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Pancreas regeneration

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Abstract

The pancreas is made from two distinct components: the exocrine pancreas, a reservoir of digestive enzymes, and the endocrine islets, the source of the vital metabolic hormone insulin. Human islets possess limited regenerative ability; loss of islet β-cells in diseases such as type 1 diabetes requires therapeutic intervention. The leading strategy for restoration of β-cell mass is through the generation and transplantation of new β-cells derived from human pluripotent stem cells. Other approaches include stimulating endogenous β-cell proliferation, reprogramming non-β-cells to β-like cells, and harvesting islets from genetically engineered animals. Together these approaches form a rich pipeline of therapeutic development for pancreatic regeneration.

The pancreas is central to the control of energy consumption and metabolism and is composed of two morphologically and functionally distinct components: the exocrine pancreas (acinar cells and ductal cells) and the endocrine pancreas (islets of Langerhans).Exocrine acinar cells produce an array of digestive enzymes, including lipases, proteinases, and amylases, which are secreted into pancreatic ducts and flow into the small intestine to break down fats, proteins, and carbohydrates for absorption. The endocrine islets represent less than 5% of total pancreatic mass but nevertheless number more than a billion cells in humans. Each of the five major types of islet cell synthesizes and secretes a principle hormone: insulin (β-cells), glucagon (α-cells), somatostatin (δ-cells), pancreatic polypeptide (PP cells), and ghrelin (ε-cells). Insulin and glucagon are released directly into the blood circulation through a dense intra-islet vascular network and have essential roles in the regulation of blood glucose levels

Distinct diseases afflict the exocrine and endocrine pancreas. Pancreatitis and pancreatic cancers, the majority of which are ductal carcinomas, originate from the exocrine pancreas whereas diabetes and rare pancreatic neuroendocrine tumours arise from the endocrine islets. Diabetes has been estimated to afflict well over 300 million people worldwide and is a major and growing health problem in the modern world. Complications resulting from long-

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term diabetes include kidney failure, peripheral vascular disease, stroke, and coronary artery disease; together, these complications create enormous medical and social burdens as well as causing premature deaths. The majority of diabetic patients suffer from type 2 diabetes (T2D), a disease attributed to insulin resistance by peripheral organs including liver, fat, and muscle. Recent genetic linkage studies and histological analyses have shown that patients with T2D also have significantly fewer islet β-cells than healthy individuals[1–4]. Type 1 diabetes (T1D), which makes up about 5–10% of all diabetes cases, is an autoimmune disease in which β-cells are selectively destroyed, leading to a severe insulin deficiency that must be treated with daily insulin injections for survival. Together, these diseases account for a large and growing patient population with pancreatic β-cell deficiency.

There is a long history of investigations into pancreatic regeneration, going back nearly a century[5]. The epidemic of diabetes in recent decades has spurred numerous studies on pancreas development, homeostasis, and regeneration. Animal studies have suggested that the exocrine pancreas possesses an intrinsic capacity for regeneration and thus can make a rapid and full recovery from exocrine diseases such as acute pancreatitis. By contrast, the endocrine islets have limited regenerative capacity in adults. Indeed, it remains unclear whether the adult human pancreas can spontaneously regenerate β-cells in any physiologically meaningful way. Substantial β-cell loss therefore results in permanent endocrine deficiency and irreversible diabetes. There is an increasing consensus that a regenerative medicine approach will be helpful, even essential, in treating certain forms of diabetes including T1D and possibly the subset of T2D in which there is substantial β-cell loss. Learning how to enhance or induce the intrinsic regenerative ability of endocrine islets and devising new strate-gies to produce insulin-secreting β-cells will have profound implications for developing therapeutic treatment for diabetes. Here we summarize our current understanding of pancreatic endocrine and exocrine regeneration and review the different strategies for therapeutic regeneration and repair.

Regeneration of the endocrine pancreas

The majority of studies on pancreas regeneration have focused on endocrine islets, owing to their central importance in diabetes. Historically, studies of islet regeneration relied on rodent injury models, including pancreatectomy, pancreatic duct ligation, and chemical ablation of islet cells. In pancreatectomy, removal of up to 90% of the rat pancreas does not affect glucose homeostasis, suggesting a large reserve capacity, as 10% of the islet mass is sufficient to maintain blood glucose control[6–8]. By contrast, resection of 50–60% of the pancreas in humans triggers insulin-dependent diabetes[9,10]. Young rodents show tissue growth and sprouting from the cut surface after pancreatectomy[6,7]. Observations of rare samples from children also suggest tissue growth after pancreatectomy[11]. The capacity for this type of regeneration, however, declines sharply in adult animals and is absent in adult humans[8,10,12].

