Pancreatic β-cell failure, clinical implications, and therapeutic strategies in type 2 diabetes

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Abstract

Pancreatic β-cell failure due to a reduction in function and mass has been defined as a primary contributor to the progression of type 2 diabetes (T2D). Reserving insulin-producing β-cells and hence restoring insulin production are gaining attention in translational diabetes research, and β-cell replenishment has been the main focus for diabetes treatment. Significant findings in β-cell proliferation, transdifferentiation, pluripotent stem cell differentiation, and associated small molecules have served as promising strategies to regenerate β-cells. In this review, we summarize current knowledge on the mechanisms implicated in β-cell dynamic processes under physiological and diabetic conditions, in which genetic factors, age-related alterations, metabolic stresses, and compromised identity are critical factors contributing to β-cell failure in T2D. The article also focuses on recent advances in therapeutic strategies for diabetes treatment by promoting β-cell proliferation, inducing non-β-cell transdifferentiation, and reprograming stem cell differentiation. Although a significant challenge remains for each of these strategies, the recognition of the mechanisms responsible for β-cell development and mature endocrine cell plasticity and remarkable advances in the generation of exogenous β-cells from stem cells and single-cell studies pave the way for developing potential approaches to cure diabetes.

Keywords: Pancreatic β-cell regeneration; Diabetes; Insulin; Dedifferentiation; Transdifferentiation; Stem cell

Introduction

Diabetes is the most common multidimensional metabolic disorder in humans. It is estimated that over 537 million adults worldwide suffered from diabetes in 2021, and this number is expected to increase to 783 million by 2045.[1] Type 2 diabetes (T2D) characterized by peripheral insulin resistance and progressive β-cell dysfunction is the most prevalent form of diabetes, and its progression may increase the risk of various micro- or macrovascular complications, such as retinopathy, nephropathy, foot ulcers, cardiomyopathy, and cerebral or peripheral vascular disease.[2] Presently, although many different classes of antihyperglycemic therapies can improve glucose tolerance and temporally maintain blood glucose homeostasis, no real cure exists for T2D.[3,4] Most patients with late-stage T2DM are still dependent on insulin injection,[2] which is often associated with side effects even life-threatening hypoglycemic episodes.

Pancreatic β-cells located in the islets of Langerhans are responsible for insulin synthesis as well as storage and secretion, playing an essential role in the regulation of blood glucose levels. β-cell failure, either functional decline or mass reduction, may lead to the disruption of glucose homeostasis and eventually the onset and progression of diabetes.[5] At the early stage of T2D, pancreatic β-cells adaptively compensate for insulin resistance by expanding their mass and augmenting insulin production. As the disease develops, the β-cell compensatory response fails and functional β-cell mass loss occurs.[6] Therefore, targeting pancreatic β cells by maintaining or restoring their mass and function with various endogenous and exogenous approaches serves as a promising strategy for diabetes treatment.[7]

In this review, we first introduce the endogenous regulation of insulin biogenesis and secretion and then highlight recent findings on the mechanisms driving β-cell alteration during the progression of T2D. Finally, we focus mainly on advances in therapeutic strategies for better prevention and treatment of T2D by targeting β-cells.

Normal β-cell Physiology

Pancreatic β-cells are essential for metabolic homeostasis, particularly the homeostatic glucose setpoint by producing

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an appropriate amount of insulin. Under physiological conditions, insulin synthesis and secretion are tightly controlled by nutrient availability.

Insulin biogenesis

Insulin is initially produced as preproinsulin, which is composed of a signal peptide (SP) followed by an entire proinsulin, including a B-chain at the N-terminus, an A-chain at the C-terminus, and an intermediate connecting C-peptide. Preproinsulin is transported into the endoplasmic reticulum (ER) lumen via both cotranslational and posttranslational translocation routes.[8] Once delivered to the ER lumen, the SP domain is rapidly removed from the preproinsulin molecules to yield proinsulin, which undergoes folding and acquires three disulfide bonds at A20–B19, A7–B7, and A6–A11. Folded proinsulin dimerizes in the ER, and cleavage of the C-peptide by prohormone convertases (PC1/3 and PC2) and carboxypeptidase E converts proinsulin into mature insulin. Upon translocation into the Golgi complex, the proinsulin dimers begin to form zinc-containing hexamers that are then loaded into immature secretory granules. Alongside insulin maturation, the secretory granules also progressively mature by refining their composition, such as luminal acidification, lipid composition changes, membrane cholesterol enrichment, membrane adaptor protein exposure, removal of unwanted cargoes and soluble components, and formation of Zn^{2+}-mediated dense insulin cores.[9] Thus, the granules acquire the competence needed for insulin storage and stimulus-response secretion. Ultrastructurally, a β-cell possesses approximately 10,000 dense-core secretory granules with Zn_2-$insulin_6$ complex vesicles in these dense-core granules. Each granule contains 300,000 or more insulin molecules, which corresponds to 8–9 fg insulin. Only a small fraction of (1–5%) granules are distributed into a "ready releasable" pool that is docked to the cell membrane, and they are secreted on demand without any further modification; Major (95–99%) insulin granules are stored in a reserve pool and they undergo a series of preparatory reactions before acquiring release competence.[5]

Insulin secretion

Although a range of metabolic fuels including amino acids, α-ketoacid ketoisocaproate, and fatty acids can stimulate insulin secretion, glucose is the primary physiological stimulator for insulin release.[5] In addition to multiple metabolic signals, intraislet local signals, including autocrine transmitters of β-cells and paracrine signals between β-cells and other adjoining cells, notably α-cells and δ-cells, also govern islet function and insulin secretion.

Insulin secretion is regulated by metabolic signals

Pancreatic β-cells sense changes in the blood glucose level and secrete appropriate insulin to maintain blood glucose in a homeostatic range. A prominent feature of insulin secretion is its two-defined phasic pattern, displaying a transient first phase with a rapid rise in insulin secretion followed by a prolonged second phase of lower but sustained insulin secretion. The first phase involves a triggering pathway that is activated by the glycolytic and oxidative metabolism of glucose. Elevated circulating blood glucose (>8–10 mmol/L) is transported into β-cells through the membrane glucose transporter. Intracellular glucose is metabolized to generate pyruvate via a glycolytic pathway. Afterward, pyruvate is transported into mitochondria and is oxidized via the tricarboxylic acid cycle to produce a larger amount of adenosine-5′-triphosphate (ATP), which is subsequently transferred into the cytoplasm by the ATP/adenosine diphosphate (ADP) transport complex. The increase in cytoplastic ATP induces the closure of ATP-sensitive potassium (K_{ATP}) channels, giving rise to cellular depolarization followed by an activation of voltage-gated Ca^{2+} channels (VGCC) and a resultant rapid Ca^{2+} influx. This in turn prompts the assembly of a tetrameric complex consisting of Sec1/Munc18-like (SM) and three soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins.[10] The SM/SNARE quaternary complex forms a molecular zipper structure that bridges the insulin secretory granules to the plasma membrane of maximum Ca^{2+} concentration. Along with additional Ca^{2+} sensors, such as synaptotagmin (Syt), double C2 domain (DOC2) and Ca^{2+}-dependent activator protein for secretion (CAPS), the SM/SNARE complex facilitates the exocytosis of insulin from a "readily releasable" pool. Insulin granule exocytosis is further potentiated during the second phase, which relies on an amplifying pathway that is independent of the K_{ATP} channel and is predominantly activated by the accumulation of metabolic intermediates, such as citrate and malate.[11] The second phase of insulin secretion can be maintained during the entire postprandial state, causing the release of membrane-associated primed granules and cell-internal insulin into the storage pool.

