Targeting β-cell dedifferentiation and transdifferentiation: opportunities and challenges

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Abstract

The most distinctive pathological characteristics of diabetes mellitus induced by various stressors or immune-mediated injuries are reductions of pancreatic islet β-cell populations and activity. Existing treatment strategies cannot slow disease progression; consequently, research to genetically engineer β-cell mimetics through bi-directional plasticity is ongoing. The current consensus implicates β-cell dedifferentiation as the primary etiology of reduced β-cell mass and activity. This review aims to summarize the etiology and proposed mechanisms of β-cell dedifferentiation and to explore the possibility that there might be a time interval from the onset of β-cell dysfunction caused by dedifferentiation to the development of diabetes, which may offer a therapeutic window to reduce β-cell injury and to stabilize functionality. In addition, to investigate β-cell plasticity, we review strategies for β-cell regeneration utilizing genetic programming, small molecules, cytokines, and bioengineering to transdifferentiate other cell types into β-cells; the development of biomimetic acellular constructs to generate fully functional β-cell-mimetics. However, the maturation of regenerated β-cells is currently limited. Further studies are needed to develop simple and efficient reprogramming methods for assembling perfectly functional β-cells. Future investigations are necessary to transform diabetes into a potentially curable disease.

Introduction

Diabetes mellitus (DM) is an epidemic chronic disease characterized by impaired glucose homeostasis, leading to hyperglycemia and multiple complications such as cardiopathy, neuropathy, nephropathy, and retinopathy. The prevalence of DM has increased dramatically in recent decades and is projected to rise to 642 million people by 2040 (1), with profound impacts on quality of life, demands for health services, and economic costs (2). There are two common syndromes, type 1 (T1D) and type 2 diabetes (T2D), characterized by an absolute or relative deficiency of β-cells, respectively (3). A previously held belief stipulated that the fate of fully differentiated β-cells is fixed and that gradual β-cell death due to glucotoxicity was a final outcome of DM (4). Treatment ultimately depended on supplemental insulin and islet transplantation, which alleviated disease severity by reducing or normalizing glycemic levels without curing DM (4). Long-term insulin treatment carries risks of hypoglycemic episodes, weight gain, and an increased incidence of cancer (5). The effectiveness of islet transplantation is limited by a shortage of donor islets and immune rejection (6). Therefore, new therapeutic strategies are urgently needed to prevent and treat this highly prevalent metabolic disorder.

The study of β-cell maturation and physiology demonstrates the heterogeneity and plasticity of mature β-cell phenotypes and function (7). The three main β-cell
phenotypes in diabetes are dedifferentiated, senescent, and transdifferentiated types (8). Glucotoxicity leads to \( \beta \)-cell dedifferentiation during hyperglycemia and reduces the expression of \( \beta \)-cell enrichment genes such as key transcription factors and genes that encode insulin; glucose metabolism, protein processing, and secretory pathways; as well as upregulation of genes that are suppressed or expressed at low levels in normal \( \beta \)-cells, including forbidden and progenitor cell genes (9). Under various stress conditions, mature \( \beta \)-cells may lose their differentiated phenotypes and return to a less differentiated or even a progenitor cell state. \( \beta \)-cell dedifferentiation is a potential adaptive mechanism to escape cell death during physiologic stress (10). Exploring the mechanisms of dedifferentiation may inform new strategies for the reversal of dedifferentiation and the restoration of \( \beta \)-cell functionality. We focus on the possible mechanisms of hyperglycemia-induced dedifferentiation that may result from the cascade of metabolic, oxidative, and endoplasmic reticulum stresses; and on epigenetic changes due to chronic stress. We also review current antidiabetic strategies and potential future research directions that may identify a time window for alleviating the \( \beta \)-cell stress response; thus preserving \( \beta \)-cell mass, regaining cell maturation, improving cellular function, and delaying disease progression.

Current research is focused on methods to increase \( \beta \)-cell numbers, maturity, function, and post-transplantation survival in addition to protecting existing \( \beta \)-cells, thus providing potential breakthroughs in the treatment of DM (11). \( \beta \)-Cell deficiency could be reversed by promoting cellular replication and redifferentiation during the early stages of DM (12). However, the application of these methods has been limited due to low \( \beta \)-cell proliferation rates, instability, and high heterogeneity. Transdifferentiation (13), defined as the phenotype switch between different cell types, obviates the shortcomings of the aforementioned methods and provides a safe and efficient approach to regeneration. The focus herein is on the eventual differentiation of non-\( \beta \)-cells into a \( \beta \)-cell phenotype. At present, most studies focus on the transdifferentiation of pancreatic non-\( \beta \)-cells; hepatic and biliary cells; gastrointestinal cells into \( \beta \)-cells through genetic programming; cytokines; and small molecules. These cells comprise the leading candidates because of their common endodermal origin with \( \beta \)-cells, abundant populations, and high conversion efficiency. The development of bionic technology offers an expanding range of options for \( \beta \)-cell regeneration.

Benefiting from excellent reviews of this field, we focus on the state of knowledge of \( \beta \)-cell dedifferentiation and transdifferentiation, as well as the highlights of exciting new research. Optimized protocols to augment functional mature \( \beta \)-cells will guide future precision medicine studies of improved treatment strategies for patients with DM and may even result in potential cures.

**\( \beta \)-Cell dedifferentiation**

Stress response is an adaptation to environmental changes. Moderate stress responses can induce effective adaptation strategies to improve survival, while an excessive stress response will cause stress injury, leading to the onset and development of a variety of organic and psychological diseases, including DM. A growing body of evidence has shown that the onset and development of DM is closely related to metabolic, oxidative, and endoplasmic reticulum stresses and to epigenetic changes caused by chronic stress. Both T1D and T2D present with a loss of \( \beta \)-cell mass and identity that ultimately impair insulin secretion. The current view is that \( \beta \)-cell loss during the development of DM may be related to \( \beta \)-cell dedifferentiation rather than apoptosis, because the relatively low rate of apoptosis may not fully explain the loss of \( \beta \)-cell mass. \( \beta \)-cell dedifferentiation is characterized by decreased expression of specific genes that maintain the characteristics and function of mature \( \beta \)-cells and by the increased expression of endocrine precursor cell genes and additional genes that are expressed at low levels in normal \( \beta \)-cells. In this section, we discuss the various stresses and mechanisms that trigger dedifferentiation, as well as the currently available methods to inhibit \( \beta \)-cell dedifferentiation (Figs 1 and 2).

**Nutritional stress**

The effects of glucose, lipid, and amino acid metabolism on pancreatic \( \beta \)-cells have received increasing attention (14). \( \beta \)-Cells gradually change from their initial adaptive stage to develop metabolic disorders and apoptosis (15). This process was previously ascribed to glucotoxicity, lipotoxicity, glucolipotoxicity, and metabolic stress; however, these proposed etiologies could not accurately reflect the state changes of \( \beta \)-cells in the processes of mixed-nutrient energy balance and imbalance. At present, nutritional stress is considered the most appropriate terminology (16). Transitory metabolic imbalances stimulate insulin synthesis and release (17). Once the balance is broken, usually in the context of a poor lifestyle, long-term overnutrition, and aging, \( \beta \)-cells confront chronic and persistent insults that are aggravated by the individual's
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Genetic and epigenetic composition (16). Chronic metabolic stress can inhibit transcription factors such as MAF BZIP transcription factor A (MafA), pancreatic and duodenal homeobox 1 (PDX-1), neuronal differentiation 1 (NeuroD1), and insulin gene expression, thus causing β-cell dysfunction and failure, leading to T2DM (18).

In rodent and human pancreatic islets, β-cells induced by high-glucose concentrations exhibited cellular dysfunction, decreased insulin secretion, glucose-stimulated insulin secretion (GSIS), and expression of mature β-cell genes; while expressing progenitor or precursor cell-related genes (19). Elevated glucose levels and the duration of hyperglycemia are the main factors affecting β-cell dedifferentiation (20). Zinc deficiency induced by hyperglycemia may reduce the expression of key β-cell transcription factors MafA, paired box 6 (Pax6), and NK2 homeobox 2 (NKX2.2) and promote β-cell dedifferentiation (21).