A second injury model used to study pancreas regeneration is duct ligation which mimics obstructive pancreatitis. Physical ligation of the pancreatic ducts causes widespread acinar cell death, but the endocrine islets are spared and no substantial endocrine regeneration is observed[13,14]. In a third injury model, pancreatic β-cells can be specifically ablated using

streptozotocin (STZ) or alloxan, chemical toxins that structurally mimic glucose and are selectively imported into β-cells. Depending on drug dosage, the entire β-cell mass can be partially or nearly completely ablated in a few days. Extensive studies have found no convincing evidence for β-cell regeneration in adult animals following chemical ablation[12,15].

Despite the lack of substantial islet regeneration in injury models, islet hyperplasia is observed during pregnancy, in obesity, or under insulin resistance conditions in animal models[16–19]. For instance, mouse pancreatic β-cell mass increases by 3–5-fold during pregnancy, stimulated at least partly by the pregnancy hormones placental lactogen and prolactin, and involving signalling through serotonin, Menin, and FoxM1[20–23]. High-fat diet-induced obesity in mice is also accompanied by impressive increases in islet cell mass[24] Experimentally induced insulin resistance, such as liver-specific knockout of insulin receptors, induces up to a tenfold increase in β-cell mass[25]. The molecular pathways that drive these increases in β-cell mass in obesity and insulin resistance have yet to be fully elucidated.

Self-replication maintains β-cell mass

The proliferative rate of β-cells is quite high in young rodents, but declines rapidly with age[26,27]. For example, one study estimated a proliferation rate of approximately 4% per day in one-month-old rats and 0.5% per day in seven-month-old rats[28]. In addition, marked islet hyperplasia can be induced in adult animals by pregnancy or obesity. What is the source of these additional islet cells? In a milestone study, genetic lineage tracing in mice using β-cell-specific drivers showed that the major mechanism for β-cell replenishment in homeostasis or after injury was replication of pre-existing β-cells[29] (Fig. 1). The role of replication is much less clear in humans, as very few replicating human β-cells (assessed by histological staining of proliferative antigens such as Ki-67 and PCNA) can be found in pancreas samples taken during autopsy of healthy, injured, pregnant, or obese adult humans[3,10,30,31].

α-cells and δ-cells may convert to β-cells

The five principle cell type of the islets, namely β-, α-, δ -, PP, and ε-cells, appear to be highly stable in normal homeostasis or in various injury models. For instance, selective ablation of β-cells with STZ or alloxan does not significantly affect the numbers or phenotypes of other islet cells. It came as a surprise that, when a diphtheria toxin-based β-cell ablation method was used to ablate more than 99% of β-cells in mice, slow but significant recovery of β-cell mass over several months was reported[32]. Lineage tracing studies suggested that the new insulin-producing cells arose from conversion of pancreatic α- or δ-cells, depending on the age of the mice[32,33]. The molecular mechanism of this conversion between islet cell types is unknown, as is whether such conversions also occur in humans. There is no clear evidence for this type of conversion in patients with T1D, but that could be either because it does not occur or because the converted cells are eliminated by the ongoing autoimmune process. Nevertheless, these studies suggest another mechanism that can potentially regenerate part of the endocrine compartment (Fig. 1).

The search for adult pancreatic stem cells

There is a long-standing hypothesis that pancreatic stem or progenitor cells might exist in the adult animal or even human pancreas[34]. This hypothesis was initially based on histological observations of single islet cells and small islets embedded in or closely associated with adult rodent and human pancreatic ducts, suggesting the emergence of new islet cells from ducts (referred to as neogenesis)[34]. However, genetic lineage-tracing studies using exocrine drivers (*Muc1*-CreER), acinar-specific drivers (*Cela*-CreER, *Ptf1a*-CreER), and duct-specific drivers (*Sox9*-CreER, *Hnf1b*-CreER)[35–39] consistently indicated rare or no contribution from the exocrine to the endocrine compartment during normal homeostasis or in various injury models. The neogenesis hypothesis has been supported by a report that, after pancreatic duct ligation in mice, a rare population of $NGN3^+$ endocrine precursor cells appeared in ductal structures[40] and observations of $NGN3^+$ cells around islets and ducts in experimental models of α-cell to β-cell transdifferentiation[41,42]. In summary, it remains unclear whether adult pancreatic stem cells exist.

Regeneration of the exocrine pancreas

The exocrine pancreas is composed of acinar cells and duct cells. The most common injury to the exocrine pancreas is pancreatitis, a painful inflammation triggered by various environmental (injury, alcohol, high fat diet and so on) or genetic (for example, cystic fibrosis) factors[43]. Understanding of exocrine damage and regeneration comes largely from rodent studies of experimental pancreatitis, the most common of which is supraphysiological stimulation of acinar secretion by caerulein, a mouse analogue of the hormone cholecystokinin[44,45]. Caerulein treatment leads to rapid apoptosis or necrosis of acinar cells and in addition, some acinar cells lose their abundant zymogen granules and shrink considerably to resemble duct cells in a process termed acinar-to-ductal metaplasia[46]. The animals recover from acute pancreatitis rapidly. Within a few weeks, the exocrine pancreas fully regains its normal cellular architecture and function. Examination of human exocrine tissues from patients with pancreatitis also shows ductal metaplasia and cell proliferation[47,48]. Although patients with acute pancreatitis can make a full recovery, it is unclear whether their exocrine pancreas undergoes similar spontaneous repair and regeneration to that seen in animal models.