Insulin secretion is regulated by β-cell autocrine actions

Autocrine signaling refers to the effect of extracellular diffusible messengers on the same cells or nearby cells of the same type from which they have been released. Abundant evidence has indicated that autocrine signaling is involved in the regulation of β-cell function.[12]

Insulin

Pancreatic β-cells possess the necessary components of the insulin signaling pathway, such as insulin receptor (IR), insulin receptor substrate (IRS), phosphatidylinositol 3-kinase (PI3K), AKT, and mammalian target of rapamycin (mTORC1). Therefore, insulin may directly act on β-cells to regulate its own synthesis and secretion. The autocrine action of insulin on its own secretion is modulated by the microenvironments around β-cells, such as insulin or glucose concentrations and exposure time. In mouse islets, insulin secretion is stimulated by low-concentration (nanomolar) insulin but is inhibited by high concentrations (micromolar) of insulin. In rat islets, insulin secretion is inhibited after prolonged (30 min) exposure to exogenous insulin but is potentiated by acute treatment. In humans, endogenous glucose-stimulated

insulin secretion increases by 40% after a 4-h exposure to insulin.[13] Mechanistically, insulin induces Ca^{2+} release from ER stores through the activation of the IR/IRS/PI3K signaling pathway under physiological conditions, thus increasing the cytosolic Ca^{2+} concentration and triggering granule exocytosis.[14] In addition, the insulin autocrine regulation pathway may also be mediated by the PI3K-AKT-mTORC1 pathway. At high concentrations of insulin during states of insulin resistance or hyperinsulinemia, insulin may activate PI3K-dependent K_{ATP} channels, thus hyperpolarizing the cell membrane and inhibiting glucose-induced Ca^{2+} oscillations, resulting in an inhibition of granule exocytosis.[15] This positive and negative autocrine regulation of insulin coordinately accommodates insulin demand. Initially, insulin enhances its secretion in response to glucose stimulation; However, when the local insulin concentration becomes high, secretion is inhibited.

Islet amyloid polypeptide (IAPP)/amylin

IAPP or amylin is a 37 amino-acid peptide that resides in insulin secretory granules and is cosecreted with insulin in a molar ratio of approximately 1:100 in response to glucose and other stimuli. Recent studies have found that IAPP, akin to insulin, exerts a dual action on insulin secretion, potentiating basal insulin release at very low concentrations with nanomolar levels and suppressing glucose-stimulated insulin secretion at very high concentrations with micromolar levels.[16] These findings support the notion that endogenous IAPP plays an inhibitory role in insulin secretion and limits blood glucose elimination. In accordance with this notion, IAPP knockout mice display an increased insulin response and concurrent rapid blood glucose elimination, while human IAPP transgenic mice show the opposite effects. Likewise, a truncated-IAPP peptide acting as an antagonist may stimulate insulin secretion upon glucose stimulation in isolated rat islets. The complex influence of IAPP on insulin secretion might result from the interaction between calcitonin receptors and receptor activity modifier proteins (RAMPs).[17]

ATP

ATP is present in insulin granules and is cosecreted with insulin through Ca^{2+}-dependent exocytosis in isolated rat islets or human β-cells challenged with glucose, and the released ATP in turn potentiates insulin release. Human β-cells express ATP-sensing P2 purinergic receptors, which are divided into ligand-gated ionotropic P2X and G protein-coupled metabotropic P2Y receptors.[18] Extracellular ATP binds P2 receptors and induces Ca^{2+} release from intracellular stores, leading to an increase in cytoplasmic free Ca^{2+} and acute insulin release in response to glucose stimulation. ATP-potentiated insulin secretion is significantly attenuated by P2 receptor antagonists and extracellular ATP scavengers in rat islets. Moreover, both P2X and P2Y receptor agonists may potentiate insulin release upon glucose stimulation in human islets. After release from β-cells, ATP can be rapidly hydrolyzed to ADP and adenosine monophosphate (AMP). ADP may also modulate insulin secretion via different P2 receptors. The contribution of various P2 receptors to insulin exocytosis in β-cells merits further investigation.

γ-aminobutyric acid (GABA)

GABA is a neurotransmitter that is mainly produced in islet β-cells and the brain.[19] GABA is synthesized in the cytosol from the precursor glutamic acid by the enzyme glutamic acid decarboxylase. While GABA is predominantly cytoplasmic in β-cells, it is also present in insulin secretory granules and synaptic-like microvesicles in GABA transporter-expressing β-cells. Cytosolic GABA is secreted from β-cells via a nonvesicular pathway, and vesicular GABA in insulin granules is released upon glucose stimulation via Ca^{2+}-dependent exocytosis. The function of GABA in β-cells depends on its binding to its specific membrane receptor A ($GABAR_A$) or B ($GABAR_B$).[20] $GABAR_A$ is a ligand-gated Cl^- channel that controls β-cell excitability by modulating the membrane potential (V_m). In excited β-cells with a more electropositive V_m, activation of $GABAR_A$ can inhibit insulin release by clamping V_m to the equilibrium chloride potential (E_{Cl}−) or hyperpolarizing the membrane back toward E_{Cl}−. $GABAR_B$ is an inhibitory G protein (Gi)–coupled receptor that stimulates the opening of G protein-coupled inwardly rectifying K^+ channels and inhibits adenylyl cyclase, giving rise to lower cyclic AMP (cAMP) and inhibition of VGCC and concurrent reduction in insulin secretion. Mice lacking $GABAR_B$ exhibit increased insulin secretion in response to glucose stimulation and improved glucose tolerance[20]. Overall, the effect of GABA on insulin secretion is dependent on the integrated response of β-cells to metabolic, ionotropic, and metabotropic signals.

Insulin secretion is mediated by paracrine communication in islets

Paracrine communication reflects the actions of signaling molecules produced by one cell on nearby cells of a different type. In addition to the insulin-producing β-cells that account for approximately 50–75% of human and 60–80% of mouse endocrine cells, islets also contain some other endocrine cells, such as the glucagon-secreting α-cells that make up 25–35% of human and 15–20% of mouse islet mass, somatostatin-secreting δ-cells that comprise up to 22% of human and only 6% of mouse islet cell number, and pancreatic polypeptide (PP)-cells that account for <5% of human and <2% of mouse islet mass.[21] These endocrine cells are densely packed and engage in the regulation of insulin secretion by paracrine signals.[22]

