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Making β cells from Adult Cells within the Pancreas

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Abstract

Cell therapy is currently considered as a potential therapeutic alternative to traditional treatments of diabetes. Islet and whole pancreas transplantations provided the proof- of-concept of glucose homeostasis restoration after replenishment of the deficiency of β cells responsible for the disease. Current limitations of these procedures have led to the search for strategies targeting replication of pre-existing β cells or transdifferentiation of progenitors and adult cells. These investigations revealed an unexpected plasticity towards β cells of adult cells residing in pancreatic epithelium (e.g. acinar, duct and α cells). Here we discuss recent developments in β-cell replication and β-cell transdifferentiation of adult epithelial pancreatic cells, with an emphasis on techniques with a potential for clinical translation.

Keywords

diabetes; pancreas; replication; progenitors; transdifferentiation; β cells; adult cells

Introduction

Anomalies of glucose metabolism appear after quantitative or functional decline of the pancreatic β cells, leading eventually to the diagnosis of diabetes, whether type 1 (T1D), type 2 (T2D) or monogenic. The replacement of functional pancreatic β cells essentially cures diabetes as was demonstrated first with whole pancreas transplantation and then with islet transplantation; they are currently associated with insulin independence respectively in up to 30% and 44% of T1D patients 5 and 3 years after transplantation [1, 2]. However, the indications and availability of both techniques are hampered by scarcity of donors and graft failure within a few years. Pluripotent stem cells, namely embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have been regarded as alternative sources because of their theoretically unlimited supply and differentiation potential. Indeed, pancreatic progenitors derived from ESCs and iPSCs acquire glucose-responsive insulin secretion *in vivo* [3–5] and can normalize glycemia when transplanted into diabetic animals. However, the translational application of pluripotent stem cells through transplantation faces important

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Compliance with Ethics Guidelines

Conflict of Interest

Philippe A. Lysy, Gordon C. Weir, Susan Bonner-Weir declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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barriers with the risk of tumor formation *in vivo* and the need to be protected from immune attack.

In this context, pancreatic epithelial cells (duct, acinar, α and β cells) emerge as a potential alternative to pluripotent stem cells because of their demonstrated β-cell differentiation capacities and their likelihood of fewer safety concerns. Besides identifying candidate cell sources, cell therapy for diabetes requires further developments for protection of the new β cells from autoimmune destruction and/or rejection. While the complexities of immunoprotection have been described elsewhere [6], herein we discuss recent progress in exploiting the potential of the pancreas itself as a source of β cells for replacement therapy (Figure 1).

Why choose cells within the pancreas?

Regeneration and cell plasticity have both been demonstrated as occurring in rodent pancreas under specific conditions. As discussed below, new islet cells can arise from preexisting pancreatic cells of varied origin. Furthermore, the existence of facultative progenitors with *in vivo* or *ex vivo* β-cell engineering potential has been reported. Together these observations suggest the possibility that the formation of new β cells from cells residing within the adult pancreas has therapeutic potential. Having a reservoir of endocrine progenitor cells in the organ itself allows for either *in situ* or *ex vivo* expansion and transdifferentiation approaches to increase β-cell mass. Since pancreatic epithelial cells all arise from a common progenitor [7], they share similar epigenetic profiles [8, 9] that could facilitate their transdifferentiation towards β cells.

Pancreatic epithelial cells have a natural advantage over pluripotent stem cells due to the stability of their differentiation status after isolation or *in vitro* culture. Experience with transplantation of epithelial cells (hepatocytes [10], islets [11], corneal cells [12]) confirms this stability even after years of follow-up. In contrast, clinical translation of pluripotent stem cell-derived β-like cells awaits better definition of the differentiated products [13, 14] to avoid the transplantation of precursor cells with tumorigenic potential. For all expanded cells, attention must be paid to chromosomal abnormalities and epigenetic changes associated with risk of transformation that might occur after their expansion in culture as described with cells of mesenchymal origin [15].

What is a good candidate for β-cell engineering?