Two distinct modes of regeneration have been proposed to occur in models of pancreatitis[49] (Fig. 2). In the classical regeneration mode, new acinar cells are produced from proliferation of pre-existing acinar cells[35,50,51]. In the second regeneration mode, the degranulated and duct-like acinar cells are believed to ‘redifferentiate’ and revert back to a normal and functional acinar state. The dedifferentiated acinar cells have not been tracked with a lineage marker and their redifferentiation has been inferred by indirect means. Mechanistic studies in animal models have identified several genes and pathways required for the exocrine regenerative response in pancreatitis. Deletion of key components of the Hedgehog, Notch, and Wnt pathways from acinar cells severely disrupts exocrine regeneration[52–54], as does deletion of the acinar-restricted transcription factors NR5A2 and PTF1A[55,56]. The dedifferentiated state of acinar cells appears to represent a vulnerability in which environmental and genetic factors could conspire to induce neoplastic transformation

towards the deadly pancreatic cancers[57,58]. The mechanisms that control regeneration versus neoplastic transformation are not yet understood.

Strategies to produce new endocrine islet cells

Whereas adult mouse pancreatic islets show robust regeneration under physiological challenges such as obesity, insulin resistance, or pregnancy, it is uncertain whether the adult human pancreas can deploy an adaptive regenerative response and, even if it does, these responses are evidently not able to make a significant physiological impact. At the same time, the clinical need for β-cell regeneration therapy is enormous. Approximately 2.5 million people in the USA (and more than 20 million worldwide) suffer from T1D and many millions more patients with T2D have pancreatic β-cell deficiency. Both patient populations could benefit from therapies that restore functional β-cell mass, freeing them from daily insulin injections and avoiding the serious complications that develop from imprecise dosing. The need for β-cell regeneration in patients with T1D is particularly pressing as this disease preferentially affects children and the severe lack of β-cells in T1D can cause life-threatening fluctuations in blood glucose[59,60].

Decades of clinical studies have established that cadaveric islet transplantation can be beneficial in patients with T1D, with some patients remaining free from insulin use for years[61,62]. Nevertheless, clinical cadaveric islet transplantation is used only on a small scale owing to the lack of suitable cadaveric islets and the requirement for long-term immune suppression to combat auto- and alloimmunity. To treat larger populations of patients, it would be beneficial to have a reliable and standardized source of human islets for transplantation, ideally without the need for immunosuppression. Alternatively, therapeutic interventions that stimulate endogenous islet regeneration could be used. In response to the enormous unmet medical need, several research efforts are now underway to evaluate strategies to produce new islets in vitro or stimulate islet regeneration in vivo (Fig. 3).

Differentiation of pluripotent stem cells

Decades of developmental studies in frogs, fish, and mice have mapped out the key steps and critical signalling events that lead from a fertilized egg to the formation of mature islets in early childhood[63–65]. This deep understanding of pancreatic development was put to the service of regenerative medicine in 1998, when human embryonic stem cells (hES cells) were successfully cultured and opened the door to developing methods of deriving pancreatic islets from hES cells[66]. This advance was followed in 2006 by the groundbreaking discovery that induced pluripotent stem cells (iPS cells) can be derived from somatic cells such as skin fibroblasts, providing a pathway for generating patient-specific cell products[67].

In the first major studies of deriving pancreatic endocrine cells from hES cells, a step-wise protocol was devised using combinations of signalling molecules to guide hES cell differentiation through four successive stages (definitive endoderm, pancreatic epithelium, endocrine progenitors, and β-like cells)[68,69]. The first differentiations of human stem cells into islet cells produced a population of cells with mixed hormone expression, but not mature or true human β-cells[68]. These studies, together with the decades of cellular and

genetic studies of pancreatic development in animal models, created a blueprint for in vitro differentiation protocols that have been applied to pluripotent stem cells.

More recently, efforts have been directed towards the production of endocrine islets that can respond to glucose. More complicated differentiation protocols have been devised, with additional steps, optimized cocktails of inducing factors and chemicals, and the use of three-dimensional culture methods, which yield cellular clusters with remarkable morphological and functional resemblance to pancreatic islets[70,71]. Transplantation of these in vitro-derived cell clusters led to further functional maturation in vivo and robust rescue of experimental diabetes in mouse models[70,71].