β-cells (MIN6) cultured in a high-glucose environment exhibited reduced expression of vitamin D receptor (VDR). Vitamin D3 treatment can prevent β-cell dedifferentiation and increase the expression of genes encoding essential transcription factors such as Pdx1, MafA, and VDR. Notably, the expressions of insulin 1 (Ins1) and insulin 2 (Ins2) are also increased (22). β-Cell absorbed free fatty acids (FFA) (including triglyceride hydrolyzed by lipoprotein lipase), very low-density lipoprotein, and low-density lipoprotein produced by endocytosis attenuate glucose toxicity (22). Thus, circulating lipid levels appear to influence not only glucose toxicity but also β-cell adaptation to hyperglycemia. Hyperglycemia and hyperlipidemia interact to impair β-cell function (23, 24). Circulating glucose and lipid levels are elevated prior to the onset of obesity-related T2D, so a reasonable suggestion is that excessive levels of these two nutrients are pathogenic. In the context of overnutrition associated with T2D, disorders of lipid homeostasis associated with hyperlipidemia and hypercholesterolemia, and often elevated plasma FFA, precede the onset of T2D in both human clinical experience and in rodent models. Multiple in vitro studies using β-cell lines (INS, MIN6, or HIT cells) or isolated rodent and human islets have shown synergistic toxicity of elevated glucose and FFA on β-cell function and survival (25, 26). A recent 6-year Canadian follow-up study showed a strong negative association between total plasma FFA levels and β-cell function (27). Similarly, another study showed that elevated plasma FFA is strongly associated with decreased β-cell function in children and adults and impaired insulin secretion rather than insulin sensitivity (28). Reduced in vivo and in vitro GSIS is associated with changes in the glyceride/fatty acid cycle (29, 30, 31, 32, 33, 34). In lipid-treated MIN6 cells, inhibition of ID1 expression can reduce the expression of islet stress genes and increase insulin secretion. ID1 is a negative regulator of insulin secretion, and its expression plays a crucial role in the etiology of β-cell dedifferentiation under conditions of glucose intolerance, insulin secretion dysfunction, and increased lipid load, providing a molecular link between chronic lipid-induced damage and β-cell dedifferentiation and dysfunction (35). None of these models demonstrated β-cell apoptosis or reduced cell mass, suggesting that β-cell

![Figure 1](https://ec.bioscientifica.com)

β-Cells lost the mature phenotype under various stressors and dedifferentiated as an adaptive response to avoid apoptosis.
dysfunction, rather than cell death, promotes the onset of DM (36). The effects of glucose and lipids on β-cells in different experiments were discriminating. Another study suggested that β-cell dedifferentiation could be induced in lipotoxic conditions with or without hyperglycemia (37). This may be related to the composition and quantity of FFA. Nutritional stress in β-cells may result not only from glucose and lipids but also from amino acids, especially branched-chain amino acids (BCAA), including leucine, isoleucine, and valine. These three amino acids, as well as tyrosine and phenylalanine, were increased while glycine was decreased in obese hyperinsulinemic patients (14, 38, 39). Elevated plasma levels of BCAA and aromatic amino acids (tyrosine and tryptophan) are associated with obesity, insulin resistance, and susceptibility to T2D.

The β-cell dysfunction induced by disordered amino acid metabolism is possibly mediated through the continued activation of the mammalian target of rapamycin (mTOR) signaling and consequent mitochondrial dysfunction (40). An increasing body of evidence suggests that reduced nutritional stress can improve β-cell function (37). Low-calorie diets (41), hypoglycemic drugs (42), dietary additives (43), or bariatric surgery (44, 45) have led to diabetic remission and/or improved insulin secretion in a significant proportion of T2D patients. We suggest that in

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Figure 2
Mechanisms of β-cell dedifferentiation. (A) Reduced expression of raptor-induced suppression of the mTORC1 signaling pathway increased the expressions of β-cell-specific disallowed genes (e.g. Hk1, Dlk1, Pdgfra, Oat, and Mylk). (B) Stress is a major contributor to β-cell dedifferentiation via activating Nf-κB signaling, which compromises β-cell identity and thus decreases insulin secretion. (C) The JNK pathway is activated under diabetic conditions such as stress and cytokine release, accompanied by Pdx1 nuclear translocation and suppression of insulin and GLUT2 gene expression. (D) The p38 MAPK pathway mediates the degradation of endogenous MafA during hyperglycemia. The dotted and solid boxes represent signaling pathway inhibition and activation, respectively. 4E-BP1, 4E binding protein 1; elf4F, eukaryotic initiation factor 4F; Hk1, hexokinase 1; Oat, ornithine aminotransferase; Pdgfrα, platelet-derived growth factor receptor α; Mylk, myosin light chain kinase; IKK, IkB kinase; IkB, nuclear factor-kappaB inhibitor alpha; NIK, NF-κB inducing kinase; RTK, receptor tyrosine kinase; TNF, tumor necrosis factor; TRAF2, TNF receptor-associated factor 2; RIP1, receptor-interacting protein 1; MEK, MAPK kinase; MEKK, MEK kinase; TRADD, TNFR1-associated death domain-containing protein; FADD, Fas-associated death domain-containing protein; TAK-1, TGF-beta-activating kinase 1.

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most cases, these benefits are derived from the reversal of pancreatic β-cells dedifferentiation (37, 46).

**Endoplasmic reticulum stress**

Endoplasmic reticulum (ER) stress is caused primarily by reactive oxygen species (ROS) accumulation, toxic substances, and genetic mutations. Stressors usually originate from changes in the cellular internal environment and include protein misfolding, aggregation of misfolded proteins, calcium homeostasis disorders, and other ER dysfunctions (47). In prediabetes, complexes of misfolded proteins accumulate in the ER of β-cells, suggesting that the disruption of ER balance is an early event in the development of T2D (48). There is solid evidence that ER stress contributes to β-cell dysfunction in both T1D and T2D (49, 50, 51). In the context of DM, chronic ER stressors such as hyperglycemia, hyperlipidemia, hypoxia, and pro-inflammatory cytokines (TNF-α, IL-1) lead to the gradual loss of β-cell-specific transcription factors, including Pdx1, MafA, and forkhead box O1 (FoxO1), and the acquisition of endocrine progenitor cell markers such as neurogenin3 (Ngn3) and octamer-binding transcription factor 4 (Oct4) (52, 53, 54). In this setting, ER homeostasis will collapse and initiate the unfolded protein response (UPR). The UPR is a protective mechanism that maintains the balance between synthesis and degradation; supports correct folding and function; sustains protein homeostasis, or proteostasis (55). Many factors in pancreatic β-cells can disrupt the UPR balance to trigger ER stress. These include genetic mutations, cytokines, infections, excess nutrients, islet amyloid polypeptide (IAPP), and insulin resistance (IR) and can result in DM (50, 56, 57, 58). This disruption of the adaptive UPR promotes diabetic progression and β-cell dedifferentiation (59). Combinatorial signals from the three core components of the UPR (protein kinase R-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6)) initially trigger transcriptional programs that upregulate genes encoding many of the aforementioned ER-resident protein-folding machines that play important roles in insulin biosynthesis (60, 61). Consequently, further research is needed to clarify which steps of the ER stress process are easily targeted, because activation of key proteins of the UPR may be beneficial in the treatment of T2D (62). In fact, some of the therapeutic effects of drugs currently used to treat DM may stem from their ability to regulate ER stress, target protein folding, and modulate the UPR signaling pathway. These processes may provide targets for future drug candidates such as chemical chaperone molecules 4-phenylbutyric acid and taurine-deoxycholic acid to promote correct protein folding and cellular function (63, 64).