Insulin secretion is regulated by paracrine signals of α-cells

Glucagon secreted by islet α-cells is traditionally thought of as a counter-regulatory hormone to preventing hypoglycemia by stimulating hepatic glycogenolysis and gluconeogenesis.[23] However, new studies have also revealed that glucagon stimulates insulin secretion, which is dependent on the dose and duration of glucagon exposure as well as the nutritional status. In overnight-fasted

mice, exogenous glucagon increases blood glucose levels without changes in insulin levels, while administration of glucagon along with glucose diminishes the rise in blood glucose levels and concurrently increases insulin levels. In fed mice, a challenge of 20 μg/kg glucagon increases blood glucose levels without impacting insulin levels, while an administration of 1 mg/kg glucagon decreases blood glucose and increases insulin levels.[22] Mechanistically, glucagon binds to the β-cell glucagon receptor (GCGR), resulting in the activation of adenylyl cyclase and the subsequent generation of cAMP. The increase in intracellular cAMP activates protein kinase A (PKA), which subsequently phosphorylates the SUR1 subunit of the K_{ATP} channel and Cav1/2 of the Ca^{2+} channel, and thereby inactivates the K_{ATP}-channel and activates the Ca^{2+}-channel, resulting in Ca^{2+} influx and insulin secretion. Additionally, cAMP may also bind to EPAC2A, which is a guanine nucleotide exchange factor to facilitate interaction with RAP1, a small GTPase. The EPAC2A/RAP1 complex activates the ryanodine receptor (RyR) on the ER, resulting in Ca^{2+} release from the ER. The increase in the cytoplastic Ca^{2+} concentration enhances recruitment of insulin granules to the cell membrane. There is also evidence that EPAC2A interacts with Rim2α, Rab3, and Piccolo, which are all needed for cAMP-regulated granule exocytosis. Furthermore, EPAC2 can also interact with the K_{ATP} channel to inhibit the functioning of the K_{ATP} channel and potentiate insulin release.[22] However, glucagon is only a modulatory signal, which has no stimulatory effect on insulin secretion in the absence of glucose.

In addition to glucagon, glucagon-like peptide 1 (GLP-1) is also an α-cell–derived insulin secretion-stimulating signal. Both glucagon and GLP-1 are derived from the common precursor proglucagon, which is divergently processed to GLP-1 by prohormone convertase (PC) 1/3 or glucagon by PC2.[24] Islet α-cells normally express PC2 for the production of glucagon, and they are also capable of expressing PC 1/3 and producing GLP-1 under certain circumstances. GLP-1 specifically binds to the β-cell GLP-1 receptor (GLP1R) and stimulates insulin exocytosis through a signaling cascade closely aligning with the signaling events of glucagon. In human β-cells, GLP1R is approximately twice as highly expressed as GCGR.[5] At physiological plasma concentrations (<50 pmol/L), each ligand specifically binds to its cognate receptor, but glucagon is also able to activate GLP1R at nanomolar concentrations, whereas GLP-1 does not act on GCGR.[25] The potential effects of both glucagon and GLP1 on insulin secretion either in isolated islets *ex vivo* or in mice and humans *in vivo* are glucose-concentration dependent,[26] and deciphering their relative contributions to the regulation of insulin secretion is an ongoing line of investigation.

Effects of δ-cell-derived somatostatin on insulin secretion

Islet δ-cells primarily secrete the somatostatin-14 isoform (SS-14), which is derived from the posttranslational process of prosomatostatin.[27] In addition to SS-14, prosomatostatin is also the precursor of the somatostatin-28 isoform (SS-28), which is mainly generated by gastrointestinal D-cells and accounts for 65% of the total body somatostatin.[27] Various nutrients, such as glucose, leucine and arginine, may stimulate somatostatin secretion in a K_{ATP}-channel-dependent way similar to that of β-cell insulin secretion. Somatostatin activates somatostatin receptors on α- or β-cells, leading to inactivation of adenylyl cyclase and thereby reduction in intracellular cAMP levels and cAMP-induced exocytosis of glucagon and insulin.[28] Somatostatin may also inhibit VGCC and activate G-protein-activated inward rectifier K^+ channels, which induce membrane repolarization and inhibit electrical activity, resulting in less release of glucagon and insulin. Such paracrine inhibition of β-cells would ensure that insulin release is restrained in a timely manner to counterbalance nutrient stimulation, thus avoiding hypoglycemia caused by excess insulin release.

Pathogenesis of T2D: Insulin Resistance and Pancreatic β-cell Damage

Hyperglycemia accompanied by obesity, particularly visceral adiposity, causes insulin resistance, and more insulin is needed to override the ineffectiveness of insulin. Pancreatic β-cells detect this requirement and adaptively augment insulin synthesis and secretion through compensatory expansion of β-cell mass, restoring glucose homeostasis.[6] Ultimately, with increasing time, the number of β-cells as well as their secretory function progressively decline, and glucose homeostasis is impaired, eventually causing diabetes.

Insulin resistance

Insulin exerts its physiological effects by binding to plasma membrane–bound receptors of target cells. Although IRs are ubiquitously distributed in diverse tissues, the role of insulin in glucose homeostasis is typified by insulin's direct effects on skeletal muscle, liver, and white adipocytes.[29] Insulin resistance is a pathological condition in which target tissues are unable to respond normally to plasma insulin levels and thus fail to coordinate the glucose-lowering response.[29] Since insulin stimulation of glucose consumption is primarily involved in skeletal muscle, and because muscular glucose uptake and glycogen synthesis are highly dependent on intact insulin action, muscle insulin resistance could affect whole-body glucose homeostasis and is indeed a principal component of T2D. Insulin in the liver promotes net glucose disposal by coordinately suppressing gluconeogenesis and glycogenolysis and activating the deposition of glucose as glycogen.[3] Defective suppression of hepatic gluconeogenesis in insulin resistance and thereby the increase in glucose production are key drivers of fasting hyperglycemia that defines T2D. Meanwhile, insulin also fails to control hepatic glycogen metabolism under insulin-resistant conditions, and fasting and postprandial hepatic glycogen content is indeed lower in T2D patients. Insulin action in white adipose tissue strengthens the suppression of hepatic gluconeogenesis through the inhibition of triglyceride lipolysis, which decreases both the glycerol substrate of gluconeogenesis and acetyl-CoA of pyruvate carboxylase allosteric activator.[29] Therefore, adipocyte insulin resistance will increase

hepatic glucose production and thereby contribute to the pathogenesis of T2D.

Decreased β-cell mass and function in T2D progression

Upon peripheral insulin resistance, pancreatic β-cells adaptively enhance insulin production to meet the elevated metabolic demand. However, long-term overnutrition and insulin resistance will lead to prolonged hyperinsulinemia, which desensitizes IRs and further deteriorates insulin resistance. Once β-cells cannot adequately respond to insulin resistance, they lose their compensatory ability and switch to a pathological response, resulting in progressive loss of cell mass and/or function and thereby diabetes onset. In fact, multiple autopsy studies have quantified β-cells in T2D, and it is assumed that β-cell mass is reduced by approximately 40–60% in most people with T2D.[30] Importantly, even people with impaired fasting glucose also show approximately 40% deficit in β-cell volume density, indicating that a decline in β-cells occurs early in the prediabetic stage. In addition to mass reduction, β-cell functional deficits are also evident in T2D patients. Along with the progressive loss of insulin-producing cells, the remaining β-cells concurrently make functional adaptations in response to increased insulin demand. Over time, β-cells become progressively maladaptive and are eventually defective in secretory function. As described above, β-cell mass is reduced by approximately 40–60% in T2D patients, while their insulin secretion capacity is decreased by 50–97%, indicating a contribution of β-cell function to insulin insufficiency.[31] Moreover, dysfunctional β-cells are unable to efficiently process proinsulin to mature insulin, further magnifying the degree of insulin deficits. Human studies have demonstrated that these deficits are present not only in overt T2D patients but also in people with glucose intolerance or impaired fasting glucose.