In addition to endocrine islets, pancreatic progenitor cells, some of which have the capacity to produce mature hormone-producing cells, have emerged as a candidate cell therapy product[69]. Preclinical studies showed that when hES cell-derived $PDX1^+$ progenitors are transplanted into mice, some of these cells undergo growth and differentiation in vivo into functional β-cells that can reverse diabetes[69,72]. These advances have led to phase I and phase II clinical trials of pancreatic progenitors. More fully differentiated functional human islet clusters are set to enter trials in a few years. For both approaches, the in vitro-derived islet clusters contain both β-cells and other islet cell types (α-cells and δ-cells) that are known to fine-tune the function of β-cells. There are additional cell types present in native islets, including vascular cells and fibroblast-like cells, and incorporating these additional cells into clusters for transplantation may offer some benefit.

Despite marked advances in producing pancreatic endocrine cells from hES cells, important challenges remain. These include perfecting the differentiation protocols for manufacturing at a large scale, eliminating unwanted cells from the final product and, of course, providing protection against immune rejection. Both allo- and autoimmune rejection can in principle be avoided by physical protection in a small device: for example, encapsulation in alginate or a more durable biomaterial. Alternatively, it may be possible to reduce the immune attack by genetic modification of the transplanted cells and/or manipulating the immune system of the recipient.

Patient-specific cell products can be derived from iPS cells, which should avoid immune rejection and be immunologically compatible with the patient from whom the iPS cell was derived. This is, of course, primarily relevant for replacing β-cells in patients with T2D, as patients with T1D suffer from autoimmunity. Manufacturing patient-specific products, however, presents its own challenges, as it will require optimization of differentiation conditions for every batch of iPS cells, adding substantial costs and operational burden to the process.

β-cell replication

Stimulating β-cell proliferation is a simple and intuitive solution to replenishing β-cell mass. Indeed, a large number of growth factors and mitogenic agents have been shown to promote β-cell proliferation in animal models. These include parathyroid hormone-related protein, hepatocyte growth factor, glucagon-like peptide, insulin-like growth factors, gastrin, epidermal growth factors, platelet-derived growth factor, adenosine kinase inhibitors, and

others[16–18,73–75]. However, these agents have generally failed to promote significant proliferation of human β-cells. Substantial proliferation of human β-cells appears to occur naturally only in early childhood (mostly the first year of life)[76–79]. In all, the weight of evidence indicates that human β-cells are resistant to proliferative stimuli.

There are structural and molecular differences between mouse and human islets. For instance, β-cells are concentrated in the core of mouse islets but are more evenly distributed in human islets. Human β-cells also express several factors, such as MAFB, that are absent from mouse β-cells[80], and use GLUT1 rather than GLUT2 as the main glucose transporter[81,82]. In addition, although rodent β-cells are capable of substantial proliferation and growth in pregnancy, obesity, and insulin-resistant states, such proliferation is limited at best in adult humans. The failure of adult human β-cells to proliferate is puzzling, as they possess the necessary molecular elements that control cell cycle reentry (including cyclins, cyclin-dependent kinases (CDKs), E2F factors, and others). Direct manipulation of this molecular machinery can force human β-cells to proliferate; genetic mutations in cell cycle genes can also give rise to rare pancreatic endocrine hyperplasias such as insulinoma in humans[83–85]. Nevertheless, many of the cell cycle factors appear to be sequestered in the cytoplasm of mature β-cells[86,87]. It is unclear why this is the case or under what circumstances the cell cycle factors could be induced to traffic into the nucleus. Broad molecular and epigenetic changes occur as β-cells mature and age, with well-documented loss of EZH2 and BMI1, an increase in cell-cycle inhibitors such as $P16^{INK4a}$ and $p18^{INK4c}$, and epigenetic changes[15,88,89]. Some of these changes seem to improve β-cell function[90], but they may broadly suppress the ability of β-cells to respond to proliferative stimuli. There is longstanding evidence that insulin and glucose, both of which are elevated in obesity or insulin resistance, may directly stimulate β-cell proliferation[91–93]. But it remains unclear whether these are the key signals that drive islet hyperplasia.

A potentially important advance has come from high-throughput compound screens that identified inhibitors of dual specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A) as reagents that can potently stimulate proliferation of cultured human β-cells in vitro and transplanted human β-cells in vivo. This provides the first concrete molecular target to manipulate human β-cell proliferation[94–96]. In addition, other pathways involved in human β-cell proliferation, such as calcineurin and SerpinB1, are being identifed[97,98]. To advance these reagents into clinics will require controlling the cell type specificity, targeting the intervention to islets, and ensuring that reagents that impinge on conserved cell cycle machineries do not raise the problem of tumour formation.