**Oxidative stress**

Oxidative stress and metabolic disorders are considered the two most important pathogenic factors of insulin secretion disorder (65). The development of T2D results from a variety of cellular changes associated with oxidative stress and impaired redox signaling, usually caused by lipotoxicity and glucotoxicity due to continued overfeeding (66). Oxidative stress is not only closely related to lipotoxicity and glucotoxicity but is also associated with inflammation, ER stress, hypoxia, and mitochondrial damage in the promotion of β-cell dedifferentiation (66, 67, 68). Oxidative stress impairs pancreatic β-cell maintenance and function (69), which is widely believed to be associated with the onset of DM and diabetic complications. The consequent increase of oxidized biological components leads directly not only to pathologies such as inhibited insulin secretion but also to the induction of new cellular responses, namely programmed cell death, i.e., apoptosis. These phenomena occur in parallel after cumulative oxidative stress reaches a certain threshold (66). In T1D, ROS promote autoimmune responses, cytokine release, and inflammation-induced impairment of β-cell function (70). Drews et al. suggested that oxidative stress-mediated loss of cellular function (e.g. impaired secretion and increased insulin resistance) plays an important role in the pathogenesis of both T1D and T2D (36). Oxidative stress-related β-cell dysfunction induces cytoplasmic transposition and inactivation of transcription factors MafA, Nkx6.1, and Pdx1 in the islets of T2D patients (54, 71). The expression of oxidative-stress-related genes increased the release of pro-inflammatory cytokines and upregulated the expressions of pancreatic progenitor cell-specific transcription factors such as SRY-Box transcription factor 4 (SOX4), SRY-Box transcription factor 9 (SOX9), inhibitor of DNA binding (ID2), and vimentin in islet cells cultured for 3 days. These findings indicated that cells dedifferentiated under oxidative stress in vitro (72). Oxidative stress leads to dephosphorylation and inactivation of these pathways by stimulating the activity of phosphatases such as protein tyrosine phosphatase 1B (PTP1B) and SH2-containing tyrosine-protein phosphatase, thus inhibiting insulin effect (73). Free radicals not only have the aforesaid direct effects but can also indirectly activate various intracellular signaling pathways, such as mitogen-activated protein kinase (MAPK) and nuclear factor-κB (NF-κB), which further exacerbate oxidative stress and contribute to the progression of diabetic complications (74-76).
pathways such as mTOR, nuclear factor-kappa b (NF-κb), p38 mitogen-activated protein kinases (p38 MAPK), stress-activated protein kinase/c-Jun NH (2)-terminal kinase (JNK/SAPK), hexosamine pathways, protein kinase C (PKC), and advanced glycation end product/receptor for AGE (AGE/RAGE) interaction (74). The inactivation of mTOR signaling reduced β-cell mass and insulin secretion. The mTOR complex 1(mTORC1) was suppressed under oxidative stress, suggesting that the mTOR pathway has a potential role in β-cell dedifferentiation under oxidative stress (75, 76). p38 inhibition can reduce β-cell apoptosis and protect cellular function (77). Administration of antioxidants to β-cells may restore free radical scavenging potential and reduce oxidative stress, thereby increasing PDX-1, Ins-1, ngn3, GLUT, and IRS-1 expressions, thus promoting β-cell regeneration and subsequent pancreatic insulin release (78). Oxidative stress in the setting of diabetes activates the JNK pathway, which impairs insulin signaling, thereby increasing IRS1 serine phosphorylation and decreasing both IRS1 tyrosine phosphorylation and IRS1-associated PI3K activity. JNK activation reduces the phosphorylation of FoxO1, which in turn inhibits the expression of the insulin transcription factor Pdx1, thus lowering insulin levels and ultimately impairing β-cell function (79, 80). Activation of the p38 MAPK and JNK signaling pathways contributes to β-cell dysfunction in the pathogenesis of T2DM (81).

Commonly used antidiabetic drugs include metformin, thiazolidinediones, α-glucosidase inhibitors, insulin, glucagon-like peptide-1 (GLP-1) receptor agonists, dipeptidyl peptidase-4 inhibitors, and sodium-glucose cotransporter type 2 inhibitors (SGLT2is); which may enhance the regulation of adaptive responses to multiple stressors. Metformin has direct and indirect antioxidant and anti-inflammatory properties, inhibits the PERK/CHOP signaling pathway, reduces the ER stress response, and also prevents lipotoxic β-cell apoptosis (82, 83). Pioglitazone attenuates β-cell oxidative stress, inflammation, and ER stress by inhibiting NF-κb activation (84). Rosiglitazone prevents oxidative stress by regulating NF-κb activity through a PPARα-dependent mechanism (85). Exendin-4 augments cellular defenses by inducing the ER chaperone BIP and the anti-apoptotic protein JUNB (86), and also prevents β-cell dysfunction and apoptosis by inhibiting the activation of JNK and p38 MAPK signaling (87, 88). SGLT2i attenuates the oxidative stress mediated by AGE-RAGE (89). Drugs that are currently used to treat diabetes exhibit excellent performance in stabilizing glycemic levels; however, the morbidity caused by diabetic complications obliges the development of novel alternatives and the creation of new preventive protocols for patients at high risk of insulin resistance (24, 63).

The discovery of safe and effective antioxidants could yield novel therapeutic options for the treatment of DM. Significantly, vitamin E, contained in nuts, reduces cellular oxidation by reacting with lipid radicals produced in the lipid peroxidation chain. Vitamin C, obtained from green leafy vegetables and fruits, acts synergistically with vitamin E to quench ROS through antioxidant activity (90). Several herbal derivatives (i.e. curcumin, cinnamon, garlic, and resveratrol) may have potential roles in maintaining β-cell function and inhibiting oxidative injury through their antioxidant properties (90).

In addition, the interaction of multiple cellular stressors induces epigenetic changes that can disrupt cellular function and trigger β-cell dedifferentiation. DNA methylation, histone modification, and noncoding RNA (ncRNA)-mediated gene regulation are examples of epigenetic mechanisms (91). A role of ncRNAs in epigenetic inheritance has been suggested recently (91). The function of ncRNAs, including miRNAs and long noncoding RNAs (lncRNAs), in β-cell dedifferentiation, has attracted increasing attention (91). lncRNA is abundant in β-cells and plays a vital role in differentiation (92). β-cell-specific lncRNAs interact with transcription factors to orchestrate transcription networks. lncRNAs could represent therapeutic targets to mitigate β-cell dysfunction. MIR-24 may trigger β-cell dedifferentiation (93). Ectopic MIR-24 expression in Min6 cells and primary islets increases Ngn3 and SOX9 expression and also inhibits its direct target Ire1α, which consequently reduces Xbp1 and Atf4 expressions. MIR-302 upregulation simultaneously suppresses the expression of several β-cell identity genes such as NeuroD1, peroxisome proliferator-activated receptor α (PPARα), and lysine acetyltransferase 2B (Kat2B), suggesting a role of MIR-302 as a therapeutic target to prevent β-cell dedifferentiation (94). Defects in β-cell-specific lncRNAs cause DM in humans (95). MEG3, an lncRNA associated with normal β-cell function, acts as a unique controller to decrease MapA and Pdx1 expressions. A crucial mechanistic role of PLUTO in preventing β-cell dedifferentiation is highly likely (9). PLUTO promotes Pdx1 expression by facilitating binding between the Pdx1 promoter and its enhancer cluster (95). Novel lncRNAs will probably be characterized in the near future to further elucidate their regulation of pancreatic development and β-cell function (96). The role of lncRNAs in β-cell dedifferentiation is a fascinating research topic that deserves further exploration.
Although existing therapies improve glycemic control effectively, the increasing prevalence of serious diabetic complications suggests that targeting existing β-cell populations is inadequate. The exploitation of new resources to produce β-like cells may generate solutions to replenish depleted β-cells. Transdifferentiation offers a promising option.

β-Cell transdifferentiation

Cellular transdifferentiation, also known as lineage reprogramming, can be used to regenerate β-cells (97). Since hepatic, gastrointestinal, and pancreatic exocrine cells are derived from common endodermal progenitor cells, the transdifferentiation of developmentally related cells into β-cells can be easily accomplished. Because of identical developmental transcription mechanisms, similar epigenetic landscapes, and unique locations of endogenous cells, only a small portion of the epigenome needs to be rearranged, thus providing an attractive process for cellular reprogramming (98). The efficacy of particular technical strategies and the feasibility of using specific cell types for transdifferentiation are key questions that have been explored in exciting new studies of pancreatic β-cell regeneration (11) (Fig. 3 and Table 1).