Even though the acquisition of *bona fide* β-cell functionality is the ultimate goal of β-cell engineering procedures, additional issues must be addressed before a cell source can be considered for cell therapy. These include the need to isolate the candidate cells in a reliable and minimally invasive manner to collect or expand the cells to produce a clinically relevant mass, to cryopreserve the cells in a cell bank for elective procedures, to maintain genetic stability of the expanded cells during *in vitro* expansion and after transplantation [16], and to prepare the cells in a good manufacturing practice-compliant facility. The need to have full β-cell functionality is perhaps the most stringent prerequisite but it might not be absolute. Indeed providing patients with diabetes with cells capable of insulin secretion, even without fine-tuned glucose regulation, might be helpful for lowering daily insulin requirements and improving glycemic control in some difficult to control individuals.

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What cell types are candidates?

A. β cells

Replication of endogenous β cells—The β cell has proven to be a major determinant of the regeneration potential of the pancreas in rodents after birth [17]. In humans, an important increase in β cell mass occurs by replication of preexisting β cells in the neonate but fades after 2 years of age [18, 19]. In the adult, β-cell replication in some studies appeared to be a rare event [19, 20] although Reers *et al.* systematically observed proliferative Ki67$^+$ β-cells in human donor pancreases [21] with the number of Ki67$^+$ β cells decreasing with age. In a recent study [22], we found 0.22±0.03% Ki67$^+$/insulin$^+$ cells in surgically resected pancreases from older humans, suggesting that Ki67 positivity may be artifactually low in autopsy and cadaver donor pancreas, meaning that β-cell turnover may be much higher and important than generally thought.

As seen in rodents, the human endocrine pancreas seems to respond with a compensatory increase in β-cell mass to conditions with higher metabolic demand and insulin resistance, such as obesity [23, 24] and pregnancy [25, 26]. In the latter, β-cell turnover is believed to result partly from replication, increased insulin secretion, and neogenesis [25, 26]. Activation of serotonin synthesis (through activity of tryptophan hydroxylase 1) is one of the pathways implicated in the changes in β-cell growth during pregnancy [27]. Obesity and pregnancy appear to be associated with similar molecular controls of β-cell replication, including upregulation of *FoxM1* expression [28, 29] and inhibition of *menin* [30], each playing opposite roles in cell cycle activity. Recently, decreased miR-338-3p levels were also identified as a β-cell expansion stimulus in both obese and pregnant rodents [31].

Thorough understanding of the mechanisms that govern the β-cell cycle in human pancreas is needed to identify a pathway that can be selectively targeted for therapeutic purposes [32, 33]. The feasibility of inducing human β-cell proliferation has been shown using virus-based over-expression of cyclin-dependent kinase (cdk)-4 and cyclin D1 *in vitro* [34], or cdk-6, with or without cyclin D1, *in vitro* and in grafts [35]. Although such strategies offer opportunities for *ex vivo* islet expansion, specific β cell targets are needed for *in vivo* interventions. Also, non-viral stimulating agents are sought for clinical application, as small molecules having the advantage of being inexpensive and readily available, making them suitable for high-throughput screening methods [36, 37]. Accordingly, recent screens identified new compounds promoting β-cell replication through interactions with the adenosine pathway [38, 39].

Besides cell cycle proteins, other metabolic pathway gatekeepers are currently considered as putative targets for molecular induction; among these are the enzymes glycogen synthase kinase-3 β (GSK-3β) and glucokinase (GCK). GSK-3β is a widely expressed and constitutively active serine/threonine kinase implicated in signaling pathways, such as the Wnt/β-catenin- and the phosphoinositide 3-kinase (PI3K)/Akt, both of which have important roles in β-cell function and proliferation [40, 41]. For example, GSK-3β activity indirectly induces proteasome degradation of β-catenin, leading to reduced β-cell replication [42], so its inhibition may have stimulating effects on β-cell mass. Since GSK-3β inhibitors such as paullones tend to interact with CDK1/cyclin B, more selective inhibitors are being developed, such as 9-cyano-1-aza-paullone or CHIR99021 that induced proliferation of primary rat β cells [43]. Since GSK-3β has such important roles in the entire organism, the development of β-cell specific GSK-3β stimulators for *in vivo* use will be challenging.