Reprogramming of non-β-cells to β-like cells

Observations of rare events in developmental cell fate changes go back many decades[99]. In the well-documented example of newt lens regeneration, removal of the lens leads to proliferation of pigmented epithelial cells surrounding the lens and regeneration of a new lens[100]. Molecular studies of master regulators of cell lineages such as MYOD further cemented the notion that powerful genetic factors could dictate cell fate choices[101]. And somatic cell nuclear transfer has demonstrated the potential of nearly every nucleus to be reprogrammed to another cell state[102]. Accordingly, there has been great interest in using

master regulators of β-cell development to convert non-β-cells into insulin-producing cells. An early example is the induction of insulin expression from cultured mouse liver cells[103]. Other studies confirmed that insulin expression can be induced in non-β-cells, but these cells do not take on the morphological, molecular, and functional properties of pancreatic β-cells and are not reprogrammed to a β-like cell state[104–108].

A combinatorial screening strategy showed that a combination of three developmental regulators of β-cells, NGN3, PDX1, and MAFA (referred to as NPM factors), could efficiently convert pancreatic acinar cells into β-like cells after delivery into the adult mouse pancreas using adenoviral vectors[109]. The induced β-like cells achieved long-term stability and acquired the ability to reverse diabetes[110]. Further screening identified gastrointestinal epithelial cells as another cell type that could be converted into β-like cells[111]. Cells from the antral stomach appear to be particularly amenable to such conversion[112]. In a separate study, conditional deletion of FOXO1 from $NGN3^+$ intestinal endocrine progenitors also led to the formation of insulin-producing cells in the gut[113]. These studies together suggest that gastrointestinal epithelial cells are a potential source of functional insulin-expressing cells by reprogramming. Other examples of reprogramming mouse cells include cytokine-mediated conversion of acinar cells to insulin-expressing cells, conversion of duct cells to insulin-expressing cells byFBW7 deletion, and conversion of hepatocytes to insulin-producing cells by TGIF2[114–116].

Extreme β-cell loss can trigger spontaneous conversion of pancreatic δ- and α-cells into β-cells[32,33]. Although the molecular mechanisms of these conversion events remain unknown, genetic deletion of ARX, a regulator of α-cell development, or forced expression of PAX4, a regulator of β-cell development, can convert α-cells into β-cells in mouse models[117,118]. A unique population of insulin-producing cells at the periphery of the islets has recently been proposed to be an inter-mediary in the transition from α-cells to β-cells[119]. Further studies have identified γ-aminobutyric acid (GABA) signalling as a potential facilitator of the reprogramming event. Long-term GABA treatment in mice led to impressive increase in β-cell mass[42].

Despite proof-of-concept demonstrations of β-cell reprogramming in animal models, efforts to reprogram human cells have been less successful. Several studies have suggested that human α-cells in islets can be reprogrammed to become β-cells[42,120]. Changes in α-cell to β-cell ratios and the appearance of cells positive for both glucagon and insulin have provided some evidence for such conversions; however, in the absence of lineage tracing, direct evidence is still lacking. Other cell types such as pancreatic acinar cells, ductal cells, gall bladder cells, and intestinal cells have also been used to generate insulin-expressing cells, but these cells did not form long-term stable grafts after transplantation, suggesting incomplete cell fate conversion or an unstable epigenetic state[121–124]. At this point, the main challenge in translating approach into the clinic is to define reliable methods for efficient production of human β-like cells that can develop stable and functional transplants. Besides the in vitro reprogramming approach, in vivo reprogramming in human patients targeting pancreatic α-cells, acinar cells, or gastrointestinal epithelial cells may also be feasible. It will be challenging, however, to optimize in vivo reprogramming protocols for therapeutic use in humans.

Islets from genetically engineered animals

There has been long-standing interest in islet xenotransplantation, and several exploratory clinical xenotransplantation studies with pig islets were conducted decades ago[125–127]. However, severe immune rejection of xenograft materials by the human immune system and the presence of large numbers of pig retroviruses that may jump species pose substantial obstacles. Recent advances in genetic engineering have led to reconsideration of the possibility of using organs grown in pigs. Using CRISPR–Cas9 technology, a pool of 62 known pig retroviruses was deleted from pig skin cells[128,129], which in principle could be used to make pig iPS cells, and subsequently, genetically 'clean' pigs as islet donors. Future clinical use of pig islets will depend on advances in encapsulation technology to protect the cells from human immune reactions while ensuring long-term survival and functionality.

Growing human pancreatic tissue in animals

The idea of growing human organs in animals for therapeutic use may seem futuristic. However, advances in stem cell technology and the identification of master regulators of organ formation have spurred efforts to explore this idea using animal models[130–132]. For example, genetic deletion of PDX1 in rats leads to specific loss of the entire pancreas due to a failure to create the embryonic pancreas. Injection of mouse embryonic stem cells into *Pdx1*$^{-/-}$ rat blastocysts created mouse–rat chimaeric animals in which all organs were made up of a mixture of mouse and rat cells except for the pancreas, which was derived from mouse cells. Thus, a mouse pancreas was grown in the body of a rat. The mouse islets from these rats can be harvested and transplanted back to diabetic mice to cure their diabetes[132]. This proof-of-concept experiment between two distinct rodent species provided a glimpse of what the future might hold for growing human organs in animal species. Nevertheless, this idea is still in its infancy. Preliminary studies have suggested that standard hES cells cannot make significant contributions to animal chimaeras[133]. Further mechanistic understanding may see the development of new methods to reduce species incompatibility and approaches that minimize or eliminate indiscriminate contribution of human cells to chimaeric animals. Aside from these technological challenges, societal consent is likely to be needed to move this technology towards clinical application.