Transdifferentiation of pancreatic non-β-cells to β-cells

Pancreatic non-β-cells, such as α, δ, acinar, and duct cells, share developmental histories and have similar epigenetic profiles. Because they may share common pathways during transdifferentiation into β-cells (97), we summarize their processes here. α-cell to β-cell transdifferentiation was observed upon β-cell loss in mice from puberty to adulthood (13). Near-total in vivo ablation of β-cells can be induced by diphtheria toxin (99), pancreatic duct ligation (PDL), and partial pancreatectomy (PPX) (98). PDL and PPX promote β-cell transdifferentiation not only from α-cells but also from duct (100) and acinar cells (101) in murine models. However, cell reprogramming is technically difficult. Current transdifferentiation strategies lack uniform methods. Efficiencies are usually low, and reprogrammed cells may exhibit unstable or immature phenotypes (97). Regulation of transcription factor expression and the use of several drugs offer more promising options. MafA and Pdx1 are essential transcription factors that interact with other factors to regulate transdifferentiation. The ablation of the Aristaless-related homeobox gene (Arx) (102) or the overexpression of PAX4 (103), MafA, and Pdx1 (104) promotes the transdifferentiation of β-cells from α-cells. Transdifferentiation from adult murine pancreatic duct (105)
| Cell types       | Instructive strategies                                                                 | Advantages                                                                 | Limitations and challenges                                                                 | Functioning validity (weeks) | Involved mechanisms                                                                 | Refs                  |
|------------------|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|------------------------------|-------------------------------------------------------------------------------------|-----------------------|
| Pancreatic non-β-cells | Injury (PDL, PPX); Transduction of pancreatic transcription factors MafA, PDX1 and Ngn3; Administration of cytokines EGF, CNTF, nicotinamide, LIF; Peptide (GLP-1) | Similar developmental background to β-cells in vivo; Large proportion and flexible plasticity; Share quantity of transcription factors; Corresponding metabolic mechanism and hormone secretion | Low conversion rate; Weak proliferative capacity and stability; Immature morphology and biological function; The reprogramming effectiveness and efficiency with or without gene manipulation need to be improved | 2                            | PI3K/Akt pathways; MAPK/STAT3 signaling pathways; Erk1/2 signaling pathways       | (101, 111, 112, 113)|
| Acinar cells     | Injury (PDL, PPX); Ectopic overexpression of MafA, PDX1 and Ngn3; NeuroD1, Pdx6 needed for human ductal reprogramming; Administration of cytokines TGFα, DNA methyltransferase inhibitor; Peptide (GLP-1, gastrin) | Conveniency accessible; Sufficient source and cultivate easily; Great regeneration and conversion ability; Share common characteristics including responsiveness to glucose, and mass of specific transcription factors | Efficient viral transfection strategies accompanied with safety concerns; Exploring cytokine or chemical induced safe and effective transition is prospective | 4–16                        | Wnt signaling pathway                                                              | (119, 120, 121)      |
| Duct cells       | Ectopic expression of PDX1 and NeuroD1; Down-regulating the expression of HNF1α and HNF4α; Specific factors (GLP-1R, Notch inhibitors, TGF-β inhibitors) | Conveniently accessible | Limited source of cells; Transient transformation; Expand cell source and optimize reprogramming protocol need to be investigated | 3–4                          | Notch signaling pathway                                                             | (122, 123, 124, 125)|
| Liver cells      | Ectopically overexpressing PDX1 and NeuroD1; Down-regulating the expression of HNF1α and HNF4α; Specific factors (GLP-1R, Notch inhibitors, TGF-β inhibitors) | Conveniently accessible | Limited source of cells; Transient transformation; Expand cell source and optimize reprogramming protocol need to be investigated | 3–4                          | Notch signaling pathway                                                             | (122, 123, 124, 125)|
| Biliary cells    | Transduction of pancreatic transcription factors Pdx1, NeuroD1, Ngn3, MafA or Pdx1/VP16; Excision of Hes1 | Conveniently accessible | Limited source of cells; Transient transformation; Expand cell source and optimize reprogramming protocol need to be investigated | 3–4                          | Notch signaling pathway                                                             | (122, 123, 124, 125)|
| Gastrointestinal cells | Ectopic expression of Pdx1, MafA, and Ngn3; Excision of transcription factor FoxO1; Peptide (GLP-1); ‘Small intestinal organ’ | Simple, non-invasive, easy to access; Sufficient source and cultivate easily; Great regeneration and conversion ability; Similar glucose sensitive system and secretion mechanism with islet cells | Low conversion rate            | 1–3                          | PI3K/Akt/FoxO1 pathways                                                           | (126, 128, 129)      |
| Intestinal cells | Ectopic expression of Pdx1, MafA, and Ngn3; Excision of transcription factor FoxO1; Peptide (GLP-1); ‘Small intestinal organ’ | Simple, non-invasive, easy to access; Sufficient source and cultivate easily; Great regeneration and conversion ability; Similar glucose sensitive system and secretion mechanism with islet cells | Low conversion rate            | 1–3                          | PI3K/Akt/FoxO1 pathways                                                           | (126, 128, 129)      |
| Glandular cells  | Ectopic expression of Pdx1, MafA, and Ngn3; ‘Stomach mini-organs’                     | Similar glucose sensitive system and secretion mechanism with islet cells | Similar glucose sensitive system and secretion mechanism with islet cells              | 3–6                          | P13K/Akt pathways                                                                 | (134, 136, 138)      |
and acinar cells \( (106) \) to β-cells can be induced by \( \text{MafA}, \text{Pdx1} \), and \( \text{Ngn3}/\text{NeuroD} \), while induction of \( \text{Pax6} \) was also needed to reprogram human ductal cells into β-cells \( (107) \). GLP-1 \( (108) \) may transform α-duct, and acinar cells into cells with a β-cell-like phenotype. GABA \( (109) \) and artemisinins \( (110) \) promote the transdifferentiation of α-cells into β-cells. EGF in combination with ciliary neurotrophic factor (CNTF), nicotinamide, and leukemic inhibitory factor (LIF) \( (111, 112, 113) \) can also induce transdifferentiation of pancreatic acinar cells to insulin-producing cells in culture. Other growth factors such as gastrin and transforming growth factor-α (TGF-α) \( (114) \) as well as the DNA methyltransferase inhibitor 5-aza-2-deoxycytidine \( (115) \) induced \( \text{Pdx1} \) expression in ductal cells to promote endocrine differentiation. These processes may be related to the PI3K/AKT/FOXO1 \( (116) \) and MAPK/STAT3 signaling pathways \( (117) \). The aforementioned results indicate that overexpression of endocrine genes, inhibition of exocrine genes, and treatment with cytokines or small molecules are promising strategies for β-cell regeneration. Results are variable due to the different models and methods; conversion rates are often low. More data are needed to identify safe and effective transcription factors and drugs to promote transdifferentiation for clinical use.

Transdifferentiation of hepatocytes and biliary cells to β-cells

Due to the proliferative ability and tissue specificity of liver tissue, as well as the common endodermal origin shared between hepatocytes and pancreatic cells, the clinical manipulation of genetic factors in combination with small molecules targeting specific pathways could render the human liver an ideal source of functional insulin-producing cells \( (118) \). Some hepatocytes display ectopic expression of \( \text{Pdx1} \). These hepatocytes are typically located near central veins and seem predisposed to transdifferentiation into β-cells \( (119) \). Ectopic overexpression of \( \text{Pdx1} \) and \( \text{NeuroD1} \), downregulation of the expressions of hepatic transcription factors \( \text{HNF1α} \) and \( \text{HNF4α} \), the reprogramming of hepatocytes into insulin-producing cells, and activation of the Wnt signaling pathway are necessary conditions for maintaining this plasticity \( (119, 120) \). Other factors such as GLP-1R agonists, Notch inhibitors, and transforming growth factor-β (TGF-β) inhibitors could enhance hepatocyte transdifferentiation \( (121) \). A notable finding is that the plasticity of the extrahepatic biliary tree enables intrahepatic biliary epithelial cells to express \( \text{Pdx1}, \text{NeuroD}, \text{Pdx1}/\text{VP16} \), the insulin gene \( \text{Ins} \), and \( \text{Glut2} \) \( (122) \). In \( \text{Hes1} \) knockout models, biliary epithelium differentiated into pancreatic exocrine and endocrine cells that formed acinar and islet-like structures in bile ducts with upregulated expression of \( \text{Ngn3} \) \( (123, 124) \). Murine gallbladder epithelial cells were reprogrammed into β-like cells through the overexpression \( \text{Pdx1}, \text{Ngn3}, \) and \( \text{MafA} \), that led to increased expressions of pancreatic endocrine genes (insulin, \( \text{NeuroD1}, \text{Nkx2.2}, \) and \( \text{Isl1} \) \( (125) \)). The overexpression of critical transcription factors combined with suppression of inhibitory factors may potentially enhance the efficiency of cell reprogramming.