Mechanisms Leading to β-cell Failure

Several hypotheses for the explanation of β-cell failure have been proposed, including genetic background, age, amylin cytotoxicity, β-cell identity loss, ER and oxidative stress, mitochondrial dysfunction, and islet inflammation. Better recognition of the molecular mechanisms underpinning β-cell dysfunction could identify new therapeutic targets or lead to more specific approaches to diabetes management.

Genetics

Genetics plays an important role in T2D, and genome-wide association studies show that the majority of T2D-associated genomic polymorphisms are linked to disturbances in β-cell function or mass.[32] Although many genetic variants associated with T2D are located in intergenic regions, some are specifically in certain genes. The most consistently associated genes include cyclin-dependent kinase 5 (CDK5) regulatory subunit-associated protein-like 1 (CDKAL1), which modifies $tRNA^{Lys}$ at position 37 to strengthen amino acid incorporation and proinsulin processing; Zinc transporter 8 (SLC30A8), which is responsible for zinc homeostasis in insulin secretory granules; Melatonin receptor 1B (MTNR1B), which is specifically expressed in β-cells, and its activation leads to decreased cAMP levels and insulin secretion; And peptidylglycine α-amidating monooxygenase (PAM), which amidates the bioactive peptides in β-cell secretory granules, and loss of PAM function causes reduced insulin exocytosis in response to glucose. In addition, some associated variants are found to be in transcription factors that are essential to β-cell development and insulin production, including mutations in NK6 homeobox 3 (NKX6-3), GATA-binding factor 6 (GATA6), hepatocyte nuclear factor 4α (HNF4α), glucokinase (GCK), hepatocyte nuclear factor 4α (HNF1α), pancreatic and duodenal homeobox protein 1 (PDX1), neuronal differentiation 1 (NEUROD1), and hepatocyte nuclear factor 1β (HNF1β), which are also the cause of monogenic forms of diabetes.[33] Moreover, recent single-cell epigenomic analysis revealed that the T2D-associated genetic variant rs231361 at the noncoding region of the potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*) locus regulates β-cell chromatin accessibility and insulin synthesis.[34] Overall, these results provide evidence that both β-cell function and development are responsible for T2D pathogenesis. Further analysis should identify more β-cell function-associated genes and functionally validate their therapeutic potential.

Aging

A substantial body of data has shown that aged individuals display a gradual deterioration in the regulation of glucose homeostasis, and this metabolic alteration is partially due to inadequate β-cell compensatory adaptation to peripheral insulin resistance.[35] In fact, β-cell proliferation rates decline rapidly following the first 2 years of life and thereafter maintain an unaltered very low replicative rate. To accompany the decrease in β-cell division in aged human β-cells, the mitogenic transcription factor FOXM1, cell cycle activators including cyclin A1 and A2 (CCNA1 and CCNA2) and cyclin-dependent kinases (CDK1, CDK4, and CDK6), and proliferation-regulating platelet-derived growth factor (PDGF) receptor (PDGFR) are downregulated. Conversely, the cell cycle inhibitor CDKN2A (also known as $P16^{INK4a}$) and its upstream activator transforming growth factor-beta (TGFβ) are upregulated during the process of aging.[36] Furthermore, the islet blood vessel network, which is essential to the production and spread of mitogenic signals for β-cell proliferation, is decreased in elderly subjects.[35] In addition, telomeric DNA lengths decrease gradually with aging, and eventually, telomere shortening triggers β-cell proliferation arrest and senescence.[35] Of note, recent studies suggest that aging correlates with a noticeable reduction in global m6A mRNA methylation, and depletion of m6A in β-cells decreases β-cell proliferation and insulin secretion.[37] Furthermore, aged β-cells may acquire markers of senescence and secrete a senescence-associated secretory phenotype, which accelerates adjacent β-cell senescence and dysfunction in a paracrine manner.[35]

Apart from proliferation, β-cell identity may be altered in older individuals. Recent accumulating evidence in rodents has shown that aged animals and senescent islets

tend to express less β-cell transcription factors (*Pdx-1*, *Nkx6.1*, *FoxO1*, and *MafA*) and functionally important genes (*Ins1*, *Ins2*, *Slc2a2*, and *Slc30a8*), along with more expression of β-cell dedifferentiation markers (*Sox9* and *Aldh1a3*).[38] Similarly, in the human pancreas, PDX1 expression is markedly downregulated with aging.[39] Remarkably, a single-cell RNA-seq analysis revealed that the number of bihormonal cells that simultaneously expressed both insulin and glucagon mRNA increased with age, and the presence of such transcriptionally noisy cells suggests a fate drift between α- and β-cells in the aging pancreas, resulting in a loss of β-cell identity and function. Likewise, these transcriptionally noisy cells associated with aging are also observed in pancreatic islet cells from cynomolgus monkeys.[40]

Oxidative stress

Pancreatic islets of T2D patients and rodent models are often subjected to oxidative stress, which arises when intracellular reactive oxygen species (ROS) generation exceeds the antioxidant responses of the cell. β-cells express substantially less antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase, rendering them highly vulnerable to oxidative stress.[41] Under hyperglycemic conditions, the continuously increased cellular glucose augments β-cell glycolysis and pyruvate production. However, pyruvate in β-cells cannot be metabolized via the lactate pathway due to the disallowed expression of lactate dehydrogenase (LDH) and monocarboxylate transporter-1. Thus, glucose in β-cells is almost oxidized through mitochondrial oxidative phosphorylation, leading to an increased production of ATP along with elevated ROS generation.[42] Of note, the increase in intracellular Ca^{2+} induced by glucose oxidative metabolism triggers endogenous ROS production through PKC-dependent activation of NADPH oxidase, which in turn stimulates superoxide anion ($O_2^{\bullet-}$) formation by transferring electrons from intracellular NADPH to extracellular molecular oxygen. Thereafter, $O_2^{\bullet-}$ is either transported into the cytosol to initiate intracellular signaling or dismutated to H_2O_2 by extracellular SOD. Furthermore, the insulin-maturing process is unavoidably accompanied by ROS generation. As the formation of three proper disulfide bonds is crucial for insulin biofunction, and each formation of a disulfide bond produces one molecule of ROS, it is anticipated that three molecules of ROS are produced with each production of one molecule of insulin. Thus, a 50-fold increase in insulin biosynthesis under hyperglycemic conditions inevitably results in the production of millions of ROS molecules per minute in every β-cell.[41]

Biologically, low physiological concentrations of ROS act as second messengers to participate in diverse cellular processes, and transient and moderate ROS can stimulate β-cell proliferation and potentiate glucose-stimulated insulin secretion by inducing ryanodine receptor-mediated Ca^{2+} release.[43] However, long-term exposure to excessive amounts of ROS causes β-cell impairment. ROS may activate stress-induced mitogen-activated protein kinases (MAPKs) such as JNK and p38 MAPK, which in turn potentiate the degradation of β-cell key transcription factors PDX-1 and V-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MAFA).[42] In addition, ROS accumulation can stabilize hypoxia-inducible factor-1α (HIF-1α), which is a transcription factor of some glycolytic enzymes, such as pyruvate dehydrogenase kinase 1 (PDK1) and LDH, resulting in excessive expression of LDH and PDK1.[42] Consequently, pyruvate is primarily converted to lactate rather than acetyl-CoA for mitochondrial oxidative phosphorylation, leading to a reduction in ATP production and insulin secretion. ROS may also damage β-cells by inducing mitochondrial injury and its associated abnormal oxidative metabolism.[41] Aside from the breakage of mitochondrial DNA and protein denaturation, oxidative stress is also believed to induce aberrant mitochondrial fusion by suppressing mitochondrial fission 1 protein (FIS-1), thus forming elongated giant mitochondria with impaired function to change the metabolic pattern of β-cells.