Redifferentiating β-cells

Pancreatic β-cells become dysfunctional under a variety of stress conditions, such as prolonged hyperglycaemia and hyperlipidaemia (T2D), pancreatic inflammation due to chronic pancreatitis or pancreatic cancers (type 3c diabetes), or autoimmune-induced inflammation (T1D). Severe distress could lead to β-cell degranulation and down-regulation of β-cell genes. Recent studies have suggested that the loss of β-cell properties may represent dedifferentiation characterized by upregulation of genes that are typically expressed in embryonic islet progenitors (such as *Neurog3*)[134]. It is unclear whether dedifferentiation is a common characteristic of dysfunctional β-cells, and whether the dedifferentiation process, if it exists in humans, can be reversed. We do know that dysfunctional β-cells can recover in patients with T2D with proper management, such as diet, exercise, or intensive insulin therapy. If pharmacological means can be found to 'redifferentiate' the dedifferentiated β-cells, it could constitute a new therapeutic approach

for diabetes and may be viewed as a distinct form of regenerative therapy, one that does not involve creation of new cells per se[135]. This therapy would be most relevant for T2D but could conceivably be helpful for early stage T1D as well.

Challenges of developing cell therapy for T1D

Developing cellular products to treat T1D faces the unique challenge of autoimmunity[59,136]. To protect the new β-cells, one can use immunosuppressants, the standard treatment for T1D patients that receive cadaveric islet transplants. However, many of these drugs are known to be toxic to β-cells, not to mention reducing the patient's immune capacity. An alternative way to protect transplanted β-cells is encapsulation with engineered materials. Encapsulation physically separates β-cells from immune cells but it also separates β-cells from blood vessels, thus altering the kinetics of glucose sensing and oxygen and nutrient delivery, and potentially compromising the survival and function of the encapsulated cells. Innovative encapsulation materials are being developed to address these issues[137–140]. An alternative way to protect β-cells in patients with T1D is to modulate the immune system. Different immunotherapy regiments have been shown to attenuate or even completely arrest autoimmune attacks in the non-obese diabetic (NOD) mouse model[141–143]. Unfortunately, in clinical trials, these agents did not demonstrate a significant benefit for patients.

The current cell therapies for T1D require the use of encapsulation devices or immunosuppressive agents, with drawbacks and risks. Looking ahead, it would be ideal to find ways to produce islets that naturally resist autoimmunity. How might this be done? One important clue comes from studies of patients with longstanding T1D (such as the Joslin Medalist study), which made the surprising finding that a considerable number of these patients have detectable insulin production with preservation of glucose responsiveness, suggesting that some β-cells may evade autoimmunity and continue to function[144,145]. Another possibility is that new β-cells may be continuously produced in some patients. The autoimmune attack within the pancreas itself is also not uniform. Rather, some islets or even entire pancreatic lobes have been observed to escape immune destruction while surrounded by lobes depleted of β-cells[60]. These data suggest that human β-cells may be heterogeneous, and that a subpopulation of β-cells may resist the autoimmune attack. These clinical observations are consistent with accumulating evidence that mouse and human β-cells in normal islets are heterogeneous in their molecular signatures or proliferativepotential[146–148]. A subset of β-cells has also been proposed to serve as 'hubs' for initiating pulsatile insulin release[149]. At present, it is unclear whether the β-cells found in patients with T1D are newly created in response to autoimmunity or are for some reason resistant to immune elimination. Focused studies of these β-cells in human samples and deeper understanding of the heterogeneity of human β-cells may eventually yield molecular targets that allow the production of functional insulin-secreting cells that resist autoimmunity. β-Cells derived from reprogramming of α-cells have been shown to resist autoimmunity in mouse studies, providing another potential path to study and possibly produce autoimmune-resistant β-cells[120]. Finally, it may be possible to genetically modify β-cells so that they are able to avoid detection or elimination by the autoimmune cells.

Future perspectives

We have learned a great deal about how the pancreas develops during embryogenesis and the different regenerative responses the pancreas mounts to physiological challenges and injuries. These insights are now being used to formulate regenerative strategies by differentiating stem cells, reprogramming non-β-cells, and other approaches. Fundamental research into pancreas development and homeostasis will continue to provide new insights that inspire alternative therapeutic approaches. For instance, studies of islet formation during embryogenesis may help to refine protocols for differentiating hES cells into 3D islet clusters; deeper understanding of how islets transition from the immature to mature state in postnatal development should facilitate efforts to produce functionally mature β-cells in vitro; and investigation of signals that mediate physiological expansion of β-cell mass in obesity and insulin resistance could lead to novel β-cell proliferation reagents without significant tumorigenic risks.