Transdifferentiation of gastrointestinal cells to β-cells

The gastrointestinal tract is a highly regenerative organ system rich in endocrine cells that are highly similar to pancreatic β-cells and is also an immune-privileged site. Based on these advantages, the gastrointestinal tract can be used as a site for either transdifferentiation to produce β-like cells or for the engraftment of regenerated cells that mimic β-cell function \( (126, 127) \).

By screening adult cell types capable of becoming insulin-producing cells \( \text{in vivo} \), we found that due to the ectopic expression of \( \text{Pdx1}, \text{MafA}, \) and \( \text{Ngn3} \) in the intestinal crypts, intestinal cells can form β-like cells and may represent an accessible and abundant source of functional insulin-producing cells \( (128) \). Under the influence of \( \text{Pdx1}, \text{MafA}, \) and \( \text{Ngn3} \) (PMN), enterocytes are capable of acquiring β-like characteristics that include the abilities to process preproinsulin into its mature form (with the release of C-peptide); to upregulate genes encoding the β-cell KATP channel subunits \text{Kir6.2} \) and \text{Sur1}, and to display distinctive β-granules \( (128) \). Ablation of the \( \text{FoxO1} \) transcription factor in enteroendocrine cells produces functional β-like cells \( (126) \). GLP-1 treatment induces insulin production in developing enterocytes, and to a lesser extent, in adult enterocytes both \text{in vitro and in vivo}; this process is mediated by the activation of \( \text{Ngn3} \) and its downstream genes \( (128, 129) \).

However, the success rate of intestinal epithelial transdifferentiation is low, the transformation of enterocytes is relatively incomplete and the lifespan of intestinal insulin+ cells is shorter than that of sinus insulin+ cells due to \( \text{CDX2} \), the intestine-specific cell surface marker, which prevents enterocytes from reprogramming into effective β-cells \( (130) \). Although these studies have revealed the feasibility of producing β-like cells from the intestinal epithelium, intestinal insulin+ cells cannot be regarded as completely regenerated β-cells because of their deficient \( \text{NXX6.1} \) expression and unstable phenotype \( (131, 132, 133) \). A new framework that promotes a complete
reprogramming of intestinal epithelial cells to become fully functioning β-cells is urgently needed.

However, the stomach represents a potential source of reprogrammable cells that may be transdifferentiated to produce insulin-secreting cells (134). The transformation mechanisms of different gastric cell types are varied. Based on the regenerative capacity of the antrum, native stem cells can supplement the increased demand for antral insulin+ cells and provide an excellent source of functional β-like cells. Antral endocrine cells mediated by PMN can be effectively reprogrammed into insulin+ cells, with robust expressions of key β-cell genes such as Sur1, and Glp1R, resulting in substantially improved glucose responsiveness. The gastric corpus contains a small amount of Ngn3-derived endocrine cells which differ from those of the antrum or intestinal tract and are not derived through PMN-mediated β-cell transformation (134, 135, 136). To summarize, cell plasticity and mechanisms of transdifferentiation differ in various segments of the gastrointestinal tract. The advantages of easy accessibility and transformability of gastrointestinal cells have attracted increasing attention. Further developments in biotechnology are expected to yield functional β-cells.

Bioengineered approaches

The in situ induction of β-cells from the native gastrointestinal tract may be limited due to a physiological environment that may disrupt normal endocrine homeostasis. In addition, the functionality of reprogrammed gastrointestinal cells remains to be determined. Unresolved issues include whether they express all key beta cytokines, whether their physiological function is complete, and whether they can reliably control insulin production in response to various physiologic stimuli. The development of regenerative engineering offers a potential replacement option described as organoids or functional 3D structures assembled with cell types from different sources. This strategy is widely used in regenerative medicine for tissue replacement or repair (137). However, the tissue-engineered stomach represents a versatile in vivo tool. ‘Stomach mini-organs’ that contain genetically engineered antral tissue enable both the formation and protection of transformed cells to constitute a new β-cell reservoir (134). Bioengineered gastric spheres isolate newly derived β-mimetic cells from the native organ, maintaining the physiological stability of the endocrine cell population in the intestinal tract, while on the other hand protecting the deposited β-mimetic cells from inappropriate glucose responses under stress (138).

Recent advances in genetic engineering have expanded the accessibility of gastric organoids. The potential research (139) and therapeutic applications of genetically engineered organoids are substantial.

Emerging advances in synthetic biology have enabled the construction of specialized cells capable of performing vital functions. Conditioned media and the addition of a glucose sensing medium have been employed to synthesize β-cell-mimetic designer cells (140). We engineered a glucose-inducible transcriptional system by coupling a β-cell-mimetic cascade of glycolysis-mediated calcium entry to a synthetic excitation transcription coupling system in human embryonic kidney 293 (HEK-293) cells. These engineered cells are capable of glucose sensing and concentration-dependent expression of insulin and GLP-1. Injection of microencapsulated cells increased insulin secretion and improved hyperglycemia in diabetic mice (141). A semi-autonomous light control system stimulated the secretion of glucose-lowering hormones by photoactivated HEK293 cells implanted in diabetic mice, thus restoring glucose homeostasis (142, 143). Glucose-sensing devices loaded with biomimetic cells displayed glucose-induced insulin release comparable to endogenous β-cells; these constructs may be modified to secrete therapeutic proteins such as GLP-1 required for T2D therapy. However, the elucidation of the long-term in vivo effects of biomimetic cells will require additional studies.

Future research could explore the potential advantages of combining bioengineering (utilizing polymers such as PTFE or polycaprolactone, and/or microencapsulation of β-cells in materials such as alginate, polyacrylate, collagen or agarose) (144); gene-editing tools (CRISPR-Cas9) (145, 146); immune tolerance induction (147, 148); the adaptation of cell lines for enhanced mass production in bioreactors; optimization of biological process parameters and bioreactor environments; the promotion of cell growth and differentiation to augment biological function. The efficient mass production of cells that are stable, functionally mature, and that can mitigate functional deficiencies will accelerate the development of long-term cell replacement therapy for DM.

Acellular bioengineered constructs may offer alternatives to avoid design complexities and bioincompatibilities of reprogrammed cells. These non-living biomimetic assemblies include vesicles that carry drug payloads in cell membrane-cloaked nanoparticles that deliver insulin in a dynamic response to hyperglycemia and could theoretically act as β-cell surrogates for DM therapy (149). A disadvantage of acellular constructs is that they only provide basic β-cell functions such as insulin

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secretion. Capacities for insulin synthesis, its modulation through amplifying signal pathways, and the fine control of relative insulin content in response to external stimuli are absent (13). However, these constructs represent an exciting advance in the search for β-cell alternatives.

Conclusion

Dedifferentiation is a key mechanism of pancreatic β-cell failure. β-cell dysfunction induced by stress is driven by a complex set of reversible environmental factors. Improved understanding of the mechanisms of β-cell dedifferentiation will inform its reversal. Augmentation of β-cell volume and mass is essential to maintain normal glucose homeostasis and treat DM.

Recent studies have shown that supplementation of endogenous β-cells by transdifferentiation of other cell types may be a better approach than the differentiation of pluripotent stem cells and induced pluripotent stem cells with reduced proliferative capacity. The transdifferentiation of endogenous cells to produce β-cell mimetics is considered a safer approach. Recent studies have reported different conversion efficiencies of various cell types. Conversion rates range from 10 to 20% in pancreatic ductal cells; 0.2 to 70% (typically 20 to 30%) in acinar cells; 30% in gastrointestinal cells; 5 to 20% in biliary cells; 10 to 30% in hepatocytes. In addition, the different combinations of islet transcription factors such as Pdx1, Ngn3, MafA, and NeuroD1 affect the efficiency of transdifferentiation, and the co-expression of islet transcription factors and EGF/TGFβ growth factors promotes insulin gene expression. Studies of the effects of GABA, artemisinin, and GLP-1 on β-cell transdifferentiation have yielded contradictory results. Drug effects need further validation because of the different cell types, animal models, study intervals, and methods used in previous studies. Numerous strategies have been developed to improve the efficiency of cellular transformation, but there are also significant challenges to be addressed. Although there are obstacles to the reconstitution of β-cell populations through dedifferentiation and transdifferentiation, the development and application of regenerative medicine may provide a wide range of options for the generation of targeted and effective DM treatments.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