GCK is the β-cell glucose sensor for insulin secretion because it is the rate-limiting enzyme for glycolysis thereby regulating mitochondrial oxidation and ATP production. Increased GCK activity can stimulate islet proliferation, as shown by a child with a *GCK* (V91L)

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mutation coding for a variant with 8.5 fold higher than normal glucose affinity [44]. The child's pancreas had hyperplastic islets (larger and with more Ki67$^+$ cells) compared to age-matched controls. The role of GCK in the regulation of β-cell mass has been further studied in mice, in which the loss of one *GCK* allele led to the loss of a compensatory increase in β-cell replication in response to a high-fat diet [45]; the proliferation could be restored with a glucokinase activator (GKA) [46]. Furthermore, this GKA augmented β-cell proliferation in wild-type mice, an effect that was blunted in Irs2$^{-/-}$ mice and by oxidative stress [47]. GKAs have a broader impact on glucose metabolism not only by stimulating insulin secretion but also by enhancing hepatic glucose uptake [48]. Some GKAs, including LY2599506, are being evaluated in clinical trials for their therapeutic potential in diabetes [49].

Glucagon-like peptide (GLP)-1 receptor agonists and DPPIV inhibitors that limit GLP-1 degradation are already widely used in T2D patients for their beneficial effects on glucose metabolism [50]. In rodents, use of these agonists, e.g. exendin-4 and liraglutide, resulted in increased β-cell mass [51, 52] leading to hopes for similar beneficial effects on β-cell mass in humans. However, the potential effect of exendin-4 as a proliferation stimulus of transplanted human islets has been observed only with young donors [53] and was not confirmed in our series [22]. As of yet, no lasting effect after drug washout suggesting significant restitution of β-cell mass has been reported for this class of drugs.

Studies on β-cell fate during development of diabetes may provide new clues for β cell mass restoration. Recently, Talchai *et al.* described β-cell dedifferentiation in diabetic mouse models (*db/db* and insulin-resistant GIRKO mice) and proposed a central role of this mechanism in the β-cell failure observed in diabetes [54]. These authors observed an increased proportion of 'empty' chromogranin A$^+$/hormone$^-$ cells in hyperglycemic mice and suggested that re-differentiation of these empty endocrine cells might be as useful as replication of pre-existing β cells for increasing the β-cell mass in type 2 diabetes. Previously impressive changes in expression of genes key to the β-cell phenotype (e.g. transcription factors, glucose metabolism genes, stress genes) have been shown to result from exposure to chronic modest hyperglycemia and correlated with β-cell dysfunction [55, 56], but it is unclear whether any of these β cells reverted to an endocrine progenitor as recently suggested [54]. Moreover, it remains to be seen if such dedifferentiated cells can be found in islets of individuals with T2D.

In T1D, *in situ* replication of β cells would require immunomodulation to limit destruction of newly formed β cells. However, even with ongoing autoimmunity, the possibility to maintain the β cell mass to a minimum level could help stabilize the disease, as seen during the partial remission phase. Subjects with T2D and monogenic diabetes, not having a threat from autoimmunity, should represent better candidates for β-cell replication strategies. One concern is that the metabolic syndrome in T2D would be expected to make the β cells work harder, but failure from such stress should take years to cause problems.

Ex vivo expansion of β cells—Under specific *in vitro* culture conditions, β cells appear to revert to an undifferentiated mesenchymal-like phenotype. The team of Efrat has exploited this propensity to expand cells from the human β-cell lineage [57, 58]. The epithelial-mesenchymal transition (EMT) cells thought to be of β-cell origin showed high proliferation capacity (up to 16 population doublings) and could be re-differentiated by inhibition of notch signaling, aggregation in serum-free media with a cocktail of nicotinamide, exendin-4, activin A, N2 and B27 supplements [59, 60]. Using lineage marking, previously dedifferentiated β cells were shown to be responsible for the re-differentiation capacity, which was attributed to their epigenetic memory [8]. The authors reported a re-differentiation yield of up to 25% of all EMT cells, which exceeds the yields

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described with ESCs [3, 4] and represents an 8–32 fold increase in the number of insulin$^+$ cells starting from the uncultured islets. Preliminary studies on transplantation of these re-differentiated EMT cells showed an *in vivo* functional ability to partially reduce the blood glucose of streptozotocin-diabetic mice.

