Targeting pancreatic β-cells for diabetes treatment

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Abstract

Insulin is a life-saving drug for patients with type 1 diabetes, however, even today no pharmacotherapy can prevent the loss or dysfunction of pancreatic insulin-producing β-cells to stop or reverse disease progression. Thus, pancreatic β-cells have been a main focus for cell-replacement and regenerative therapies as a curative treatment for diabetes. In this Review, we highlight recent advances towards the development of diabetes therapies that target β-cells to enhance proliferation, redifferentiation, protection from cell death and/or enable selective killing of senescent β-cells. We describe currently available therapies and their mode of action, as well as insufficiencies of GLP-1 and insulin therapies. We discuss and summarize data collected over the last decades that support the notion that pharmacological targeting of β-cell insulin signaling might protect and/or regenerate β-cells as an improved treatment of patients with diabetes.

(I) Introduction:

This year marks the 101th anniversary of the discovery of the life-saving drug insulin by Sir Frederick G. Banting and Charles H. Best[1,2]. The discovery of insulin in 1921 was one of the most important findings of the 20th century in biomedical research, as it enabled control of an otherwise deadly disease. Since then, there has been astounding progress in the understanding of the molecular action of insulin. Insulin is secreted by the β-cells of the pancreatic islets of Langerhans in response to elevated blood glucose levels[3,4]. β-cell failure and insulin resistance in peripheral organs, such as muscle, liver and adipose tissue causes onset and progression of type 2 diabetes (T2D)[3,5–7]. Both hypersecretion and deficiency of insulin, as well as insulin resistance are early warning signs of T2D. Therefore, insulin and insulin-like growth factor 1 (IGF1) signaling has been a focus of study in recent decades.

Although there is no consensus among researchers if insulin resistance or insulin hypersecretion is the root cause of diabetes[8], it is clear that loss of regulated insulin secretion due to β-cell dysfunction or β-cell loss marks the onset of overt diabetes. Insulin, IGF1, and IGF2 ligands are known to regulate growth, proliferation and metabolism of almost all cells in the mammalian body, including pancreatic β-cells. The effect of insulin/IGF signaling on β-cells has been studied in both *in vitro* and *in vivo* model systems and it is crucially important for β-cell function, survival and compensatory proliferation[9–13], which makes this pathway an attractive target for diabetes therapy.

In this Review, we will first introduce the islet of Langerhans, the endogenous niche of the β-cells and their heterogeneity within the islet. Over the last decades, the insulin/IGF signaling has emerged as an important regulator for β-cell function, growth, and proliferation. Loss-of-function of both insulin and IGF1 receptors in pancreatic β-cells has been reported to cause overt diabetes, illustrating the importance of this pathway for β-cell function. We will summarize both *in vitro* and *in vivo* studies with a focus on β-cell specific perturbation of insulin/IGF signaling that has been reported to cause β-cell failure and/or development of diabetes. We also described the autocrine actions of insulin hormone on the pancreatic β-cells and how β-cells shield themselves from constitutive insulin/IGF pathway activation. Targeting pancreatic β-cells to control

hyperglycaemia by promoting β-cell proliferation and by preserving functional β-cell mass is gaining attention for developing new strategies for diabetes treatment.

(II) Comparing architecture and cellular heterogeneity of mice and human islets

Given the high prevalence of diabetes, the structure of the human pancreas has been studied at large but limited to biopsies or donor samples and islet isolations. Therefore, in most cases, the data available come from mouse studies. The cellular composition of the adult mouse islet comprises of 75% β-cells, 20% α-cells and 5% δ-cells as compared to 55% β-cells, 35% α-cells and 10% δ-cells in human islets[14,15] (Figure 1).

The arrangement of α- and β-cells in mouse and human islets are very distinct[16]. Mouse islets have the majority of β-cells located in the islet core that contact other β-cells. In contrast, human islets have a greater or similar proportion of α-cells to β-cells and β-cells more frequently have direct contact to α-cells[15,17,18]. Mouse islets are dependent on homotypic cellular communication between β-cells, resulting in the formation of a functioning syncytium with coordinated islet activity, whereas human islets are rich in heterotypic islet cell contacts[14,18,19].

It is well documented that murine β-cells are in proximity to other β-cells but only a small percentage are in direct contact to α-cells. The implications for paracrine interactions between α- and β-cells for the tight regulation of glucose homeostasis under healthy and diabetic conditions are still under investigation. It is known that glucose homeostasis is dependent not just on β-cells, but also on the overall balance of insulin and other counter-regulatory hormones, such as glucagon. Single cell RNA sequencing (scRNA-seq) technologies have revealed cellular heterogeneity within islet endocrine cell types that constitute diverse cell functions[20–22]. β-cells exist as heterogeneous subpopulations depending on islet architecture, but also maturation, physiological and pathological state[23,24]. Combining pharmacology with scRNA-seq and diabetes disease models have the power to define de- and redifferentiation trajectories and mechanism-of-action of drugs to define intervention points. These techniques have uncovered that insulin therapy not only avoids β-cell glucotoxicity by glucose uptake into metabolic tissues, but also directly acts on β-cells to trigger β-cell redifferentiation. Understanding β-cell

heterogeneity in health and disease and specifically identifying pathways that protect from stress-mediated cell death or pathways that can restore β-cell function will be crucial[25].

(III) Targeting β-cells for causal diabetes therapy

(a) Improving *in vivo* β-cell insulin signaling: What do we know?