References

1 Zheng Y, Ley SH & Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nature Reviews: Endocrinology 2018 14 88–98. (https://doi.org/10.1038/nrendo.2017.151)
2 Harding JL, Pavkov ME, Magliano DJ, Shaw JE & Gregg EW. Global trends in diabetes complications: a review of current evidence. Diabetologia 2019 62 3–16. (https://doi.org/10.1007/s00125-018-4711-2)
3 Nasteska D, Viloria K, Everett L & Hudson DJ. Informing β-cell regeneration strategies using studies of heterogeneity. Molecular Metabolism 2019 27S S49–S59. (https://doi.org/10.1016/j.molmet.2019.06.004)
4 Berreton MJ, Rohm M & Ashcroft FM. β-Cell dysfunction in diabetes: a crisis of identity? Diabetes, Obesity and Metabolism 2016 18 (Supplement 1) 102–109. (https://doi.org/10.1111/dom.12732)
5 Mannucci E. Insulin therapy and cancer in type 2 diabetes. ISRN Endocrinology 2012 2012 240634. (https://doi.org/10.5402/2012/240634)
6 Rother KJ & Harlan DM. Challenges facing islet transplantation for the treatment of type 1 diabetes mellitus. Journal of Clinical Investigation 2004 114 877–883. (https://doi.org/10.1172/JCI23235)
7 Remedi MS & Emfinger C. Pancreatic β-cell identity in diabetes. Diabetes, Obesity and Metabolism 2016 18 (Supplement 1) 110–116. (https://doi.org/10.1111/dom.12727)
8 Salinno C, Cota P, Bastidas-Ponce A, Tarquis-Medina M, Lickert H & Babii H. β-Cell maturation and identity in health and disease. International Journal of Molecular Sciences 2019 20 5417. (https://doi.org/10.3390/ijms20115417)
9 Bensellam M, Jonas JC & Laybutt DR. Mechanisms of β-cell dedifferentiation in diabetes: recent findings and future research directions. Journal of Endocrinology 2018 236 R109–R143. (https://doi.org/10.1530/JEO-17-0516)
10 Efrat S. Beta-cell dedifferentiation in type 2 diabetes: concise review. Stem Cells 2019 37 1267–1272. (https://doi.org/10.1002/stem.3059)
11 Aguayo-Mazzucato C & Bonner-Weir S. Pancreatic β-cell regeneration as a possible therapy for diabetes. Cell Metabolism 2018 27 57–67. (https://doi.org/10.1016/j.cmet.2017.08.007)
12 Wang P, Fiaschi-Taesch NM, Vasavada RC, Scott DK, García-Ocaña A & Stewart AE. Diabetes mellitus – advances and challenges in human β-cell proliferation. Nature Reviews: Endocrinology 2015 11 201–212. (https://doi.org/10.1038/nrendo.2015.9)
13 Nair GG, Tzanakakis ES & Hebrok M. Emerging routes to the generation of functional β-cells for diabetes mellitus cell therapy. Nature Reviews: Endocrinology 2020 16 S06–S18. (https://doi.org/10.1038/s41574-020-0375-3)
14 Moule VS, Ghislain J & Poitout V. Nutrient regulation of pancreatic β-cell proliferation. Biochimie 2017 143 10–17. (https://doi.org/10.1016/j.biochi.2017.09.017)
15. Brun T, Jiménez-Sánchez C, Madsen JGS, Hadadi N, Duhamel D, Bartley C, Oberhauser I, Trajkovski M, Mandrup S & Maechler P. AMPK profiling in rodent and human pancreatic beta-cells under nutrient-rich metabolic stress. *International Journal of Molecular Sciences* 2020 21 3982. (https://doi.org/10.3390/ijms21113982)

16. Prentki M, Peyot ML, Maseli P & Madiraju SRM. Nutrient-induced metabolic stress, adaptation, detoxification, and toxicity in the pancreatic β-cell. *Diabetes* 2020 69 279–290. (https://doi.org/10.2337/db19-0014)

17. Flame D, Berger V, Kruheffer M, Orntoft T, Pipeleers D & Schuit FC. Critical role for cataplerosis via citrate in glucose-regulated insulin release. *Diabetes* 2002 51 2018–2024. (https://doi.org/10.2337/diabetes.51.7.2018)

18. van der Meulen T & Hoising MO. Role of transcription factors in the transdifferentiation of pancreatic islet cells. *Journal of Molecular Endocrinology* 2015 54 R103–R117. (https://doi.org/10.1530/JME-14-0290)

19. Weir GC, Gaglia J & Bonner-Weir S. Inadequate beta-cell regulation of glucose metabolism and beta cell function in the prospective metabolism and islet cell relationships. *Diabetes* 2009 58 2369–2379. (https://doi.org/10.2337/db09-0152)

20. Moin ASM & Butler AE. Alterations in beta cell identity in type 1 diabetes. *Diabetes* 2010 59 2178–2187. (https://doi.org/10.2337/db10-0536)

21. Nolan CJ, Leahy JL, Delghingaro-Augusto V, Mohi J, Soni K, Peyot ML, Fortier M, Guay C, Lamontagne J, Barbeau A, et al. Beta cell compensation for insulin resistance in Zucker fatty rats: increased lipolysis and fatty acid signalling. *Diabetologia* 2006 49 2120–2130. (https://doi.org/10.1007/s00125-006-0305-5)

31. Delghingaro-Augusto V, Décar S, Peyot ML, Latour MG, Lamontagne J, Paradis-Isler N, Lacharité-Lemieux M, Akakpo H, Birot O, Nolan CJ, et al. Voluntary running exercise prevents β-cell failure in susceptible islets of the Zucker diabetic fatty rat. *American Journal of Physiology: Endocrinology and Metabolism* 2012 302 E234–E246. (https://doi.org/10.1152/ajpendo.00360.2011)

32. Fontés G, Zarrouki B, Hagman DK, Latour MG, Semache M, Roskens V, Moore PC, Prentki M, Rhodes CJ, Jetton TL, et al. Glucolipotoxicity age-dependently impairs beta cell function in rats despite a marked increase in beta cell mass. *Diabetologia* 2010 53 2369–2379. (https://doi.org/10.1007/s00125-010-1850-5)

33. Peyot ML, Pepin E, Lamontagne J, Latour MG, Zarrouki B, Lussier R, Pineda M, Jetton TL, Madiraju SR, Joly E, et al. Beta-cell failure in diet-induced obese mice stratified according to body weight gain: secretory dysfunction and altered islet lipid metabolism without steatosis or reduced beta-cell mass. *Diabetes* 2010 59 2506–2514. (https://doi.org/10.2337/db09-1452)

34. Tiano JP, Delghingaro-Augusto V, Le May C, Liu S, Kaw MK, Khuder SS, Latour MG, Bhattacharya K, Najjar SM, et al. Estrogen receptor activation reduces lipid synthesis in pancreatic islets and prevents β-cell failure in rodent models of type 2 diabetes. *Journal of Clinical Investigation* 2011 121 3331–3342. (https://doi.org/10.1172/JCI44564)

35. Akerfeldt MC & Laybutt DR. Inhibition of β1 augments insulin secretion and protects against high-fat diet-induced glucose intolerance. *Diabetes* 2011 60 2506–2514. (https://doi.org/10.2337/db11-0083)

36. Sun T & Han X. Death versus dedifferentiation: the molecular bases of beta cell mass reduction in type 2 diabetes. *Seminars in Cell and Developmental Biology* 2020 103 76–82. (https://doi.org/10.1016/j.semcdb.2019.12.002)

37. White MG, Shaw JA & Taylor R. Type 2 diabetes: the pathologic basis of reversible β-cell dysfunction. *Diabetes Care* 2016 39 2080–2088. (https://doi.org/10.2337/dc16-0619)

38. Lynch CJ & Adams SH. Branched-chain amino acids in metabolic signalling and insulin resistance. *Nature Reviews: Endocrinology* 2014 10 723–736. (https://doi.org/10.1038/merodo.2014.171)

39. Owei I, Umekwe N, Stentz F, Wan J & Dagogo-Jack S. Amino acid signature predictive of incident prediabetes: a case-control study nested within the longitudinal pathobiology of prediabetes in a biracial cohort. *Metabolism: Clinical and Experimental* 2019 98 76–83. (https://doi.org/10.1016/j.metabol.2019.06.011)

