., Clarke, W.L. and Habener, J.F. (1997) Early-onset type-II diabetes mellitus (MODY4) linked to IPF1. J. Clin. Invest. 97, R41–R48 https://doi.org/10.1172/JCI7469

22 Stoffers, D.A., Ferrer, J., Clarke, W.L. and Habener, J.F. (1997) Early-onset type-II diabetes mellitus (MODY4) linked to IPF1. Nat. Genet. 17, 138–139 https://doi.org/10.1038/ng1097-138

23 Gannon, M., Ables, E.T., Crawford, L., Lowe, D., Offield, M.F., Magnussen, M.A. et al. (2008). pxd-1 function is specifically required in embryonic beta cells to generate appropriate numbers of endocrine cell endometrics and maintain glucose homeostasis. Dev. Biol. 314, 406–417 https://doi.org/10.1016/j.ydbio.2007.10.038

24 Ahlgren, U., Jonsson, J., Jonsson, L. and Simu, K. (1998) Edlound H. beta-cell-specific inactivation of the mouse lpf1/Pdx1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. Genes Dev. 12, 1763–1768 https://doi.org/10.1101/gad.12.12.1763

25 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. U.S.A. 97, 1607–1611 https://doi.org/10.1073/pnas.97.4.1607

26 Schwitzgebel, V.M., Scheel, D.W., Conners, J.R., Kalamaras, J., Lee, J.E., Anderson, D.J. et al. (2000) Expression of neurogenin3 reveals an islet cell precursor population in the pancreas. Development 127, 3533–3542 https://doi.org/10.1242/develop.127.16.3533

27 Jenny, M., Uhl, C., Roche, C., Duluc, I., Guillermin, V., Guillermont, F. et al. (2002) Neurogenin3 is differentially required for endocrine cell fate specification in the intestinal and gastric epithelium. EMBO J. 21, 6338–6347 https://doi.org/10.1093/emboj/cdf649

28 Sheets, T.P., Park, K.E., Park, C.H., Swift, S.M., Powell, A., Donovan, D.M. et al. (2018) Targeted mutation of Ngn3 gene disrupts pancreatic endocrine cell development in pigs. Sci. Rep. 8, 3582 https://doi.org/10.1038/s41598-018-22050-0

29 Rulkstalis, J.M. and Habener, J.F. (2009) Neurogenin3: a master regulator of pancreatic islet differentiation and regeneration. Islets 1, 177–184 https://doi.org/10.4161/is.8.3.38248

30 Vanhoose, A.M., Samaras, S., Artert, I., Henderson, E., Hang, Y. and Stein, R. (2008) MafA and MafB regulate Pdx1 transcription through the Area II control region in pancreatic beta cells. J. Biol. Chem. 283, 22612–22619 https://doi.org/10.1074/jbc.M802902200

31 Artert, I., Hang, Y., Mazur, M., Yamaamoto, T., Guo, M., Lindner, J. et al. (2010) MafA and MafB regulate genes critical to beta-cells in a unique temporal manner. Diabetes 59, 2530–2539 https://doi.org/10.2337/db10-0190

32 Hang, Y. and Stein, R. (2011) MafA and MafB activity in pancreatic beta cells. Trends Endocrinol. Metab. 22, 364–373 https://doi.org/10.1016/j.tem.2011.05.003

33 Nishimura, W., Bonner-Weir, S. and Sharma, A. (2009) Expression of MafA in pancreatic progenitors is detrimental for pancreatic development. Dev. Biol. 333, 108–120 https://doi.org/10.1016/j.ydbio.2009.06.029

34 Conrad, E., Dai, C., Spaeth, J., Guo, M., Cyphert, H.A., Scoville, D. et al. (2016) The MAFB transcription factor impacts islet alpha-cell function in rodents and represents a unique signature of primate islet beta-cells. Am. J. Physiol. Endocrinol. Metab. 310, E91–E102 https://doi.org/10.1152/ajpendo.00285.2015
Arntz, I., Bianchi, B., Raum, J.C., Guo, M., Kaneko, T., Cordes, S. et al. (2007) MafB is required for islet beta cell maturation. Proc. Natl Acad. Sci. U.S.A. 104, 3853–3858 https://doi.org/10.1073/pnas.0700013104