One of the earliest studies by Iversen and Miles in 1972 found that insulin has an inhibitory autocrine effect in the isolated and perifused canine pancreas[26] and it was later demonstrated to diminish endogenous insulin synthesis[27]. To investigate the short- and long-term effect of eliminating insulin receptor (IR) signaling *in vivo*, an extremely valuable mouse model was generated using the Cre-LoxP system to conditionally inactivate the *IR* gene in a tissue-specific and β-cells selective manner (βIRKO)[9,28]. Using this model, it was shown that mice lacking IR in β-cells have blunted first-phase insulin secretion, loss of the glucose transporter (Glut2), insufficient functional β-cell mass, and profound glucose intolerance, a phenotype similar as seen in T2D (Figure 2)[9,28,29]. Furthermore, targeted deletion of IGF1 receptor (IGF1R) in β-cells using the rat Ins2 promoter driven Cre recombinase (βIGF1R-KO) showed no effect on β-cell mass[30]. β-cell double knockouts of IR and IGF1R enhanced apoptosis accompanied by reduced β-cell mass, hyperglycemia, and glucose intolerance[11].

George *et al.* have shown that overexpression of IGF1 in β-cells induces β-cell replication and proliferation and allows transgenic mice to recover β-cell mass[31]. Mice lacking IR in β-cells do not show compensatory β-cell mass increase that accompanies a high-fat diet[28]. The βIRKO mice were generated with a rat Ins2 promoter-driven Cre (RIP-Cre) recombinase, which was subsequently shown to also recombine in the brain owing to endogenous Ins2 expression in the brain and thymus[32–35]. In another study, mouse Ins1 promoter-driven tamoxifen-inducible Cre-recombinase IR knockout (MIP-βIRKO) were generated to study the loss of β-cell IR during pancreatic development and have identified an islet compensatory overgrowth phenotype[36]. In contrary, Ins1-Cre allele was used recently to delete IR specifically in β-cells of both male and female mice. Deletion of IR induces glucose-stimulated insulin secretion by enhancing action potential firing and Ca^{2+}-oscillation frequency, causing improved glucose tolerance in insulin sensitive animals[37]. A model was suggested in which insulin inhibits its own secretion which has

been shown to have physiological consequences for glucose tolerance. Multiple different conditions including Cre-driver lines, genetic backgrounds of mice, diets, housing conditions, microbiome, or glucose concentrations could contribute to observed differences between different β-cell specific IR knockout mouse models [9,28,29]. Furthermore, it was reported by Brouwers et al, that the human growth hormone (*hGH*) minigene (added to constructs to increase transcription efficiency) could also express bioactive growth hormone[38]. The glucose intolerance phenotypes in mice lines like *MIP*-CreERT^{1Lphi} (expressing the *hGH* minigene) should be interpreted with proper "Cre-only" controls when assessing proliferation, cell survival or β-cell mass. Alternatively, the mice lines *Ins1*-Cre^{Thor} (driving constitutive Cre expression in the β-cells) and *Ins1*-CreERT^{Thor} (tamoxifen-inducible Cre) are best selections for studying β-cell-specific expression as both these mice lines use a knock-in approach to the *Ins1* gene locus and eliminate the *hGH* minigene problem[39].

Despite the controversy about the function of IR in positive or negative feedback regulation of glucose-stimulated insulin secretion, it is clear that any disruption of signaling components downstream of the insulin/IGF-receptors have been reported to cause dysregulation of β-cell mass and development of diabetes in mice. For example, ablation of pyruvate dehydrogenase kinase 1 (PDK1) in pancreatic β-cells result in progressive hyperglycemia due to loss of β-cell mass (Figure 2)[40]. In addition, mice with reduced Akt activity in their islet β-cells exhibit impaired glucose tolerance due to defective insulin secretion[41]. Downstream, Akt phosphorylates forkhead box O (FoxO) transcription factor, leading to its nuclear exclusion and inactivation. A gain-of-function mutation of *FoxO1* targeted to pancreatic β-cells results in metabolic abnormalities similar to T2D[42]. Growth factors and nutrients are important regulators of β-cell mass and function. Tuberous sclerosis complex 2 (Tsc2) is crucial for this process, since it is a converging point for both growth factor and nutrient signals in β-cells. Disruption of Tsc2/mTOR (mammalian target of rapamycin) pathway in pancreatic β-cells has been shown to induce expansion of β-cell mass by increased proliferation and cell size and also improves glucose tolerance in mice[43]. Altogether, these studies strongly suggest that both insulin/IGF-signaling and its downstream components are crucially important for β-cell growth, proliferation and

function and any perturbations (genetic or pharmacological) would lead to β-cell failure and onset of diabetes.

1. Autocrine action of insulin on β-cells:

Insulin itself is known to have an autocrine effect on pancreatic β-cells. The first molecular evidence of autocrine insulin action on β-cells came from two studies in 1995, where the authors have reported activation of β-cell insulin receptor and its downstream targets by secreted insulin[44,45]. Mice lacking different components of the insulin signaling pathway have demonstrated both positive and negative actions of insulin on the function and survival of pancreatic β-cells[46].

Positive autocrine action involves secretion of insulin from β-cells in response to glucose via activation of its gene transcription[47–51]. This positive feedback loop provides a physiological mechanism to enhance insulin production in the face of increased glucose concentrations. Positive autocrine action of insulin also inhibits β-cell apoptosis and increases β-cell proliferation, differentiation, and survival[52–54]. In contrast, insulin has negative autocrine effects when β-cells are exposed to very high concentration of insulin. Short-term inhibitory action of insulin on its own synthesis allows β-cells to stop insulin secretion when blood glucose reaches normal physiological levels[26,55–57]. This inhibition prevents excessive synthesis and secretion of insulin into the bloodstream. A critical unanswered question is what the physiological concentration of insulin around β-cells is?