B. Duct cells

During pancreas development, the ductal epithelium gives rise to all pancreatic epithelial lineages, i.e. duct, acinar and endocrine cells. As shown in rodent studies particularly with lineage-tracing techniques, new islet cells can develop from progenitors through a process called neogenesis in postnatal life and in models of pancreas injury [61]. The existence of facultative β-cell progenitors in the duct epithelium was elegantly shown by Xu *et al.* [62], with the induction of Ngn3$^+$cytokeratin$^+$ cells as soon as 3 days after partial duct ligation (PDL) in mice. Seven days after PDL in *Ngn3-nlacZ* reporter mice, cells co-stained with the lineage marker β-gal$^+$ and islet hormones, indicating endocrine differentiation of the Ngn3$^+$ progenitors. Then using *Ngn3-eGFP* mice and FACS-sorted eGFP$^+$ cells 7 days after PDL, the eGFP$^+$ non-granulated cells or Ngn3$^+$ progenitors, which represented only 0.04% of the total number of sorted pancreatic cells, had an expression profile of pancreatic progenitors and were shown to differentiate into glucose-responsive β-like cells when cultured with *Ngn3^{null}* embryonic pancreas explants.

The capacity of duct cells to give rise to new β cells in the adult mouse has become controversial with contradictory results even when similar Cre-loxP lineage tracing strategies were employed [63–69]. While several studies [63–65] found no endocrine cells marked for duct lineage after birth or after injury, other studies did [66–68]. Lineage tracing is considered the “gold-standard” for tracing progeny, but there are caveats as with any technique, including cell heterogeneity within the ductal tree [70], distinct reprogramming potential of subpopulations, perdurance of tamoxifen [71], or a low efficiency of some tracing strategies. The discrepancy of findings from two well-done studies using Sox9 to drive inducible Cre illustrate this point: in one [63] Kopp *et al.* found a few non-β-endocrine cells derived from the Sox9$^+$ ducts in early postnatal life but no labeled acinar cells, in the other Furuyama *et al.* observed plentiful labeling of acinar cells after the pulse-chase was initiated during postnatal period [64] and only 1% labeled pancreatic endocrine cells when tracing was initiated on the first postnatal day. Both studies traced the fate of Sox9$^+$ populations, stressing that accuracy and interpretability of current lineage tracing approaches are not without issues.

In the human pancreas, it is much more difficult to assess neogenesis but using the definition of neogenesis as hormone-positive cells “budding” from ducts, the frequency of insulin positive cells within the duct has been reported as about 0.5% insulin$^+$ duct cell in healthy patients [18, 21] and tends to increase in situations of high metabolic demand, as obesity [72] and pregnancy [26]. However, there is considerable uncertainty about how this natural propensity for duct cells to transdifferentiate into β cells might be exploited *in vivo* for clinical purposes.

Ex vivo, unequivocal demonstration of the β-cell differentiation potential of human duct cells has been made with purified CA19-9$^+$ populations [73]. The *ex vivo* exploitation of duct cells has several advantages over other pancreatic epithelial cells: duct cells are relatively more resistant to handling and shipping, the cells can be reliably purified based on cell surface markers, and they attach in culture, facilitating the possibility of 2D differentiation and enhancement of proliferation signals. However, as with many epithelial cell types, duct cells lack the capacity for sustained proliferation and tend to lose their phenotype *in vitro* [74, 75]. We developed a system to induce proliferation of purified CA19-9$^+$ duct cells by forcing their natural tendency to dedifferentiate [76]. Using specific

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culture conditions, we obtained highly proliferative cultures that showed typical features of EMT. These undifferentiated EMT cells were found to respond to *in vitro* β-cell differentiation strategies by acquiring β-cell specific characteristics in gene expression and human insulin secretion, even though glucose-stimulated insulin secretion (GSIS) was lacking. Whereas further work is needed to provide a full picture of the capacity of these duct-derived cells for β-cell engineering, our work suggests that these expanded human duct cells can be differentiated into cells with a β cell-like phenotype.

Another aspect of research on duct cell differentiation is their potential to give rise to β-cell lineage. In their work on Pax4-induced α-to-β transdifferentiation, Collombat *et al.* showed the capacity of duct cells to reprogram to α cells through the activation of Ngn3 expression [77]. Pax4-induced α-to-β conversion led to a glucagon deficiency that triggered neogenesis of α cells from the duct epithelium. Lentiviral-based knockdown strategies confirmed the central role of Ngn3 in this duct-to-α cell neogenesis.