Rall, L.B., Pictet, R.L., Williams, R.H. and Rutter, W.J. (1973) Early differentiation of glucagon-producing cells in embryonic pancreas: a possible developmental role for glucagon. Proc. Natl Acad. Sci. U.S.A. 70, 3478–3482 https://doi.org/10.1073/pnas.70.12.3478

Murtaugh, L.C. (2007) Pancreas and beta-cell development: from the actual to the possible. Development 134, 427–438 https://doi.org/10.1242/dev.02770

Cazor-Castellano, I. and Stewart, A.F. (2005) Molecular engineering human hepatocytes into pancreatic beta cells for diabetes therapy. Proc. Natl Acad. Sci. U.S.A. 102, 7761–7762 https://doi.org/10.1073/pnas.0503261102

Hao, E., Tyberg, B., Itkin-Ansari, P., Lakey, J.R., Geron, I., Monsnov, E.Z. et al. (2006) Beta-cell differentiation from nonendocrine epithelial cells of the adult human pancreas. Nat. Med. 12, 310–316 https://doi.org/10.1038/nm1367

McKimpson, W.M. and Accili, D. (2019) Reprogramming cells to make insulin. Proc. Natl Acad. Sci. U.S.A. 116, 2274–2277 https://doi.org/10.1073/pnas.1910414116

Li, W., Nakamichi, M., Zurnsteig, A., Shear, M., Wright, C., Melton, D.A. et al. (2014) In vivo reprogramming of pancreatic acinar cells to three islet endocrine subtypes. eLife 3, e01846 https://doi.org/10.7554/eLife.01846

Zhao, Q., Brown, J., Kanarek, J., Rajagopal, J. and Melton, D.A. (2008) In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature 455, 627–632 https://doi.org/10.1038/nature07314

Sapir, T., Shemesh, M., Keidar-Levy, I., Blumenfeld, T., Cohen, H., Skutelsky, E. et al. (2005) Cell-replacement therapy for diabetes: generating functional insulin-producing tissue from adult human liver cells. Proc. Natl Acad. Sci. U.S.A. 102, 7964–7969 https://doi.org/10.1073/pnas.0405277102

Barker, N., van Es, J.H., Kujala, P., van den Born, M., Cozijnsen, M. et al. (2010) Lgr5(+ve) stem cells drive self-renewal in the stomach and generate the entire gut epithelium. Cell Stem Cell 7, 19–29 https://doi.org/10.1016/j.stem.2010.03.009

Quesada, I., Tuduri, E., Ripoll, C. and Nadal, A. (2008) Physiology of the pancreatic alpha-cell and glucagon secretion: role in glucose homeostasis and islet cell development. Cell Markers 18, 61–76 https://doi.org/10.1159/000105079

Wei, R., Gu, L., Yang, J., Yang, K., Liu, J., Le, Y. et al. (2019) Antagonistic glucagon receptor antibody promotes alpha-cell proliferation and increases the functional beta-cell mass in diabetic mice. Proc. Natl Acad. Sci. U.S.A. 116, 8713–8718 https://doi.org/10.1073/pnas.1904673116

Zhang, Q., Ramracheya, R., Lahmann, C., Tarasov, A., Bengtsson, M., Braha, O. et al. (2013) Role of KATP channels in glucose-regulated glucagon secretion. Cell Metab. 17, 326–339 https://doi.org/10.1016/j.cmet.2013.05.003

Sangaran, C.B., Jover, R., Heimberg, H. and Tosh, D. (2015) In vivo reprogramming of pancreatic alpha cells towards a beta cell phenotype following ectopic HNF-Alpha expression. Mol. Cell. Endocrinol. 399, 50–59 https://doi.org/10.1016/j.mce.2014.09.009

...
67 Zhang, Y., Fava, G.E., Wang, H., Mauvais-Jarvis, F., Fonseca, V.A. and Wu, H. (2016) PAX4 gene transfer induces alpha-to-beta cell phenotypic conversion and confers therapeutic benefits for diabetes treatment. *Mol. Ther.* **24**, 251–260. https://doi.org/10.1038/mt.2015.181