Since insulin is secreted from β-cells, pancreatic cells must be constitutively exposed to locally secreted insulin. If so, mechanisms must have evolved to shield the pancreatic islets and β-cells from insulin auto- and paracrine action and constitutive pathway activation in islets of Langerhans. Moreover, as β-cells are the source of insulin, concentrations are likely high enough to trigger the high affinity IR and lower affinity IGF1R simultaneously. It is therefore intriguing to know what are the molecular mechanisms causing insulin/IGF signaling desensitization in β-cells. A few arguments have been put forward in order to address this question. For example: (1) it has been reported that the IR gets internalized into endosomal compartments upon binding to its ligand insulin, leading to reduced availability at the plasma membrane and thus

contributing to the desensitization process[58], and (2) prolonged and/or exposure to high concentration of insulin effectively desensitize insulin receptor substrate (IRS) signaling pathway via promoting IRS1/2 degradation as well as downregulate expression of insulin receptor as a feedback mechanism[59]. However, these studies were not β-cell specific and therefore insulin signaling feedback mechanisms may differ in a cell- and context-dependent manner.

2. Protective feedback mechanisms desensitize β-cell insulin signaling

Prolonged exposure of insulin to β-cells is also reported to cause insulin resistance and development of diabetes[60–62]. However, it is well known that β-cells express insulin/IGF receptors as well as its downstream components suggesting that insulin/IGF signaling plays a crucial part in β-cell function. This observation indicates that different feedback mechanisms might operate in different tissues, depending on the following criteria: 1) concentration of insulin surrounding those cells and/or 2) duration of signaling amplitude i.e., short term and prolonged exposure to insulin.

It was reported that insulin secretion is enhanced from isolated mouse islets at low insulin concentrations (0.05 - 0.1 nM), whilst insulin secretion is inhibited at an insulin concentration above 250 nM[57,63]. Efforts have been made to directly measure the pulsatile insulin secretion from the portal vein in humans which estimated an approximately 5-fold increase in insulin concentration in the portal vein compared to arterialized vein[64]. In a mathematical modelling study to estimate local insulin concentration within the islet extracellular space, the total insulin concentrations in its monomeric form needed for activating IR was in the picomolar range (around 300 pM), regardless of glucose stimulation[65]. Therefore, insulin concentration must be high in the islet milieu and the islet cells must be exposed consistently to high concentrations of insulin. In such a scenario, it would not be surprising if pancreatic islet cells needed an additional mechanism to desensitize insulin signaling compared to cells in peripheral tissues, such as muscle, liver and adipose tissue. Moreover, islet cells likely also evolved effective feedback mechanisms during islet neogenesis or diabetes progression, when islet architecture, cell-type composition (endocrine, mesenchymal, endothelial and immune cells), β-cell

state and function as well as IR and IGF1R ligand concentrations (insulin, IGF1 and IGF2) are dynamically changing.

In 2021, a novel insulin inhibitory receptor (in short 'inceptor') was described that calibrates insulin action in β-cells[65,66]. Inceptor is highly expressed in the pancreas and negatively regulates insulin/IGF-signaling in mouse β-cells *in vivo* to control glycemia. Insulin/IGF receptor signaling is crucial for β-cell proliferation, homeostasis and function. The idea that inceptor shields β-cells from insulin auto- and paracrine activation is supported by the findings that mice lacking inceptor have an increased β-cell insulin/IGF signaling as well as increased β-cell proliferation and mass compared to their wild-type controls. Furthermore, inceptor interacts with insulin/IGF-receptor to facilitate clathrin-mediated endocytosis of activated receptor complexes in β-cells (Figure 3). Thus, inceptor provides an additional mechanism to desensitize the insulin signaling pathway in β-cells, which are the source of insulin. Importantly, monoclonal antibodies targeted against the ectodomain of inceptor have been shown to blocked the interaction between Inceptor and IR, leading to the retention of both receptors on the plasma membrane to sustain the activation of insulin/IGF-signaling in mouse and human β-cells (Figure 3). Therefore, inceptor might constitute a molecular target to sensitize insulin/IGF-signaling in pancreatic β-cells to enhance function, proliferation and survival for diabetes therapy. The identification of new regulators of insulin/IGF signaling in β-cells further increases our understanding that insulin/IGF signaling plays a key role in β-cell biology and provides novel targets for pharmacological intervention.

(b) Controlled enhancement of β-cell proliferation

During the fetal and neonatal stages of development, pancreatic β-cells readily replicate after which the proliferation rate rapidly declines. The bulk of insulin-positive β-cells emerge around embryonic day 12.5 in mice and gestational weeks 8–9 in humans during embryonic development and in the fetal stage are generated by the endocrine progenitor cells[67].

In humans, β-cells mass in adults roughly constitutes 2% of total pancreatic mass, corresponding to 1–2 g of tissue[68]. Human β-cells mainly replicate during the initial period

of life which defines the β-cell mass in adulthood[69–73]. Due to the ethical challenges and restraints of investigating the human pancreas *in vivo*, analysis of human β-cell mass and turnover is limited. As a result, most of the research is restricted to post-mortem tissue examination or *in vitro* studies of cells derived from cadaveric pancreases. Because human pancreatic samples are intrinsically varied, reliable findings must be drawn from a large number of donors[69,74,75].

Using mass cytometry, Wang *et. al.* in 2016 identified proliferative subpopulations of β-cell from human donors, revealing heterogeneity within proliferating β-cells[76]. An important question would be – what are the signals and triggers that allow a subpopulation of β-cells to divide but others not? For instance, less mature, dedifferentiated or naïve β-cells could still have the potential to divide? It will thus be important to understand if the progression of type 1 or 2 diabetic condition triggers β-cell dedifferentiation and allows the cells to proliferate and compensate for metabolic needs.