ER stress

Insulin is translated and formed into its native structure in the ER of β-cells. Approximately 1 million proinsulin molecules are produced per minute in a healthy β-cell. Thus, β-cells must adapt their ER machinery to a rise in glucose to support insulin production. As proinsulin is quite susceptible to misfolding, it is estimated that around 20% of proinsulin is misfolded.[44] Thus, under hyperglycemic conditions, the high secretory demand of insulin inevitably causes an accumulation of unfolded or misfolded proteins, which may overwhelm the ER folding capacity to trigger unfolded protein responses (UPRs) and ER stress. The UPR is initiated by the dissociation of the ER chaperone GRP78 from ER transmembrane proteins including protein kinase RNA (PKR)-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1), and ATF6, which are normally bound to GRP78 to remain inactive. Under ER stress conditions, the UPR not only inhibits protein translation via PERK-mediated inhibition of eIF2α and IRE1α-mediated mRNA degradation to attenuate ER load but also upregulates the expression of ER chaperones via ATF6 to increase folding capacity. Furthermore, the UPR can remove misfolded or unfolded proteins through the IRE1α-XBP1-mediated ER-associated degradation (ERAD) pathway and autophagy.[45] Although the adaptive UPR output can protect β-cells against ER stress, if ER stress is prolonged and unresolvable, the UPR eventually triggers proapoptotic pathways by initiating the expression of critical proapoptotic proteins, such as CHOP, RIDD, DR5, and PUMA.[46] Recent studies have implicated that some cytosolic proteins, such as RACK1 and the ABL family of tyrosine kinases, can translocate to ER membrane and interact with IRE1α, regulating ER stress-mediated β-cell apoptosis through modulation of IRE1α RNase activity.[45] In addition, the β-cell apoptosis induced by ER stress is associated with TXNIP, which is upregulated by the PERK and IRE1 pathways.[45] Moreover, recent studies have found that irremediable ER stress induces the expression of thyroid adenoma associated (THADA), which interacts with SERCA2 and RyR2 to decrease ER Ca^{2+} stores and aggravate ER stress-induced apoptosis.[47]

It is crucial to emphasize that oxidative and ER stresses are tightly entwined, and they are potent in exacerbating one

another. For example, lipotoxicity-induced ROS triggers a reduction in ER Ca^{2+} storage, leading to compromised ER folding capacity, which further aggravates ROS through ER oxidoreductase ER member 1α (ERO1α). Moreover, mitochondria and the ER actively communicate through their membrane contacts called mitochondria-associated membranes (MAMs), which also participate in Ca^{2+} distribution and ROS diffusion between these organelles.[48] Therefore, oxidative and ER stresses form a vicious cycle and profoundly impact β-cell function, and targeting both forms of stress should be more effective in disease treatment.

Inflammation

Islet immune cells are mainly composed of resident macrophages, which have the capacity to produce proinflammatory mediators, such as interleukin (IL)-1β and tumor necrosis factor α (TNF-α). Evidence from both rodents and humans clearly indicates that the number of macrophages in T2D or obese islets is increased and that they are recognized as key contributors to β-cell inflammation.[49] Transcriptomic analyses of isolated islets found that T2D is associated with overexpressed macrophage markers such as APOE and CD163L1,[50] further supporting macrophage accumulation in T2D islets. Simultaneously, several proinflammatory cytokines (IL-1β, TNF-α, and IL-24) and a few chemokines (CCL-2, 5, -7, -13, -16, -21, and -25, CX3CL-1,-11, and -12, and CCL-3, -8, and -26) are also upregulated in diabetic islet samples,[51] providing molecular evidence of islet inflammation and immune cell infiltration in T2D. Apart from local resident macrophages, β-cells can also secrete IL-1β in proinflammatory circumstances, such as prolonged exposure to high concentrations of glucose or free fatty acids.[52] Moreover, there is evidence that proinflammatory factors such as IL-1β, IL-6, and TNF-α are progressively increased from prediabetic to T2D patients.[52] These inflammatory cytokines could activate the nuclear factor (NF)-κB and JNK signaling pathways, which subsequently repress the expression of β-cell identity genes such as *Slc2a2*, *Pdx1*, and *MafA* and ultimately cause β-cell dedifferentiation. Additionally, NF-κB activation in β cells may also repress the expression of *SERCA2b*, which encodes an ER calcium pump, dampening insulin secretion by exacerbating ER stress.[52] Moreover, a recent study has shown that proinflammatory cytokines can activate proapoptotic BCL-2 proteins and induce mitochondrial stress, leading to β-cell apoptosis. In addition to the production of proinflammatory cytokines, islet-associated macrophages can decrease insulin secretion in obesity by phagocytosing β-cell insulin secretory granules, and this effect is mediated by direct contact between β-cells and intraislet macrophages. Furthermore, islet macrophages are associated with abnormal proinsulin processing during obesity, which could be attributed to their detrimental effect on the adaptive UPR in β-cells.[49]

β-cell dedifferentiation and transdifferentiation

Mature β-cells can lose their features through dedifferentiation in response to increased physiological demand, intracellular oxidative stress, ER stress, or inflammatory cytokines.[53] Upon dedifferentiation, β-cells progressively lose their specific transcription factors such as FOXO1, PDX1, MAfA, NEUROD1, and NKX6.1; Lose the "disallowed" genes, including *Ldh*, *Mct1*, and acyl-CoA thioesterase 7 (*Acot7*); and reactivate endocrine progenitor markers, such as *Ngn3*, *Aldh1a3*, and*Sox9*.[54] These alterations lead to β-cell reconfiguration in metabolism and structure, resulting in loss of β-cell characteristics and ultimately deficits in insulin production and secretion. Although there is evidence that β-cell dedifferentiation is positively correlated with β-cell deficits and T2D severity,[55] the contribution of dedifferentiation to the loss of β-cell mass and function in humans is controversial and not yet clear. One study using synaptophysin as the dedifferentiation marker identified 32% dedifferentiated β-cells in T2D patients compared with only 9% in control individuals,[54] while a subsequent study using chromogranin A as a dedifferentiation indicator found that the β-cell dedifferentiation was quantitatively small in both T2D and nondiabetic controls.[56] Similarly, one bulk mRNA sequencing study also did not observe distinct cell types among T2D islets or unaltered levels of maturation and identity genes between T2D and nondiabetic donors.[57] However, another single-cell RNA sequencing study on islets reported that the β-cell transcriptomic profiles in T2D donors resemble those in their juvenile counterparts, suggestive of partial dedifferentiation.[58] These contradictory results are possibly attributed to clinically different T2D subtypes, different patient characteristics, or distinct β-cell subpopulations, which warrant further investigation.