C. Acinar cells

Being by far the major population of cells in the pancreas, acinar cells must be examined for their capacity to generate new β cells. Acinar-to-β cell differentiation was suggested in rodents with *in vitro* differentiation assays [78] and staining studies showing cells expressing both amylase and insulin in rat pancreas after PDL [79], and in zebrafish with lineage tracing studies [80]. This potential was however challenged by a report showing no acinar-to-β cell transdifferentiation after 70% pancreatectomy, PDL, or caerulein-induced pancreatitis in mice [81]. However, with *in vitro* lineage tracing, cultured human acinar cells were shown to dedifferentiate or transdifferentiate to a ductal phenotype within a week [82], thus presenting the possibility that they could be induced to differentiate to endocrine cells.

Zhou *et al.* [83] showed the possibility to reprogram exocrine cells to β cells *in vivo* by adenoviral-mediated over-expression of key β-cell transcription factors (TFs) Pdx1, Ngn3, and MafA. Adenoviruses (Ad) were chosen as vectors due to their supposedly preferential infection of exocrine cells as compared to islet cells [84]. By 3 days after a single intrapancreatic injection of the 3 Ad-TF constructs co-expressing the reporter green fluorescent protein (GFP) in *Rag1*$^{-/-}$ non-diabetic animals, insulin-positive GFP-positive cells were found; by 10 days the reprogrammed cells had many of the characteristics of *bona fide* β cells in their expression profile and immunostaining of β-cell proteins. In streptozotocin (STZ)-treated animals, these newly formed β cells led to a significant decrease of hyperglycemia, which did not reach true normoglycemia. It still remains to be shown that these have glucose-stimulated insulin secretion and are fully reprogrammed. Others have shown the feasibility of reprogramming cells using TFs without viruses using protein transduction [85] or modified RNA transfection [86], albeit at low efficiency and by means of technically demanding protocols. With these possibilities, acinar cells remain an attractive source for new β cell formation.

Our understanding of the network of TFs governing pancreas development is growing [7], with new players being regularly identified [87]. However, there are theoretically only a few combinations of TFs exploitable for β cell engineering, as elegantly shown by Zhou *et al* in 2007 [88]. They identified using a genome-wide expression study of >1100 TFs in the developing pancreas, only 30 TFs being localized in pancreatic and endocrine progenitors by *in situ* hybridization. Even though TF-based reprogramming is intuitively powerful and specific, difficulties may arise in terms of accessibility of binding sites, influence of context-specific co-factors and presence of epigenetic remodeling complexes [89]. As for clinical translation perspectives, the cost/effectiveness of TF-based reprogramming will need to be weighed against small molecule- or growth factor-based protocols.

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D. α cells

The concept of plasticity of mature islet cells, particularly **a** cells, has led to excitement about the therapeutic potential of converting α cells to β cells. Using genetic manipulation in mice, Collombat *et al.* first showed successful conversion of adult mouse β cells into α- and PP-derivatives by misexpression of *Arx* coding for a transcription factor that specifies α cell fate [90]. Then, they showed that *Pax4* over-expression in the embryonic $Pdx1^+$ pancreatic progenitor cells or in mature $glucagon^+$ endocrine cells were sufficient for their specification into functional β cells capable of reversing STZ-induced diabetes [77]. Herrera's group confirmed these results showing that when almost all (> 99%) β cells were destroyed by selective expression of diphtheria toxin receptor downstream of the rat insulin promoter, α cells spontaneously reprogrammed into β cells [91]. In this study, the combined ablation of β and α cells prevented the reprogramming. Differential α-to-β reprogramming between *Pdx1*-enforced expression in $Ngn3^+$ endocrine progenitors and in mature hormone-expressing cells suggests a context-dependent receptiveness that may reflect prior epigenetic markings [92].

It should be pointed out, however, that while the proportion of β cells marked as having come from α cells was significant, the actual number of these cells as a function of normal β-cell mass was very low because there were so few β cells left after the killing by diphtheria toxin. It is also noteworthy that there are as yet no convincing studies showing that such conversion occurs after β-cell killing with either STZ or alloxan.