Pathways and mechanisms that regulate β-cell proliferation have been extensively studied [77–79]. Although our understanding of why the adult human β-cell is recalcitrant to proliferation when compared to other cell types is limited, there are numerous reports demonstrating upregulation of cell cycle inhibitors within β-cells. Several pathways are known to promote β-cell replication, including the IRS-phosphoinositide 3-kinase (IRS-PI3K), glycogen synthase kinase 3 (GSK3), Calcineurin/nuclear factor of activated T cells (Calcineurin/NFAT), carbohydrate-responsive element-binding protein (ChREBP), mTOR and extracellular signal-regulated kinase (Erk) pathway. Glucose, glucagon-like peptide 1 (GLP-1), and insulin have been shown to activate mitogenic signaling via the PI3K/AKT/mTOR pathway. Glucose and insulin are known inducers of β-cell replication, increasing replication in both mouse and human β-cells after glucose infusion[80,81].

One of the challenges in the field is to identify pathways to restore proliferative capacity of adult β-cells. Cell cycle entry and progression is driven by cyclin dependent kinases (CDKs). CDK inhibitors are upregulated in cells that exit the cell cycle. This is also the case in adult β-cells, where CDK4 and CDK inhibitors, such as p16, p18 and p21 are key regulators of β-cell replication. In principle, β-cell regeneration may occur as a result of

replication of pre-existing β-cells, transdifferentiation of non-β-cells into β-cells or β-cell neogenesis from ductal progenitors.

Kondegowda et al identified Osteoprotegerin as a β-cell mitogen and enhanced β-cell proliferation in young, adult, and diabetic mice increasing overall β-cell mass and delaying hyperglycemia in diabetic mice. Moreover, Osteoprotegerin induced human β-cell replication by activating cAMP-response element binding protein (CREB) and GSK3 pathways, via binding to receptor activator of NF-κB (RANK) ligand (RANKL) which essentially acts as a brake in proliferation[82]. In a high-throughput RNAi screen, Robitaille et al identified that the cell cycle inhibitors p18 and p21 are essential for maintenance of adult human β-cell quiescence and thus proposed p18 and p21 as potential targets for promoting proliferation of human β-cells[83]. Another novel class of drugs that induce robust proliferation of human β-cells are Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1A (DYRK1A) inhibitors. Inhibition of DYRK1A by small molecule inhibitors such as 5-iodotubercidin (5-IT), Harmine and GNF4877 has been shown to stimulate human β-cell proliferation[84–88]. Together, these studies demonstrate that human β-cells also possess the potential to proliferate which may offer a strategy to promote human β-cell regeneration.

(c) Protection from β-cell death

1. Cellular mechanism and mediators of β-cell death:

Apoptotic death of pancreatic β-cells is a characteristic of both type 1 and type 2 diabetes and is induced by various etiological factors including glucotoxicity, lipotoxicity, pro-inflammatory mediators and oxidative insults. The triggering mechanisms, however, remain elusive. These etiological factors individually elicit stress pathways, such as oxidative stress, endoplasmic reticulum (ER) stress, mitochondrial alteration, inflammation and disruption of protein and lysosomal degradation pathways. A combination of these stress pathways as well as the cross-talk between them contributes to β-cell apoptosis.

Pancreatic β-cells are highly sensitive to blood glucose concentrations and any changes in glucose homeostasis influence β-cell function. In fact, chronic exposure to a very high

blood glucose has detrimental effects on insulin synthesis and secretion as well as cell survival. Glucotoxicity is capable of causing dysfunction of the pancreatic β-cells through multiple mechanisms. Prolonged exposure to high levels of glucose causes a gradual decrease in the expression and activity of key β-cell transcription factors, such as pancreatic and duodenal homeobox 1 (PDX1) and MAF BZIP transcription factor A (MAFA)[89–91]. This repression affects the regulation of multiple genes implied in the function of pancreatic β-cells, including proinsulin[92–94]. Similarly, pancreatic β-cells are particularly sensitive to ER stress due to their high rates of proinsulin biosynthesis in response to glucose stimulation[95]. Glucotoxicity increases insulin synthesis, which leads to the accumulation of unfolded proteins in the ER and triggers a defense mechanism known as unfolded protein response (UPR) in an attempt to restore ER homeostasis[96]. However, unresolved UPR induces ER stress-mediated cell death[97,98]. Therefore, modulating UPR pathways might offer therapeutic opportunities to treat diabetes by promoting recovery from terminal ER stress[25].

Long-term hyperglycemia has also been shown to impair β-cell viability by inducing oxidative stress and mitochondrial apoptosis[99,100]. Altered iron metabolism has recently been shown to connect glucolipotoxicity to mitochondrial dysfunction, cytosolic reactive oxygen species (ROS) formation and apoptosis[101]. Clinical studies have shown that pancreatic islets from patients with T2D have elevated markers of oxidative stress, correlating with the degree of impairment in glucose stimulated insulin secretion (GSIS)[102].

Finally, evidence suggesting induction of non-immune mediated inflammatory pathways, like interleukin (IL)-1β, nuclear factor (NF)-kB and Fas receptors in the islets in response to chronic hyperglycemia[103,104], although other studies have failed to confirm these findings[105,106]. However, a recent study has linked both glucotoxicity and lipotoxicity to the secretion of S100A8 (a member of the damage-associated molecular pattern molecules -DAMPs) from the pancreatic islets, which promoted macrophage infiltration of the islets[107].