Apart from dedifferentiation, lineage-tracing studies indicate that β-cells lacking NKX2.2 or PDX1 or activating the α-cell transcription factor aristaless related homeobox (ARX) can be converted into glucagon-producing α-cells or somatostatin-producing δ-cells.[54] This process is referred to as β-cell transdifferentiation, which causes a reduction in insulin production, hyperglucagonemia, and hyperglycemia. Although it remains unclear whether transdifferentiation affects endogenous α-cell function and glucagon production, minimizing the transdifferentiation of β-cells to α-cells might provide a new avenue to delay or prevent the progression of diabetes. However, it is unclear whether β-cell transdifferentiation is a cause or an effect of T2D. Additionally, it is undetermined whether β-cells first differentiate into progenitor-like cells before they initiate transdifferentiation. There is evidence suggesting that transdifferentiation occurs after differentiation.[54] However, both rodent diabetes models and T2D patients have also revealed that a distinct subset of islet cells can secrete both insulin and glucagon under hyperglycemic conditions,[53,59] thus implying the existence of a more direct transdifferentiation process between endocrine cells. Further exploration is needed to understand the mechanisms that regulate the conversion between pancreatic endocrine cells.

Targeting Pancreatic β-cells for Diabetes Treatment

At present, no real cure exists for diabetes. Although a variety of pharmacological therapies are lifesaving, such

as metformin, sulfonylurea drugs, meglitinides, GLP1R agonists, dipeptidyl peptidase 4 (DPP-4) inhibitors, sodium glucose cotransporter 2 (SGLT2) inhibitors, thiazolidinediones, Ca^{2+} channel blockers, alpha-glucosidase inhibitors, and bile acid sequestrants, they often have limited glucose-lowering properties and insulin injection remains the common care for patients with late-stage T2D.[4] Although the administration of recombinant exogenous insulin can effectively maintain glycemic control, overdelivery may result in life-threatening hypoglycemic episodes. Given that endogenous insulin secreted by islet β-cells is fine-tuned and reduction in functional β-cell mass is a hallmark feature of T2D, β-cell replenishment through transplantation of whole pancreas or pancreatic islet cells or stem cell-derived β-cells or expansion of endogenous β-cells may serve as a promising approach to improve diabetes therapy [Figure 1]. Recent decades have witnessed considerable progress in the development of β-cell regenerative therapies for the treatment of diabetes.

Inducing endogenous β-cell proliferation

In principle, the induction of β-cell proliferation is the most effective and straightforward approach to regenerate β-cells, but this is quite difficult for β-cells due to their extremely low replication rate, particularly in adult humans.[60] The β-cell population in healthy individuals is largely stable throughout adult life, and long-lived β-cells are as aged as the body. However, adult β-cells are also plastic and able to reenter the cell cycle under certain pathophysiological conditions such as obesity and pregnancy, indicating their maintenance of proliferative capacity. Thus, stimulating β-cell proliferation may be a viable alternative to compensate for the severe loss of β-cells in diabetes.

Endogenous signals promoting β-cell proliferation

A number of endogenous molecules secreted by non-pancreatic organs have been shown to be involved in adaptive β-cell proliferation, including GLP-1 and its analog exendin-4, T-cell cytokines such as IL6 and IL2, hepatocyte-derived factors such as insulin-like growth factor (IGF) and serpinB1, adipocyte-derived serum factors such as leptin and adiponectin, and lactogens such as prolactin placental lactogen.[61] Additionally, multiple extracellular matrix (ECM) components including collagen, laminin, fibronectin and heparan sulfate, and ECM-sequester growth factors such as connective tissue growth factor (CTGF, also known as cellular communication network 2, CCN2), fibroblast growth factor, vascular endothelial growth factor, and hepatocyte growth factor have also been implicated in promoting β-cell proliferation by establishing a homeostatic islet microenvironment. Recently, CCN4/WISP1, which is abundant in preweaning mouse blood, was shown to promote the proliferation of endogenous and transplanted adult β-cells *in vivo*.[62] In addition to ECM components and ECM-localized factors, ECM remodeling driven by paracrine signaling from macrophages and intraislet endothelial cells is also implicated in driving β-cell proliferative activity.[63] Recent studies have demonstrated that islet macrophages from obese mice are able to promote β-cell proliferation through PDGF–PDGFR signaling[49] and the lack of PDGFR signaling in β-cells leads to compromised β-cell replication in aged mice and humans.[64]

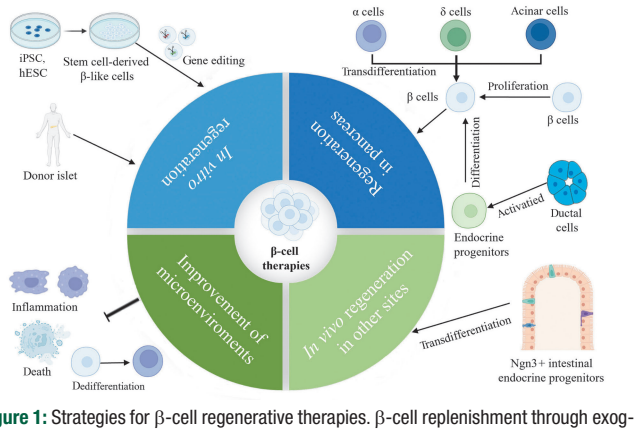

Figure 1: Strategies for β-cell regenerative therapies. β-cell replenishment through exogenous transplantation of pancreas or pancreatic islet cells or stem cell-derived β-cells and endogenous expansion of β-cells via cell proliferation or transdifferentiation. Both pancreatic endocrine cells, such as islet α-cells, δ-cells, and γ-cells, acinar cells, duct cells, and gastrointestinal tract cells, can be reprogramed into insulin-producing β-like cells. Stem cell-derived β-cells have positioned hPSCs, typically including ESCs and iPSCs, as a promising and theoretically unlimited source of insulin-producing cells. ESCs: Embryonic stem cells; hPSCs: human pluripotent stem cells; iPSCs: induced pluripotent stem cells.

β-cell intracellular signaling pathways including IRS-PI3K-mTOR, glycogen synthase kinase 3 (GSK3), carbohydrate-responsive element-binding protein (ChREBP), and extracellular signal-regulated kinase (ERK) pathways are stimulated by extracellular signals, regulating proliferative capacity in adult β cells,[65,66] For example, glucose and insulin can activate the PI3K-AKT-mTOR signaling pathway to increase β-cell replication in both mouse and human β cells. $p16^{INK4A}$ accumulation blocks β-cell proliferation in both mice and humans, a histone–lysine N/methyltransferase enzyme encoded by the enhancer of zeste homolog 2 (EZH2) can effectively repress $p16^{INK4A}$ expression to enhance β-cell replication in mice <8 months, and PDGF receptor signals may maintain EZH2 expression and EZH2-dependent β-cell replication in 14-month-old mice.[64] Osteoprotegerin, a target of prolactin receptor signaling, can bind to receptor activator of NF-κB ligand (RANKL/TNFSF11) to activate the cAMP-response element-binding protein (CREB) pathway and inhibit GSK3 to stimulate rodent and human β-cell replication.[67] SerpinB1, a liver-derived protease inhibitor, promotes the proliferation of human, mouse, and zebrafish β-cells by activating several key proteins in the insulin/IGF-1 signaling pathways including MAPK, GSK3, and PKA.[68] Likewise, reduction of the tumor repressor menin encoded by the *Men1* gene activates the MAPK pathway to increase the proliferation of mouse and human adult β cells.[69] Calcineurin–nuclear factor activated in T cells (NFAT) is a major mitogenic stimulator of β-cell replication; it promotes the cell cycle by transactivating cyclins E and A and repressing $p16^{INK4A}$, $p14^{ARF}$, and $p57^{KIP2}$.[70] Recently, it has been reported that ELAPOR1 (also known as inceptor) suppresses insulin signaling specifically in pancreatic β-cells by clathrin-mediated degradation of IR, resulting in decreased β-cell proliferation.[71] Although targeting these intracellular

signaling pathways may serve as a promising strategy to preserve β-cell expansion, many of them have been identified in rodents and hence their applicability to human cells needs to be ascertained.