In human pancreas, similar plasticity has not yet been demonstrated even though $insulin^+glucagon^+$ cells have been reported in fibrotic pancreases [93]. For therapeutic purposes, α cells may represent a putative reservoir of new β cells *in vivo* or may require *ex vivo* expansion for cell therapy. *In vivo* strategies might benefit from the natural microenvironment of α cells as islet cells already networking with each other and with other endocrine cells. *In vitro* derivation of α cells has been reported from human ESCs [94] and the feasibility to implement α-to-β reprogramming *in vitro* has been shown with a murine α-cell line that was driven towards insulin-producing cells by the small molecule BRD-7389 [95]. At this point there are only a few studies showing apparent conversion of α cells to small numbers of β cells raising concerns that this reprogramming may not prove to be a fruitful path to generating a useful number of β cells. Of note, a recent study in non-human primates found neither α-cell replication nor β-cell regeneration after STZ-provoked β-cell ablation [96].

E. Progenitors characterized *in vitro*

In the quest for pancreatic stem cells/progenitors, intracellular expression of aldehyde dehydrogenase (ALDH) has emerged as a new area of investigation after the demonstration that human bone marrow $ALDH^+$ populations are enriched in hematopoietic progenitors [97]. The association between ALDH expression and stemness is thought to result from the capacity to detoxify potentially cytotoxic metabolic products [98]. Rovira *et al.* studied the staining pattern of ALDH1 protein in adult mouse pancreas and found its expression in centroacinar cells, terminal duct epithelial cells and mesenchymal cells surrounding endocrine islets and exocrine acini [99]. They further studied the centroacinar cells, an elusive cell in the pancreas that actively proliferates after pancreas injury [100]. Centroacinar cells are small cells (10 μm in diameter) within the pancreatic epithelium residing at the junction of the terminal duct and the acinus. The function of these cells and whether they represent a unique phenotype or reflect several cell types (*e.g.* terminal duct cells) residing in the centroacinar position, is largely unknown. Using a fluorogenic ALDH1 substrate to sort the central acinar/terminal duct cells from exocrine tissue depleted from islets and large ducts, the authors observed that the $ALDH1^+$ population accounting for

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0.5% of the FACS-sorted mouse pancreatic cells was enriched for progenitor markers (Sca1, Sdf1, c-Met), and expanded to form spheres *in vitro*, either in suspension or after plating in clonal dilution. Within 5 to 7 days, 30% of the pancreatospheres expressed C-peptide; after injection into cultured embryonic dorsal pancreatic buds, $ALDH1^+$ cells contributed to both pancreatic exocrine and endocrine lineages. Furthermore, Ioannou *et al.* identified in the adult mouse rare $ALDH1B1^+$peanut $agglutinin^+$ cells identified as centroacinar cells and able to proliferate extensively and rapidly after regeneration stimuli (caerulein and STZ treatments) [101]. Both studies suggest an important role for centroacinar cells in pancreas regeneration, and further work is required to confirm this potential in humans.

In addition, the adult mouse has been shown to contain a progenitor population that can be obtained from islet or duct tissues *in vitro* using single-cell clonal culture [102]. In serum-free media, these pancreas-derived multipotent precursors (PMPs) formed colonies expressing markers of both pancreatic and neural lineages. Upon culture with Matrigel, PMPs developed into β-like cells. More recently [103], the authors identified the pancreas and not the neural crest as the source of PMPs, using *Wnt1-Cre;Z/EG* and *PDX-1-Cre;Z/EG* mice, respectively marking progeny of neural and pancreatic origin. Using *MIP-GFP* mice, they showed mouse PMPs were predominantly $insulin^+Glut2^{low}$. Similarly human PMPs were derived with an efficiency of about 2.6 generated spheres/10,000 isolated cells and expressed neural and endocrine progenitor markers. After Matrigel-induced differentiation, human PMPs contained 11.6% $insulin^+/PDX\text{-}1^+$ β-like cells, and showed modest (2 fold) higher insulin release to high glucose than low glucose. After transplantation under the kidney capsule of STZ-treated diabetic NOD-SCID mice, human PMPs lowered the hyperglycemia somewhat. The composition of grafts was not described, so it is unclear the fate of the predominant (≈ 88.4%) non-β cells present before transplantation. To facilitate prospective isolation of such progenitors, efforts are being made to identify cell surface markers for the various stages of β cell progenitors [104], much as is being done with different stages of embryonic stem cell development [13, 14].

Although these cell clusters could teach us something important about the development of islet cells, much more work must be done to show that these cells have therapeutic potential.