Diabetes is often associated with changes in lipoprotein profiles and increased free fatty acids (FFAs) concentrations[108]. Prolonged exposure to high circulating levels of FFAs

have shown to alter β-cell function and survival[94,96,109]. However, it is still an open question whether the increase in FFAs *in vivo* is enough to cause β-cell dysfunction[110–112]. Furthermore, the prolonged exposure of high FAs in hyperglycemic conditions has shown to decrease insulin gene expression[94]. Palmitate, which is a saturated FFA, is positively correlated with T2D incidence[113]. Furthermore, lysosomal degradation in pancreatic β-cells is impaired upon chronic exposure to palmitate[114,115]. Mechanistically, palmitate causes a defect in the lysosomal acidification and function[116,117], activates mTORC1[115] and ER stress-induced c-Jun N-terminal kinase (JNK) pathways[115,118]. Restoration of autophagic flux by stimulating autophagy (using the mTOR (mammalian target Of Rapamycin) inhibitor rapamycin) decreases palmitate-induced apoptosis in mouse and human β-cells[115,118–120].

Cellular senescence of pancreatic β-cells has recently received widespread attention, since it can lead to senescence and dysfunction of the neighboring cells through paracrine actions resulting in β-cell failure. Organismal ageing is associated with a gradual decline in β-cell replication and function, and such dysfunctional β-cells are thought to be involved in diabetes pathogenesis. Senescent β-cells activate a proinflammatory secretome referred to as senescence-associated secretory phenotype (SASP)[121,122].

SASP is characterized by the secretion of proinflammatory cytokines, chemokines, growth factors, extracellular matrix factors and metalloproteases by senescent cells that are highly immunogenic and mediate paracrine signaling[123–125]. A unique human and mouse β-cell SASP signature based on the proteomics analysis has recently been identified, which reveals enrichment for factors associated with inflammation, cellular stress response, and extracellular matrix remodeling across species[126].

The main purpose of SASP *in vivo* is thought to be immune surveillance and clearance of senescent cells from the tissue, which leads to resolution of inflammatory responses. However, β-cells continue to accumulate during T1D disease progression which further leads to unresolved SASP[121]. This study has revealed that during the progression of T1D in humans and non-obese diabetic (NOD) mouse models, some β-cells acquire SASP[121]. These senescent β-cells upregulate pro-survival regulator B-cell lymphoma-2 (Bcl-2), and treatment of mice with Bcl-2 inhibitors induces apoptosis and selectively clears these

senescent β-cells without altering the abundance of major immune cell types involved in the disease. Selective clearance of senescent β-cells prevents immune-modulated β-cell destruction and is sufficient to prevent diabetes in animal models. Why some senescent β-cells acquire SASP, whereas others not, is unclear. Moreover, the extrinsic and intrinsic factors triggering SASP are unknown? Emerging evidence shows that levels of DNA damage in β-cells increases with age, whereas the cellular DNA repair capacity gradually decreases, both of which predisposes β-cells to DNA-damage responsive (DDR) cellular senescence[127]. DNA-damage to β-cells in culture recapitulates some features of senescent β-cells that accumulate in T1D. Therefore, one can investigate the role of DNA damage as a model to understand the mechanism of β-cell senescence in T1D. It will also be intriguing to map transcriptional and chromatin changes in senescent β-cells with the ultimate aim to identify the key targets in order to hamper senescence phenotype.

2. Small molecule-based approach to reduce β-cell death:

Apoptotic death and senescence are the main contributors for the loss of β-cell mass in diabetes. Current treatments fail to maintain functional β-cell mass and therefore new therapies to prevent β-cell death are needed. Several key regulators have emerged in the last decade that can be used as potential therapeutic targets for diabetes treatment.

Mammalian sterile 20-like kinase-1 (MST1) has been shown to be a critical regulator of apoptotic β-cell death and function[128]. MST1 is activated under diabetic conditions and the activation of MST1 in mouse and human islets correlated with levels of β-cell apoptosis. Moreover, MST1 deficiency improves β-cell survival and function *in vitro* and *in vivo* and restores normoglycemia[128]. Therefore, MST1 inhibitors might protect β-cells from the effects of autoimmune attack in T1D and preserve β-cell mass and function in T2D.

Selective clearance of senescent β-cells using so-called senolytic compounds also hold significant promise in treating or preventing diabetes, or metabolic dysfunction more broadly. A recent study revealed the role of Bromodomain Extra-Terminal domain (BET) proteins in the transcriptional activation of senescent β-cells in the nonobese diabetic (NOD) mouse model (Figure 4). Inhibition of BET proteins using the small molecule

inhibitor, iBET-762, led to attenuated SASP gene expression, protein secretion and SASP paracrine activity from NOD islets[129]. (3) Another study demonstrated that insulin resistance accelerates β-cell senescence, leading to loss of β-cell identity and function. Treatment with a senolytic drug, ABT263, decreased the number of senescent β-cells and effectively restored β-cell function and identity, while improving glucose metabolism in insulin-resistant mice[130] (Figure 4).

Future studies should investigate the role of β-cell SASP in T1D in humans and address whether SASP components might serve as biomarkers for diabetes progression.

(d) β-cell dedifferentiation

The well-known reduction in β-cell mass over the course of T2D has long been assumed to be due to increased cell death. Interestingly, when analyzing the fate of β-cells using genetic lineage tracing studies in animal models of diabetes, it was revealed that β-cells dedifferentiate. This process involves loss of β-cell gene expression and identity, dedifferentiation to a progenitor-like phenotype and is viewed as one of the key events in the onset and progression of T2D[131,132].