In spite of major advances in our understanding of pancreas regeneration, key questions remain. To name just a few: is there convincing evidence for stem cells in the adult pancreas? How heterogeneous are pancreatic β-cells in terms of function and immunological properties? What mechanisms are used by the human pancreas for natural regeneration and repair? To answer these questions, a diverse array of model systems including rodents, zebrafish, large animals, primates, and others are likely to be informative. New technologies will play an important part in advancing these studies. Single-cell analysis will provide an unprecedented view of the heterogeneity of normal and diseased islet cells, capture rare cells relevant to endocrine regeneration or immune resistance, and define transitional states from hES cells to mature β-cells or from non-β-cells to β-like cells; live cell imaging at the single-cell level will enable direct visualization of calcium waves, insulin release, and immune interactions in intact islets in vivo; humanized mouse models and human organoids could serve as surrogates to study human pancreas biology; and human genetic studies and CRISPR–Cas technology may lead to the discovery of new factors in pancreas disease and regeneration.

Clinical trials of islet cell products derived from hES cells have begun. Other approaches, including β-cell proliferation and reprogramming, may also reach the point of therapeutic development. Each approach offers certain advantages (Table 1). Beyond the safety and efficacy of these cell products, how they will fare in the T1D autoimmune environment may be a crucial determinant for their success. We are again reminded here that the ultimate goal for T1D therapy is a cell product that will naturally resist or evade autoimmunity and requires no encapsulation or immunosuppression. Close collaboration between immunologists and β-cell biologists will be necessary to make timely progress on this goal and if we succeed, it will not only benefit patients with T1D, but also offer crucial lessons in finding cures for many other autoimmune diseases.

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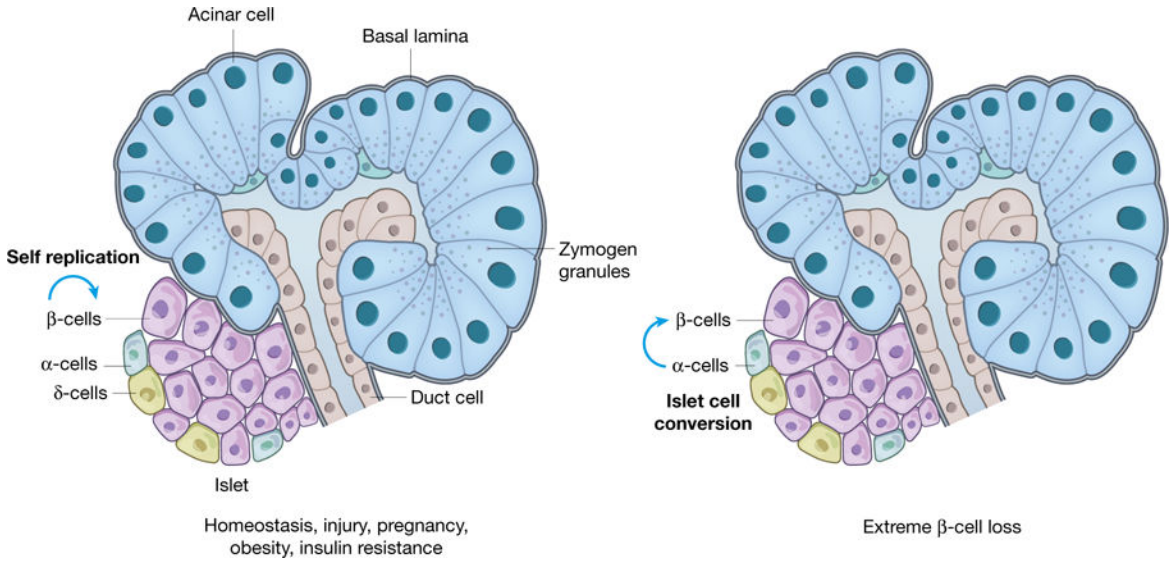

Fig. 1 |. Natural regenerative responses of the endocrine pancreas

The adult endocrine pancreas (islets of Langerhans) is made up of four major endocrine cell types with each secreting a major hormone: insulin (β-cells), glucagon (α-cells), somatostatin (δ-cells), and pancreatic polypeptide (PP cells). Animal studies have shown that β-cell replication is a major mode of regeneration and repair in homeostasis, injury, pregnancy, obesity, and insulin resistance. Conversion of islet δ- and α-cells into β-cells has been reported after extreme β-cell loss using specific ablation methods in animal models. Significant regeneration of the endocrine pancreas is largely restricted to young children and young animals. Adult animals and adult humans have little, if any, ability to regenerate the endocrine pancreas.