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Conclusion

In coming years, the pancreas may serve as a source of cells for β-cell replacement therapy. Major hurdles remain for targeting cells *in vivo* for inducing proliferation and/or transdifferentiation and for isolating and expanding cells with tropism towards the β-cell lineage. However, new technologies (e.g. protein or RNA transduction, small molecule screening, EMT-based cell expansion) offer a broad spectrum of applications, both *in vitro* and *in vivo* that may facilitate the efforts. As we learn more of the intricate molecular mechanisms that control β-cell fate and the factors that are responsible for cellular plasticity, we will have new clues for β-cell engineering.

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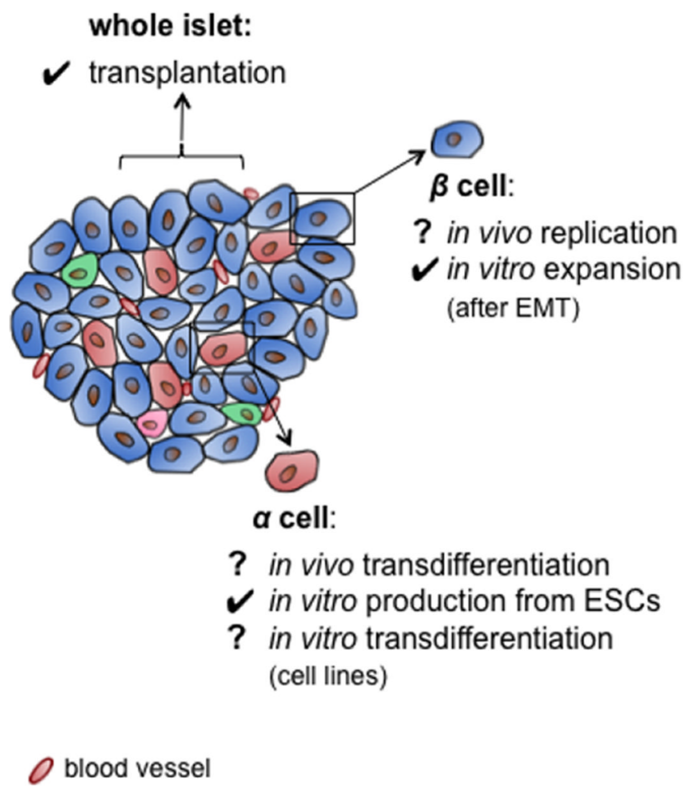

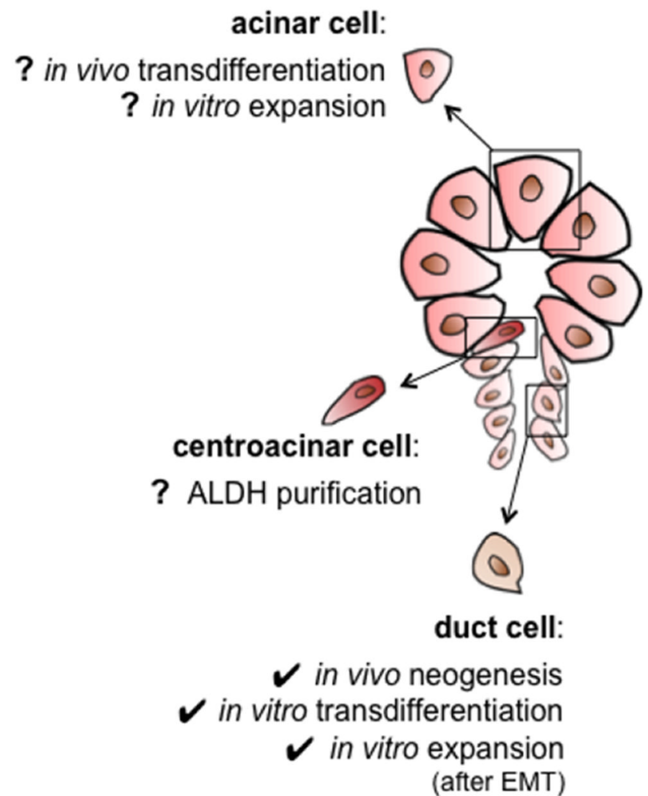

Figure 1.
Potential cell sources in the human pancreas for diabetes cell therapy. *Check marks* indicate procedures or phenomena (*i.e*. neogenesis) that were validated or observed with human cells or tissues. *Question marks* refer to experiments achieved in rodents and that await translation to human.