Over the last decade, the concept of β-cell dedifferentiation has been established and was shown to contribute to the decrease of functional β-cell mass in diabetic mice and patients with T1D and T2D. β-cell dedifferentiation has been observed in mouse models of diabetes characterized by loss of expression of insulin, maturation markers (Glut2, Urocortin 3) and increased dedifferentiation marker expression, e.g. aldehyde dehydrogenase 1 family member A3 (Aldh1a3), Gastrin, cholecystokinin (CCK), aldolase-fructose bisphosphate B (Aldob) etc.[131,133–135]. In a recent study by Sachs et al in 2020, new markers and pathways associated with β-cell dedifferentiation were identified in a scRNA-seq comparison of mouse islets from wild-type and streptozotocin (STZ)-induced severely diabetic mice[135]. Importantly, dedifferentiated β-cells could be protected from ER stress-mediated cell death by targeted delivery of GLP-1-estrogen conjugate and it was further shown that insulin treatment triggered insulin receptor pathway activation in β-cells and restored β-cell identity and function for diabetes remission in mice. Furthermore, Camunas-Soler *et al*. used Patch-seq to analyze association between gene expression profiles with each phase of exocytosis of single cells from pancreatic islets. In this study,

the authors used T2D β-cells and found that the molecules that were positively associated with exocytosis in healthy donors were increased in T2D donors[136].

Studies of pathological specimens from patients with T2D suggest that β-cell dedifferentiation also contributes to diabetes progression in humans. In one such example, the increase in hormone-negative islet cells when compared to healthy controls was accompanied by a decrease in the number of insulin-positive cells expressing the transcription factors FOXO1 and NK6 homeobox 1 (NKX6-1)[134].

In the New Zealand Obese (NZO) mouse model, it was observed that hyperglycemia associated with rapid and marked dephophorylation and activation of FoxO1. Additionally, FoxO1 dephosphorylation was followed by the reduction of the glucose transporter Glut2 and several key β-cell transcription factors, which in turn increased the apoptosis rate[137]. Similarly, activation of FoxO1 in β-cells of diabetic *db/db* mice associates with β-cell dedifferentiation and three months of caloric restriction markedly reversed these alterations and normalized glycaemia and β-cell function[138]. Moreover, pharmacological inhibition of FoxO1 has shown to mimic β-cell dedifferentiation by downregulating β-cell specific transcription factors, resulting in aberrant expression of progenitor genes. Loperamide, a small molecule that prevents FoxO inhibition, stimulates insulin protein processing and secretion by altering intracellular FoxO localization and gene expression[139]. Recently, Oppenländer *et. al.*, demonstrated that vertical sleeve gastrectomy rapidly restored normoglycemia in morbidly obese and overt diabetic db/db mice that mirrors clinical features of β-cell failure and dedifferentiation. Vertical sleeve gastrectomy, a variant of bariatric surgery, improved glycemia and functional β-cell mass primarily by β-cell redifferentiation and proliferation in diabetic db/db mouse[140].

Butler *et. al.* in 2016 evaluated the concept of β-cell dedifferentiation in human islets and found that β-cell dedifferentiation in islets from patients with T2D is quantitatively small and is not only due to degranulation or dedifferentiation of β-cells[141]. β-cell degranulation is defined by judging the amount of secretory granules per β-cell, whereas dedifferentiation is defined by the concerted downregulation of β-cell maturity and identity genes. Thus, the analysis method matters and a combined and systematic analysis on the single-cell level is warranted to clarify discrepancies between these studies.

Although, the pathophysiological significance of β-cell dedifferentiation in humans still needs further investigation, a recent Japanese study demonstrated that the proportion of dedifferentiated cells that retain the pan-endocrine marker chromogranin A without expression of the four major islet hormones was increased in islets from patients with diabetes compared to healthy individuals, and chromogranin A expression levels rose substantially during long standing disease progression[142].

(IV) Limitations of existing therapies and discovery of new antidiabetic drugs

GLP-1 receptor agonists (GLP-1RAs), such as lixisenatide, liraglutide, exenatide, dulaglutide, albiglutide, and semaglutide, are a group of glucose controlling drugs that are widely used in the treatment of T2D due to their ability to promote insulin secretion, lower glucagon secretion, and promote gastric emptying and weight loss. Their glucose lowering properties are comparable with insulin therapy[143] and these two therapies are often used in combination. GLP1-RAs are well established as glucose-lowering agents, however, currently they do not have disease modifying properties.

In 2020, the first orally administered GLP-1RA was approved for the treatment of T2D[144,145]. Another GLP-1RA, PF-06882961, was optimized to promote endogenous GLP-1R signaling at nM concentrations. PF-06882961 was shown to increase insulin levels in primates and produce dose-dependent decline in serum glucose in healthy humans[146]. GLP-1RAs promote insulin receptor and IGF receptor signaling in β-cells, by stimulating the positive feedback loop of IGF1 and IGF2 secretion[147], which highlights the potential of targeting these pathways for diabetes treatment.

In another recent study, a functional high throughput siRNA screening was performed to identify novel modulators of human β-cell insulin secretion[148]. From a total of 521 candidate genes, the study identified 23 positive and 68 negative regulators of insulin secretion and functionally validated 3 genes, namely activated transcription factor 4 (ATF4), heat-shock protein family A member 5 (HSP5A) and growth hormone secretagogue receptor (GHSR). This study verified that the depletion of ATF4 and HSP5A diminishes insulin secretion, whereas knockdown of GHSR enhances insulin secretion[148].

Furthermore, Rheb1 was identified as a critical regulator of GSIS in β-cells. Rheb1 was shown to facilitate GSIS in human islets by upregulating GLUT1 expression in mTORC1 dependent manner[149]. Depletion of insulin secretion and pancreatic β-cell mass by immune infiltration is one of the major pathophysiological features of T2D. Therefore, protection from β-cell death and preserving β-cell mass to improve glucose responsive insulin secretion are crucial for the treatment of T2D. Similarly, sortilin-derived peptides have shown to stimulate insulin secretion in response to glucose and protect β-cells against death induced by cytokines (interleukin-1β)[150]. Finally, Neratinib, an FDA-approved drug and a potent MST-1 inhibitor, has shown to restore normoglycemia and improve β-cell function, survival and mass in multiple diabetic mouse models and isolated human islets[151]. These findings may pave the way for the identification of new classes of antidiabetics.