Small molecules inducing β-cell proliferation

Currently, no drug can specifically promote islet β-cell proliferation on the market. However, a substantial number of small molecules have been discovered to induce human β-cell replication, such as 5-iodotubercidin (5-IT), harmine, GNF4877, GNF2133, INDY, leucettine-41, and CC-401.[72–74] Interestingly, all these compounds suppress the activities of dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A), which can terminate mitogenic signaling by phosphorylating NFAT and thus blocking NFAT nuclear localization.[72,73] Moreover, the combination of DYRK1A inhibitors, such as harmine, with other compounds, such as exendin-4 and liraglutide, that are GLP1R agonists,[75] as well as ALK5 that is a TGFβ-pathway inhibitor[76] induces a synergistic increase in human β-cell proliferation. In addition, some phytochemicals show strong inhibitory activity against DYRK1A, such as aristolactam BIII, licocoumarone, polyandrocarpamines, desmethylbellidifolin, and epigallocatechin-3-gallate,[77] which offer a potential alternative for promoting β-cell replication. Although DYRK1A inhibitors show a robust capacity to promote β-cell replication, their practical utility remains a concern. Considering the broad expression of DYRK1A, its inhibition could trigger unwanted proliferative responses of non-β-cells, and indeed, systemic administration of DYRK1A inhibitors activates replication of islet α-, δ-, and ductal cells.[73] Furthermore, DYRK1A inhibitors also target other signaling molecules in addition to their effects on DYRK1A, for example, harmine is also an inducer of the transcriptional regulator MYC, GNF4788 is also a suppressor of GSK-3β kinase,[74] and 5-IT is also an inhibitor of adenosine kinase[72]; Thus, a number of off-target effects are possible.

Denosumab is an FDA-approved antiosteoporosis drug that specifically inhibits human RANKL, which is known as a brake of β-cell replication.[67] Thus, denosumab acts as a partial functional equivalent of osteoprotegerin to promote β-cell proliferation. The IR antagonist S961 or the insulin and IGF receptor dual inhibitor OSI-906 potentiate β-cell proliferation independent of insulin signaling. GLP-1R agonists such as liraglutide and exenatide also promote β-cell proliferation though the cAMP/PKA, PI3K/AKT, and MAPK multiple signaling pathways. SGLT2 is a Na^+ concentration-dependent protein distributed in kidneys, and its inhibitors, such as empagliflozin and luseogliflozin, have been demonstrated to enhance β-cell proliferation and increase β-cell area in diabetic mouse models.[78] The adenosine agonist N-ethylcarboxamidoadenosine (NECA) could promote mouse β-cell proliferation and accelerate the restoration of normoglycemia following streptozotocin (STZ)-induced diabetes[79]; Thus, the components of the adenosine pathway might be therapeutically targeted for the treatment of diabetes. Most recent evidence indicates that fluoxetine, a selective serotonin reuptake inhibitor (SSRI) that is widely used as an antidepressant, is able to increase β-cell proliferation and potentiate glucose-induced insulin secretion from mouse and human islets at therapeutically relevant concentrations.[80]

With the increasing appreciation of the mechanisms involved in promoting human β-cell proliferation and the development of high-throughput screening tools, it is anticipated that more small molecules and drugs expanding functional β-cell mass will be identified. However, the determination of their specificity and efficacy *in vivo*, especially in humans over time, will bring new challenges.

Induction of cell transdifferentiation

Diverse lines of evidence have shown that pancreatic non-β-cells such as α-, δ-, acinar, and duct cells or liver cells can convert into new insulin-producing β-like cells. Therefore, the generation of new β-cells from cells not expressing insulin via cellular transdifferentiation could be a promising approach to expand functional β-cell mass.

Transdifferentiation from pancreatic non-β-cells

The conversion of α- to β-cells has been intensively studied as they develop from a common lineage trajectory. Studies in mice have shown that inactivation of ARX that is a master regulator of α-cell development, or ectopic expression of PAX4, MAFA, and PDX1 promotes α-cell to β-cell transdifferentiation.[81] Of note, the combined loss of ARX and DNA methyltransferase 1 (DNMT1) accelerates α-cell transdifferentiation to β-cells.[82] Similarly, α-cell specific MEN1 ablation leads to α- to β-cell conversion but insulinoma formation. Such a deregulation of α-cell plasticity also occurs when β-cells are almost completely ablated by diphtheria toxin receptor (DTR) in post pubescent mice.[83] Conversion to β-cells also occurs in human α-cells being reprogramed with ectopic MAFA and PDX1.[84] In addition to various transcription factors, endogenous factors such as IGF binding-protein 1 (IGFBP1) also promote α- to β-cell transdifferentiation in both mice and human islets.[85] Long-term GABA administration may trigger α- to β-cell conversion and the neo-generated β-like cells are capable of reversing STZ-induced diabetes *in vivo*.[86] Artemisinins, an antimalarial drug, has been suggested to induce α- to β-transdifferentiation by promoting the degradation of the α-cell transcription factor ARX and by stimulating GABA signaling.[87] However, such α- to β-cell conversion was not observed in studies using lineage tracing or in primary mouse islets.[88] Other interesting possibilities include GLP-1 analogs such as liraglutide and SGLT2 inhibitors such as dapagliflozin, which can induce β-cell transdifferentiation from α-cells.[89] A recent study suggested that sitagliptin and melatonin combination therapy may additively induce α- to β-cell transdifferentiation in diabetic mice.[90] In addition to α-cells, islet δ-cells and γ-cells (also known as PP cells) can also transdifferentiate into β-cells to compensate for the severe loss of β-cells.[91,92] Notably, δ- to β-cell conversion mainly occurs in prepubescent mice and relies on the suppression of mitogenic FOXO1 signaling.[91]

Besides endocrine cells, pancreatic exocrine cells such as acinar cells and duct cells retain a degree of plasticity. Studies from transgenic mice have found that overexpression of the combination of PDX1, MAFA, and Neurog3 (PMN), which are necessary transcription factors for β-cell development, or even PDX1, induces the direct conversion of acinar cells into insulin-producing β-like cells.[93] Furthermore, rodent acinar cells can also be reprogramed into insulin-producing cells following suspension culture and the addition of epidermal growth factor in combination with either leukemia inhibitory factor or nicotinamide.[94] Growth factor-induced acinar-to-β-cell conversion is nearly completely suppressed by Notch signaling, which antagonizes the expression of the proendocrine factor Neurog3, whereas Notch inhibition improves β-cell transdifferentiation, with approximately 30% of acinar cells adopting a β-like phenotype.[95] Acinar-to-β-cells conversion is also promising in humans, and ectopic expression of signal transducer and activator of transcription 3 (STAT3) and MAPK in human acinar cells can activate Neurog3 expression and induce the conversion of acinar cells to insulin-producing β-like cells.[96] Similarly, ductal epithelial cells also serve as a source of β-cells. Extensive tissue damage caused by surgical pancreatic duct ligation (PDL) or partial pancreatectomy promotes β-cell neoformation from duct cells in rat and murine models.[97] In addition, the process of duct- to β-cell progression can be stimulated by various genetic manipulations, such as overexpression of interferon-γ, simultaneous expression of gastrin and transforming growth factor alpha (TGFα) in duct cells, or deletion of the tumor suppressor ubiquitin ligase Fbw7 in pancreatic duct cells.[98]