40. Takahara T, Aminamiya Y, Sugiyama R, Maki M & Shihata H. Amino acid-dependent control of mTORC1 signaling: a variety of regulatory modes. *Journal of Biomedical Science* 2020 27 87. (https://doi.org/10.1186/s12929-020-00679-2)

41. Taylor R. Calorie restriction for long-term remission of type 2 diabetes. *Clinical Medicine* 2019 19 37–42. (https://doi.org/10.7861/clinmedicine.19-1-37)

42. Rathwa N, Patel R, Palit SP, Parmar N, Rana S, Ansari MI, Ramachandran AV & Begum R. AMPK and beta-cell markers. *Journal of Diabetes Research* 2019 2019 2019–2024. (https://doi.org/10.1155/2019/2019–2024)

43. John JA, Shah S, Singh P, Patel N, Patel H, Singh S, Shah P, et al. Amino and β-cell failure in rodent models of type 2 diabetes. *Diabetologia* 2012 55 3302–3309. (https://doi.org/10.2337/dc12-1428)

44. Delghingaro-Augusto V, Nolan CJ, Gupta D, Jetton TL, Latour MG, Peshavaria M, Madiraju SR, Joly E, Peyot ML, Prentki M, et al. Islet beta cell failure in the 60% pancreatectomised obese hyperlipidaemic Zucker fatty rat: severe dysfunction with altered glycerolipid metabolism without steatosis or a falling beta cell mass. *Diabetologia* 2009 52 1122–1132. (https://doi.org/10.1007/s00125-009-1317-8)

45. Nolan CJ, Leahy JL, Delghingaro-Augusto V, Mohi J, Soni K, Peyot ML, Fortier M, Guay C, Lamontagne J, Barbeau A, et al. Beta cell compensation for insulin resistance in Zucker fatty rats: increased lipolysis and fatty acid signalling. *Diabetologia* 2006 49 2120–2130. (https://doi.org/10.1007/s00125-006-0305-5)

46. Taylor R, Al-Mabheb A & Sattar N. Understanding the mechanisms of reversal of type 2 diabetes. *Lancet: Diabetes and Endocrinology* 2019 7 726–736. (https://doi.org/10.1016/S2213-8587(19)30076-2)

47. Taylor R, Al-Mabheb A, Ziyazhneuskiya S, Peters C, Barnes AC, Arbisala BS, Hollingsworth KG, Mathers JC, Sattar N & Lean MEJ. Remission of human Type 2 diabetes requires decrease in liver
and pancreas fat content but is dependent upon capacity for β-cell recovery. Cell Metabolism 2018 28 547.e543–556.e543. (https://doi.org/10.1016/j.cmet.2018.07.003)

56 Marselli L, Pitron A, Suleiman M, Collil ML, Yi X, Khamsi A, Carrat GR, Ratter GA, Bugliani M, Giusti L, et al. Persistent or transient human β-cell dysfunction induced by metabolic stress: specific signatures and shared gene expression with type 2 diabetes. Cell Reports 2020 33 108466. (https://doi.org/10.1016/j.celrep.2020.108466)

57 Song M & Cubillós-Ruiz JR. Endoplasmic reticulum stress responses in intratumoral immune cells: implications for cancer immunotherapy. Trends in Immunology 2019 40 128–141. (https://doi.org/10.1016/j.it.2018.12.001)

58 Arunagiri A, Haataja L, Pottekat A, Pamenen E, Kim S, Zeltser LM, Paton AW, Paton JC, Tsai B, Itkin-Ansari P, et al. Proinflammation is an early event in the progression to type 2 diabetes. eLife 2019 8 e44532. (https://doi.org/10.7554/eLife.44532)

59 Cnop M, Tovonien S, Iogil-Éstive M & Salpea P. Endoplasmic reticulum stress and eIF2α phosphorylation: the Achilles heel of pancreatic β-cells. Molecular Metabolism 2017 6 1024–1039. (https://doi.org/10.1016/j.molmet.2017.06.001)

60 Thomas SE, Dalton LE, Daly ML, Malzer E & Marciniak SJ. Obese and endoplasmic reticulum stress in β-cell dysfunction induced by metabolic stress: specific signatures and shared gene expression with type 2 diabetes. Cell Reports 2020 33 108466. (https://doi.org/10.1016/j.celrep.2020.108466)

61 Kluth O, Mirhashemi F, Scherneck S, Kaiser D, Kluge R, Neschen S, Sachdeva MM, Claiborn KC, Khoo C, Yang J, Groff DN, Mirmira RG & Cnop M. Endoplasmic reticulum stress, O1 (FOXO1) in glucose-induced β-cell failure. Diabetes/Metabolism Research and Reviews 2019 35 755–769. (https://doi.org/10.1002/dmrr.1132)

62 Siezckar M, Page MM, Panzhinskyi E, Skovor S, Mohijan M, Fernandez-Tajes J, Bruin JE, Brouard MJ, Lee JT, Xu EE, et al. Reduced insulin production relieves endoplasmic reticulum stress and induces β-cell proliferation. Cell Metabolism 2016 23 179–193. (https://doi.org/10.1016/j.cmet.2015.10.016)

63 Das I, Kryzosiak A, Schneider K, Wrabetz L, D’Antonio M, Barry N, Sigurdardottir A & Bertolotti A. Preventing proteostasis diseases by selective inhibition of a phosphatase regulatory subunit. Science 2015 348 239–242. (https://doi.org/10.1126/science.aaa4484)

64 Marín-SZ, Prins JB & McGuckin MA. Oxidative and endoplasmic reticulum stress in β-cell dysfunction in diabetes. Journal of Molecular Endocrinology 2016 56 R33–R54. (https://doi.org/10.1530/JME-15-0232)

65 Mohan S, R PRM, Brown L, Ayappan P & G RK. Endoplasmic reticulum stress: a master regulator of metabolic syndrome. European Journal of Pharmacology 2019 860 172533. (https://doi.org/10.1016/j.ejphar.2019.172533)

66 Thomas SE, Dalton LE, Daly ML, Malzer E & Marciniak SJ. Obese and endoplasmic reticulum stress in β-cell dysfunction induced by metabolic stress: specific signatures and shared gene expression with type 2 diabetes. Cell Reports 2020 33 108466. (https://doi.org/10.1016/j.celrep.2020.108466)
84 Erdmann E, Dormandy J, Wilcox R, Massi-Benedetti M & Charbonnel B. PdR activation 07: pigliotazone in the treatment of type 2 diabetes: results of the PRadactive study. Vascular Health and Risk Management 2007 3 355–370.

85 Lupi R, Del Guerra S, Bieroziklii M, Boggii U, Mosca F, Piro S, Del Prato S, et al. Lipotoxicity in human pancreatic islets and the protective effect of metformin. Diabetes 2002 51 (Supplement 1) S134–S137. (https://doi.org/10.2337/diabetes.51.2007.134)

86 Shiraki A, Oyama J, Komoda H, Asaka M, Komatsu S, Sakuma M, Kodama K, Sakamoto Y, Kotooka N, Hirase T, et al. Glucagon-like peptide 1 analog lipiglitzon reduces TNF-α-induced oxidative stress and inflammation in endothelial cells. Atherosclerosis 2012 221 375–382. (https://doi.org/10.1016/j.atherosclerosis.2011.12.009)

87 Natalicchio A, Labaruta R, Tortosa E, Biondi G, Maratou E, et al. Exendin-4 protects pancreatic beta cells from palmitate-induced apoptosis by interfering with GPR40 and the MKK4/7 stress kinase signalling pathway. Diabetesologia 2013 56 2456–2466. (https://doi.org/10.1007/s00125-013-3028-4)

88 Cunha DA, Ladrèire L, Ortil F, Igliolo-Esteve M, Guzvrn EN, Lupi R, Marchetti P, Ezizirk DL & Cnop M. Glucagon-like peptide-1 agonists protect pancreatic beta-cells from lipotoxic endoplasmic reticulum stress via upregulation of Bip and HspB. Diabetes 2009 58 2861–2862. (https://doi.org/10.2337/db09-0685)

89 Ojima A, Matsui T, Nishino Y, Nakamura N & Yamagishi S. Empaflitilozin, an inhibitor of sodium-glucose cotransporter 2 exerts anti-inflammatory and antiobiotic effects on experimental diabetic nephropathy partly by suppressing AGEs-receptor 2 activation. Hormone and Metabolic Research 2015 47 686–692. (https://doi.org/10.1055/s-0034-1395609)