(V) Advances in insulin modification and therapies:

Insulin therapy has advanced tremendously during the last century, resulting in enhanced effectiveness, safety, and convenience of administration. However, despite continuous improvements of exogenous insulin formulations, the need for more treatment flexibility, fewer hypoglycemic episodes, and improved quality of life still exists. To this end, innovative insulin formulations have been developed to accelerate the rate of absorption of rapid acting insulins while also extending and flattening the profiles of basal insulins.

These newer generation of insulin therapeutics comprise the 1) ultra-rapid prandial insulin which is similar to endogenous prandial secretion with faster onset of action with short duration. Ultrarapid insulin was created by combining chemicals that enable quicker hexamer dissociation (niacinamide, larginine, and citrate) and cause local vasodilation (treprostinil) with rapid-acting insulin[106,107], 2) ultralong basal insulin, which is designed for longer and flatter action profiles (Insulin degludec) with low risk of hypoglycemia[108], 3) hepatoselective insulin - insulin secreted from β-cells reaches the liver via the portal circulation and undergoes extraction before entering circulation[109]. In contrast, exogenous insulin does not undergo the first pass hepatic metabolism thereby displaying a suboptimal suppression of liver gluconeogenesis and over insulinization of the peripheral tissues with an increased risk of hypoglycemia, insulin resistance and weight gain. To

overcome these issues, hepatoselective insulins are currently designed to restore a normal portal-peripheral insulin gradient[110,111], 4) Glucose responsive insulin – the majority of the schemes use a polymer-based system of insulin confined within a glucose-responsive matrix-based vesicle made of glucose-reactive motif. When exposed to increased glucose concentrations/spikes, these vesicles undergo conformational changes and release insulin for systemic absorption. The goal of glucose-sensitive insulin therapies is to lower the risk of hypoglycaemia and improve treatment outcomes to benefit patients with diabetes.[112–114]

In 1983, the Diabetes Control and Complications Trail (DCCT) was conducted on patients with type 1 diabetes to examine whether intensive insulin treatment (three or more daily injections) could maintain normoglycemia compared to standard treatment (one or two injections). The study found that intensive insulin treatment reduces the occurrence of retinopathy, neuropathy and nephropathy by a range of 35% to >70%, with an adverse side effect of coma or seizure due to hypoglycaemia[115,116].

Several clinical studies have since been conducted to determine the effect of transient intense insulin treatment (TIIT) on poorly managed T2D patients to achieve normoglycaemia[117]. The rapid acquisition of glycemic control with TIIT has been found to enable many people to maintain normoglycemia following cessation of insulin therapy, using lifestyle management alone for extended periods of time. The rapid acquisition of glycemic control has been demonstrated to have a beneficial impact on β-cell function, with people achieving glycemic targets with TIIT and also having improved β-cell function[118–120]. TIIT preserves β-cell function potentially by reducing gluco-/lipotoxicity possibly via glycemic control and enabling recovery of residual β-cell function. The EARLY study investigated the use of basal insulin following failure of metformin in 1438 people with T2D and demonstrated that early basal insulin therapy was safe and effective and having a low rate of hypoglycemia[121]. Another meta-analysis of studies investigating TIIT, which included 839 participants from seven studies, found that 66.2% were in drug-free remission after three months of TIIT[122].

The downside is that many patients do not respond to basal insulin treatment and thus do not show reduced blood glucose upon this treatment. Insulin treated patients

experience hypoglycaemia many times during their lifetime which may increase the risk of cardiovascular and macrovascular events - possibly the risk increases with increased dosage. In addition, weight gain accompanies insulin treatment which is influenced by insulin regimen and the duration of the insulin therapy[152–156].

Data from the long-term clinical trials in which patients with type 2 diabetes have been treated with intensive insulin therapy have demonstrated an increasing incidence of severe hypoglycemia including the Action to Control Cardiovascular Risk in Diabetes (ACCORD), Veterans Affairs Diabetes Trial (VADT) and Normoglycemia in Intensive Care Evaluation-Survival Using Glucose Algorithm Regulation (NICE-SUGAR) study trials wherein intensive glycemic control greatly increased the incidence of severe hypoglycemia. The occurrence of severe hypoglycemic episodes in these patients increased the hazard ratio (HR) for mortality during the studies[157–160]

(VI) Conclusion and future perspectives

Undoubtedly, pancreatic β-cell survival and regeneration is an emerging paradigm in the field of diabetes research, with an increased interest in developing drugs that target β-cells with cellular specificity. Signaling processes within pancreatic islet cells are highly complex and require better understanding. Elucidating this cross talk between islet cells in which they engage nutrient sensing and in response, fine-tune and coordinate hormone secretion and β-cell well-being in health and diabetes may be key to develop future drug therapies for diabetes treatment. Furthermore, targeting β-cell ER and oxidative - stress pathways, proinsulin processing defects, senescent β-cells as well as β-cell regeneration and survival have strong potential for developing drugs for the treatment of diabetes.

Insulin has been used longer than any other medication to control hyperglycemia in diabetic patients. Although short-term intensive insulin treatment early in disease progression has recently been proposed as a promising therapeutic option with cardiovascular benefits, it comes with severe side effects, such as hypoglycemia and weight gain. Disruption of β-cell insulin/IGF signaling and/or development of β-cell insulin resistance could also contribute to β-cell dedifferentiation, dysfunction and death, which eventually leads to progression of diabetes. Overall, preclinical and clinical data suggest

that specifically targeting β-cell insulin sensitivity to protect and/or regenerate endogenous β-cell function without the unwanted side effects of insulin may provide an attractive causal therapy for improved glycemic control.