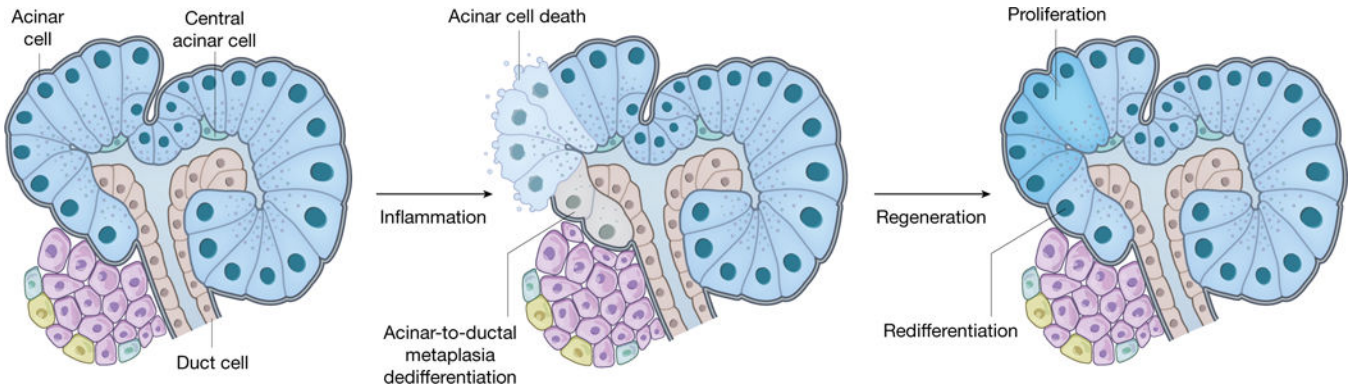

Fig. 2 |. Regeneration of the exocrine pancreas

The exocrine pancreas is composed of acinar cells that synthesize and secrete digestive enzymes, ductal cells that funnel the enzymes into the small intestine, and central acinar cells. The exocrine pancreas can regenerate spontaneously and robustly in both animals and humans. Inflammatory injuries to the exocrine pancreas such as acute pancreatitis lead to acinar cell death and acinar dedifferentiation, which is characterized by degranulation and morphological transformation into duct-like cells in a process termed acinar-to-ductal metaplasia. Once inflammation subsides, acinar cells can rapidly regenerate by self-replication and possible redifferentiation of the metaplastic duct-like cells back into a normal and functional acinar state.

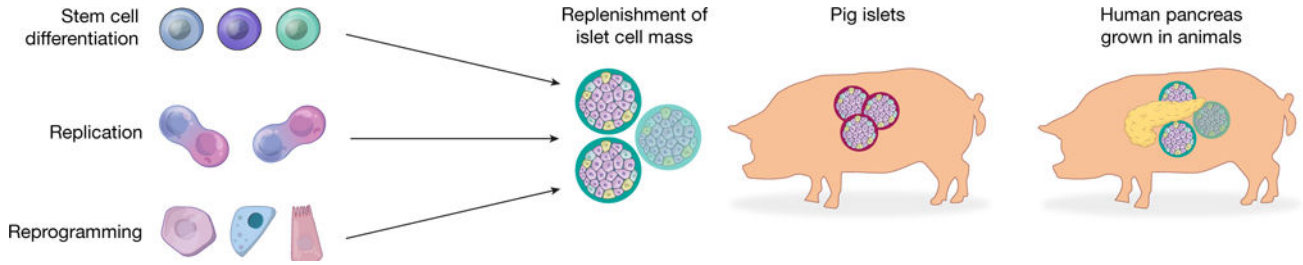

Fig. 3 |. Therapeutic strategies for regeneration and repair of the endocrine pancreas.

At present, the most advanced technology for making functional human insulin-secreting cells, and the only one to enter clinical trials, is derivation from human pluripotent stem cells. Other strategies include stimulating proliferation of residual β-cells in vivo, reprogramming of non-β-cells to β-like cells in vivo or in vitro, harvesting islets from genetically engineered pigs, and possibly growing entire human pancreata in animals followed by removal of human islets for transplantation.

Table 1 |

Comparison of current approaches to producing new β-cells

	Stem cell differentiation	Replication	In vivo reprogramming	Ex vivo reprogramming	Xenografts	Human pancreas
Stage of development	Phase I/II clinical	Proof-of-concept with human islets	Proof-of concept in animal models	Proof-of-concept With human cells	Proof-of-concept with pig cells	Concept developmet in animal models
Advantages	Unlimited supply, Standardized productin	No transplantation necessary	No transplantation necessary	Relatively simple Production process	Unlimitedsupply, lower cost	Unlimited supply
Tumour risk	Moderate (teratoma)	High	Unknown, potentially high	Moderate	Low	Low
Patient specificity	Possible with iPS cells	Yes	Yes	Possible with patient cells	No	Possible with iPScells
Issues	Complex production process	Targeted delivery required	Targeted delivery required	Stability of cell product	Targeted delivery required	Feasibility unknown, ethical concerns