68 Chakravarthy, H., Gu, X., Enge, M., Dai, X., Wang, Y., Damond, N. et al. (2017) Converting adult pancreatic islet alpha cells into beta cells by targeting both Dnmt1 and Arx. *Cell Metab.* **25**, 622–634. https://doi.org/10.1016/j.cmet.2017.01.009

69 Li, J., Casteels, T., Frogne, T., Ingvarsen, C., Honore, C., Courtney, M. et al. (2017) Artemisinins target GABAA receptor signaling and impair alpha cell identity. *Cell* **168**, 86–100 e15. https://doi.org/10.1016/j.cell.2016.11.010

70 van der Meulen, T., Lee, S., Noordeloos, E., Donaldson, C.J., Adams, M.W., Noguchi, G.M. et al. (2018) Artemether does not turn alpha cells into beta cells. *Cell Metab.* **27**, 218–225 e4. https://doi.org/10.1016/j.cmet.2017.10.002

71 Ben-Othman, N., Vieira, A., Courtney, M., Record, F., Gjernes, E., Avolio, F. et al. (2017) Long-term GABA administration induces alpha cell-mediated beta-like cell neogenesis. *Cell* **168**, 73–85 e11. https://doi.org/10.1016/j.cell.2016.11.002

72 Lee, Y.S., Shin, S., Shigihara, T., Hahm, E., Liu, M.J., Han, J. et al. (2007) Glucagon-like peptide-1 gene therapy in obese diabetic mice results in long-term cure of diabetes by improving insulin sensitivity and reducing hepatic gluconeogenesis. *Diabetes* **56**, 1671–1679. https://doi.org/10.2337/db06-1182

73 Matsuoka, T.A., Kawashima, S., Miyatsu, T., Sasaki, S., Shimo, N., Katakami, N. et al. (2017) Mafa enables Pdx1 to effectively convert pancreatic islet progenitors and committed islet alpha-cells into beta-cells in vivo. *Diabetes* **66**, 1293–1300. https://doi.org/10.2337/db16-0887

74 Xiao, X., Guo, P., Shiota, C., Zhang, T., Coudriet, G.M., Fischbach, S. et al. (2018) Endogenous reprogramming of alpha cells into beta cells, induced by viral gene therapy, reverses autoimmune diabetes. *Cell Stem Cell* **22**, 78–90 e4. https://doi.org/10.1016/j.stem.2017.11.020

75 Furuyama, K., Chera, S., van Gurp, L., Oropeza, D., Ghila, L., Damond, N. et al. (2019) Diabetes relief in mice by glucose-sensing insulin-secreting human alpha-cells. *Nature* **567**, 43–48. https://doi.org/10.1038/s41586-019-0942-8

76 Korpos, E., Kadri, N., Kappelhoff, R., Wegner, J., Overall, C.M., Weber, E. et al. (2013) The peri-islet basement membrane, a barrier to infiltrating leukocytes in type 1 diabetes in mouse and human. *Diabetes* **62**, 531–542. https://doi.org/10.2337/db12-0432

77 Henry, R.R., Wallace, P. and Olefsky, J.M. (1986) Effects of weight loss on mechanisms of hyperglycemia in obese non-insulin-dependent diabetes mellitus. *Diabetes* **35**, 990–998. https://doi.org/10.2337/db35.9.990

78 DeFronzo, R.A. and Ferrannini, E. (1987) Regulation of hepatic glucose metabolism in humans. *Diabetes Metab. Rev.* **3**, 415–459. https://doi.org/10.1002/dmr.6000303024

79 Consoli, A., Nurjhan, N., Reilly, Jr, J.J., Bier, D.M. and Gerich, J.E. (1990) Mechanism of increased gluconeogenesis in noninsulin-dependent diabetes mellitus. Role of alterations in systemic, hepatic, and muscle lactate and alanine metabolism. *J. Clin. Invest.* **86**, 2038–2045. https://doi.org/10.1172/JCI114940

80 Kahn, S.E., Hull, R.L. and Utzschneider, K.M. (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* **444**, 840–846. https://doi.org/10.1038/nature05482