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Author contributions

C.J., A., S.B. and H.L. conceived of the concepts described in this review. C.J., A., S.B. and H.L. wrote the manuscript. Figures were prepared by C.J., A. and S.B.

Competing interests

The authors declare no competing interest. C.J. work as a fulltime employee at Genentech Inc., CA 94080, USA and A. work as a fulltime employee at The Jackson Laboratory, Bar Harbor, ME 04609, USA.

Table 1:

β-Cell KO studies	Phenotypes
1. INSR–KO • β-Cell selective(9,28,29) • β-Cell specific(36)	• Glucose intolerance, Impaired GSIS, GLUT2 loss, Reduced β-Cell mass • Hyperinsulinemia in the context of glucose stimulation • Promote GSIS, Improved glucose tolerance

• β-Cell and neuron(34,35)	
2) IGF1R–KO(30)	• Hyperinsulinemia, Glucose intolerance, no change in β-Cell mass
3) INSR+IGF1R double KO(11)	• Increased apoptosis, reduced β-Cell mass, hyperglycaemia, Glucose intolerance
4) PDK1-KO(37)	• Loss of β-Cell mass, hyperglycaemia
5) Reduced AKT activity(38)	• Impaired Glucose tolerance, defective insulin secretion
6) FOXO1-gain of function(39)	• Reduced Pdx1 expression, impaired β-Cell compensation, increased hepatic glucose production
7) TSC2/mTOR disruption(40)	• Increased β-Cell mass, improved glucose tolerance

Figure 1: Comparative islet architecture overview of mice versus human under healthy and diabetic states

(a, b) Islet architecture from a healthy young mouse and a healthy adult human pancreas displaying variety of cells including β-cells (green), α-cells (red) and δ-cells (blue) and pancreatic polypeptide (PP) cells (orange). (**c, d)** Islet architecture from a diabetic mouse and diabetic human pancreas displaying morphological changes and heterogeneity in cell types. There exists a significant heterogeneity in β-cells (green-high insulin, light green-low insulin) and the spatial arrangements, interactions between islet cell types are important for overall regulation of islet function. Created with BioRender.com.

Figure 2: Effects of alteration in β-cell insulin signaling pathway components on β-cell function.

Schematic view of insulin/IGF1 signaling pathway. Binding of insulin or IGF1 ligand to Ins/IGF1 receptor activates a series of downstream signaling cascades. This interaction initiates the conformational changes and autophosphorylation of the Ins/IGF1 receptor that recruits and phosphorylates IRS proteins. IRS proteins then activate PI3-kinase which then converts phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3) on the inner side of plasma membrane. This membrane bound PIP3 activates PDK-1 which in turn phosphorylates and activates Akt and PKCs. Akt is the central molecule which mediates most of the glucose-responsive insulin signaling metabolic effects, regulating protein synthesis, gluconeogenesis, glycogen synthesis etc. Downregulation (pink color) or activation (green color) of insulin receptor and its downstream effector molecules specifically in the pancreatic β-cells have shown to cause β-cell dysfunction and/or development of diabetes.

Figure 3: Targeting inceptor to improve β-cell insulin signaling as potential diabetes therapy.

Insulin is synthesized and secreted from pancreatic β-cells in response to glucose uptake, however, it is not clear how insulin signaling is regulated in these cells. Inceptor has recently been discovered as a negative regulator of insulin signaling in β-cells, whereby it interacts with Ins/IGF1 receptor to facilitate clathrin-mediated endocytosis for receptor desensitization. In normal cells (left panel), insulin promotes interaction of the inceptor with IR via recruitment of adaptor related protein complex 2 subunit mu 1 (pAP2M1 -an active form of AP2M1) to the plasma membrane. This interaction triggers endocytosis of the inceptor along with insulin receptor, thus reducing the amount of insulin receptor on plasma membrane leading to attenuation of insulin signaling in β-cells. Deletion of inceptor or blocking the physical interaction between inceptor and insulin receptor by using

inceptor ectodomain antibodies (right panel) prevents internalization of insulin receptor through this pathway and thus enhances insulin signaling in these cells.

Figure 4: Targeting β-cells for lowering hyperglycemia.

Schematic diagram summarizing the molecular pathways and drug interventions for controlled proliferation of β-cells, protection from cell death and β-cell differentiation processes. **(a)** Osteoprotegrin enhances β-cell proliferation both in mouse and human islets by modulating CREB and GSK3 pathways via binding to RANK ligand. Similarly, Denosumab, which is a RANK ligand-specific antibody, also induces β-cell proliferation by inhibiting RANK/RANK ligand interaction. DYRK1A inhibitors such as 5IT, Harmine and GNF4877 have shown to inhibit NFATc kinases DYRK1A and GSK3b, thus preventing their nuclear export and enhancing β-cell proliferation. **(b)** Several endogenous and exogenous stress factors such as gluco/lipo-toxicity and/or DNA-damage can cause changes in gene expression and impair cellular function and proliferation of β-cells through senescence-associated secretory phenotype (SASP). Targeted removal of these senescent β-cells using senolytic drugs like iBET-762 and ABT263 has shown to preserve β-cell mass and restore insulin secretion in diabetic mouse islets. **(c)** Loperamide increases FoxO1 expression and nuclear translocation leading to upregulation of ER Ca2+ ATPase and calcium channel subunits, while down-regulating glucagon transcription. This further stimulates insulin processing and secretion by β-cells, thus maintaining β-cell identity in an in vitro dedifferentiated model. In another study, a targeted delivery of GLP-1-oestrogen conjugate to β-cells has shown to activate ER-associated protein degradation (ERAD) pathway and protects β-cells from cytokines-induced stress, thus enhancing β-cell survival and regeneration. Created with BioRender.com.

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