90 Papachristoforou E, Lambardi V, Maratou E & Makrilakis K. Association of glycemic indices (hyperglycemia, glucose variability, and hypoglycemia) with oxidative stress and diabetic complications. Journal of Diabetes Research 2020 2020 7489795. (https://doi.org/10.1155/2020/7489795)

91 Zhang H & Pollin TI. Epigenetics variation and pathogenesis in diabetes. Current Diabetes Reports 2018 18 121. (https://doi.org/10.1007/s11892-018-1091-4)

92 You L, Wang N, Yin D, Wang L, Jin F, Zhu Y, Yuan Q & De W. Downregulation of long noncoding RNA Meg3 induces insulin synthesis and secretion in mouse pancreatic beta cells. Journal of Cellular Physiology 2016 231 852–862. (https://doi.org/10.1002/jcp.25175)

93 Zhu Y, Sun Y, Zhou Y, Zhang Y, Zhang T, Li Y, You W, Chang X, Yuan L & Han X. MicroRNA-24 promotes pancreatic beta cells toward dedifferentiation to avoid endoplasmic reticulum stress-induced apoptosis. Journal of Molecular Cell Biology 2019 11 747–760. (https://doi.org/10.1093/jmcb/mjz004)

94 Sebastiani G, Grieco GE, Brusco N, Ventriglia G, Formichi C, Marselli L, Marchetti P & Dotta F. MicroRNA expression analysis of in vitro dedifferentiated human pancreatic islet cells reveals the activation of the pluripotency-related microRNA cluster mir-302s. International Journal of Molecular Sciences 2016 19 1170. (https://doi.org/10.3390/ijms19041170)

95 Akerman I, Tu Z, Noekh J, Pasquali L, et al. Human pancreatic β cell lncRNAs control cell-specific regulatory networks. Cell Metabolism 2017 25 400–411. (https://doi.org/10.1016/j.cmet.2016.11.016)

96 Arnes J & Sussel L. Epigenetic modifications and long noncoding RNAs influence pancreas development and function. Trends in Genetics 2015 31 290–299. (https://doi.org/10.1016/j.tig.2015.02.008)

97 Wei R & Hong T. Lineage reprogramming: a promising road for pancreatic β cell regeneration. Trends in Endocrinology and Metabolism 2016 27 163–176. (https://doi.org/10.1016/j.tem.2016.01.002)

98 Zhang J & Liu F. The de-, re-, and trans-differentiation of pancreatic β cells: regulation and function. Seminars in Cell and Developmental Biology 2020 103 68–75. (https://doi.org/10.1016/j.semcdb.2020.01.003)

99 Thorel F, Népoté V, Ayrii J, Kohku K, Desgraz R, Chersa S & Herrera PL. Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. Nature 2010 464 1149–1154. (https://doi.org/10.1038/nature08894)

100 Wang RN, Kloppe G & Bouwens L. Duct- to islet-cell differentiation and islet growth in the pancreas of duct-ligated adult rats. Diabetologia 1995 38 1405–1411. (https://doi.org/10.1007/BF00400600)

101 Pan FC, Bankaitis ED, Boyer D, Xu X, Van de Casteele M, Magnuson MA, Heimbeg H & Wright CV. Spatiotemporal patterns of multipotentiality in Ptf1a-expressing cells during pancreas organogenesis and injury-induced facultative restoration. Development 2013 140 751–764. (http://www.ncbi.nlm.nih.gov/pubmed/23422743)

102 Chakravarty H, Gu X, Enge M, Dal X, Wang Y, Dandm N, Conway C, Liu K, Wang J, Xing Y, et al. Converting adult pancreatic islet cells into β cells by targeting both Dnmt1 and Arx. Cell Metabolism 2017 25 622–634. (https://doi.org/10.1016/j.cmet.2017.01.009)

103 Collombat P, Xu X, Ravassard P, Sosa-Pineda B, Dussaud S, Billestrup N, Madsen OD, Serup P, Heimbeg H & Mansouri A. The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into alpha and subsequently beta cells. Cell 2009 138 449–462. (https://doi.org/10.1016/j.cell.2009.05.035)

104 Xiao X, Guo P, Shiota C, Zhang T, Couridet GM, Fischbach S, Prasad K, Fudos J, Ramachandran S, Wiktowski P, et al. Endogenous reprogramming of alpha cells into beta cells, induced by viral gene therapy, reverses autoimmune diabetes. Cell Stem Cell 2018 22 78, e4–90.e4. (https://doi.org/10.1016/j.stem.2017.11.020)

105 Assouline-Thomas B, Ellis D, Petropavlovskia M, Makhljin J, Ding J & Rosenberg L. Islet neogenesis associated protein (INGAP) induces the differentiation of an adult human pancreatic ductal cell line into insulin-expressing cells through stepwise activation of key transcription factors for embryonic beta cell development. Differentiation: Research in Biological Diversity 2015 90 77–90. (https://doi.org/10.1016/j.diff.2015.07.008)
toward engineering and transplanting the bioartificial pancreas. *Transplant International* 2020 33 1577–1588. (https://doi.org/10.1111/tri.13721)

138 Talchai C, Xuan S, Lin HV, Sussel L & Accili D. Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. *Cell* 2012 150 1223–1234. (https://doi.org/10.1016/j.cell.2012.07.029)

139 McCracken KW, Catá EM, Crawford CM, Sinagoga KL, Schumacher M, Rockich BE, Tsai YH, Mayhew CN, Spence JR, Zavros Y, et al. Modelling human development and disease in pluripotent stem-cell-derived gastric organoids. *Nature* 2014 516 400–404. (https://doi.org/10.1038/nature13863)

140 Ramasubramanian L, Kumar P & Wang A. Engineering extracellular vesicles as nanotherapeutics for regenerative medicine. *Biomolecules* 2019 10 48. (https://doi.org/10.3390/biom10010048)

141 Xie M, Ye H, Wang H, Charpin-El Hamri G, Lormeau C, Saxena P, Stelling J & Fussenegger M. β-Cell-mimetic designer cells provide closed-loop glycemic control. *Science* 2016 354 1296–1301. (https://doi.org/10.1126/science.aaf4006)

142 Ye H, Daoud-El Baba M, Peng BW & Fussenegger M. A synthetic optogenetic transcription device enhances blood-glucose homeostasis in mice. *Science* 2011 332 1565–1568. (https://doi.org/10.1126/science.1203535)

143 Shao J, Xue S, Yu G, Yu Y, Yang X, Bai Y, Zhu S, Yang L, Yin J, Wang Y, et al. Smartphone-controlled optogenetically engineered cells enable semiautomatic glucose homeostasis in diabetic mice. *Science Translational Medicine* 2017 9 eaal2298. (https://doi.org/10.1126/scitranslmed.aal2298)

144 Desai T & Shea LD. Advances in islet encapsulation technologies. *Nature Reviews: Drug Discovery* 2017 16 338–350. (https://doi.org/10.1038/nrd.2016.232)

145 Balboa D, Prasad RB, Groop L & Otonkoski T. Genome editing of human pancreatic beta cell models: problems, possibilities and outlook. *Diabetologia* 2019 62 1329–1336. (https://doi.org/10.1007/s00125-019-4908-z)

146 Mourad NI & Gianello P. Gene editing, gene therapy, and cell xenotransplantation: cell transplantation across species. *Current Transplantation Reports* 2017 4 193–200. (https://doi.org/10.1007/s40472-017-0157-6)

147 Ferreira LMR, Muller YD, Bluestone JA & Tang Q. Next-generation regulatory T cell therapy. *Nature Reviews: Drug Discovery* 2019 18 749–769. (https://doi.org/10.1038/s41573-019-0041-4)

148 Li D, Zhao B, Luo Y, Limbara S, Zhao X, Zou X, Yang W & Li Y. Transplantation of aircr-overexpressing bone marrow-derived dendritic cells delays the onset of type 1 diabetes. *International Immunopharmacology* 2017 49 13–20. (https://doi.org/10.1016/j.intimp.2017.05.023)

149 Chen Z, Wang J, Sun W, Archibong E, Kahlkoska AR, Zhang X, Lu Y, Ligler FS, Buse JB & Gu Z. Synthetic beta cells for fusion-mediated dynamic insulin secretion. *Nature Chemical Biology* 2018 14 86–93. (https://doi.org/10.1038/nchembio.2511)

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