Transdifferentiation from nonpancreatic cells

Due to their substantial plasticity and close lineage association with pancreatic cells, hepatocytes provide a promising source for functional β-cell neogenesis. Ectopic expression of pancreatic transcription factors in hepatocytes reprograms hepatocytes into insulin-producing β-like cells, and the efficiency of this plasticity is determined by the activation of the Wnt/β-catenin pathway, which stabilizes a transdifferentiation-permissive epigenome.[99] Upon ectopic expression of PDX1 in the mouse liver, certain hepatocytes typically located near the central and portal veins of the hepatic lobules display a predisposition to transdifferentiation into β-cells,[100] and additional external factors such as high glucose and hyperglycemia are necessary conditions for complete conversion into functional insulin-producing cells.[101] In addition, hepatocyte-to-β-cell transdifferentiation can be induced by GLP analogs, PDGF, Notch inhibitors, TGFβ-induced factor homeobox 2 (TGIF2), and TGFβ inhibitors.[102] Adult human hepatocytes with ectopic pancreatic transcription factors *in vitro* are also converted into insulin-producing β-like cells in response to increasing glucose within the physiological range.[102] However, only approximately 5–15% of the cells display insulin expression.

The gastrointestinal tract, in particular, the intestinal and antral stomach niches, is rich in endocrine cells with highly similar features to pancreatic β-cells and is also an immune-privileged site, rendering the gastrointestinal tract an ideal site either for being reprogramed to functional insulin-producing cells or for the engraftment of regenerated cells that mimic β-cell function.[103] Upon ectopic expression of PMN factors in intestinal crypts, intestinal cells can convert to insulin-producing and glucose-responsive β-like cells that are capable of ameliorating hyperglycemia in mice.[104] In contrast, somatic ablation of the transcription factor FOXO1 in Neurog3$^+$ enteroendocrine cells generates β-like cells that release insulin in a glucose-regulated manner and are able to reverse hyperglycemia in streptozotocin-induced diabetic mice.[103] In addition, GLP-1 treatment converts intestinal epithelial progenitors into insulin-producing cells *in vitro* and *in vivo* that depend on the hepatocyte nuclear factor 6-mediated expression of Ngn3, and the resulting β-like cells can ameliorate diabetes after implantation into STZ-induced diabetic mice.[105] Recent studies have revealed that antral stomach tissue is highly amenable to reprograming toward functional insulin-producing cells with robust expression of key β-cell genes, such as *Nkx6.1* and *Glut2*.[106] Given that cell plasticity and transformation mechanisms vary in different segments of gastrointestinal tissues, site-specific strategies are expected to improve the regenerative capacity of these tissues.

Despite the aforementioned findings in the conversion of non-β-cells into insulin-producing cells, it remains unclear whether transdifferentiated cells could preserve their new β-like features without retrogression to a prior state. In addition, it is necessary to determine whether insulin secretion in reprogramed cells is under the control of metabolic demand. Furthermore, the conversion rate remains low, which limits transdifferentiation-derived β-cells for clinical use.

Supply β-cells via exogenous pancreas or islet transplantation

Although endogenous approaches deterring the decline of β-cell mass in diabetes are of encouragement, the treatment falls short of a cure. Pancreatic β-cell replacement through whole pancreas or pancreatic islet transplantation holds the potential to truly cure diabetes. Whole pancreas transplantation has been accomplished in several thousand diabetic subjects and has achieved long-term insulin independence and good metabolic control in many individuals. Although pancreas transplantation is an effective therapy especially for patients with simultaneous kidney transplantation, the surgical risk remains an important consideration. Isolated islet transplantation has offered a more favorably safe alternative with less invasion. Since the establishment of the Edmonton protocol,[107] islet transplantation has provided a functional cure for insulin-dependent diabetes, and it is superior to exogenous insulin administration regarding stabilizing glycemic control, reducing hypoglycemic events, and restoring hypoglycemic awareness. However, islet cell transplantation faces some new challenges, notably the severe shortage of donor islets and deterioration of the cells post transplantation. First, the source of islet cells for transplantation is limited to donors who have died

from brain death and cardiac death, which severely limits the availability of donor islets. Second, isolated islets tend to undergo rapid necrosis under conventional culture conditions due to oxygen depletion, and grafted islet cells also suffer from immediate loss after transplantation due to disruption of the microvascular network and oxygen depletion in the isolation process,[108] further aggravating islet scarcity. Therefore, an excessive number of islet cells collected from two to three individual donors are needed per transplant for most islet transplant recipients.[107] Third, although immunosuppressive drugs are used, there is still a high rejection rate of islet cells after transplantation. Patients receiving multiple grafts will generate allo- and autoantibodies; Thus, the long-term effectiveness of islet allografts is not guaranteed. Finally, the lifelong use of immunosuppressants may increase the risk of infection, bone marrow suppression, kidney damage, and tumorigenesis.[109]

Generation of β-cells from human pluripotent stem cells (hPSCs)

The limited availability and quality of donor islets for transplantation treatment has fueled the search for alternative sources of insulin-secreting cells. Recent advances in the development of stem cell–derived β-cells have positioned hPSCs as a promising and theoretically unlimited source of insulin-producing cells. hPSCs typically include embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). ESCs are derived from the inner cell mass of blastocysts that occur about 4–5 days after fertilization and possess the potential to extensively self-renew and differentiate into any desired cell type in the body. In contrast, iPSCs with the same properties as ESCs are generated by genetically reprograming adult cells such as dermal fibroblasts back to an embryonic-like state using defined factors. Although ESCs represent a promising cell source for cell-based therapy and are potentially applied to treat a host of diseases, human ESCs are derived from surplus embryos, and their application in medicine has raised several ethical concerns. Pluripotent and proliferative iPSCs are derived from adult somatic cells without ethical concerns, and they retain the genetic profile of the source cells, making them a promising tool for cell therapy without immune consequences.

To truly potentiate hPSCs in β-cell regeneration, reproducible and efficient protocols that direct the differentiation of hPSCs to functional β-cells are needed. Exciting advances in the generation of hPSC-derived β-cells occurred in 2019, Velazco-Cruz *et al*[110] achieved robust dynamic insulin secretion of iPSC-derived β cells by modulating TGFβ signaling, reducing cell cluster size, and using enriched serum-free media in a three-dimensional culture system that mimics the pancreatic developmental environment. β-cells derived under these conditions can respond to multiple secretagogues in dynamic perfusion assays, and their transplantation rapidly restored the glucose tolerance of streptozotocin-treated mice. At the same time, Nair *et al*[111] employed cell sorting to reaggregate hESC-derived immature endocrine cells into β-cell enriched clusters, which more closely resemble the architecture of native islets. The resulting β-cells show dynamic insulin secretion, active calcium signaling, and metabolic maturation of mitochondria. Recently, Hogrebe *et al*[112] developed a new planar cell culture technique for generating human stem cell-derived β-cells, demonstrating that the polymerization of the actin cytoskeleton controls endocrine specification by modulating premature Neurog3 expression. Notably, β-cells generated with this protocol display dynamic insulin secretion and their transplantation into mice rapidly cures severe preexisting diabetes within 2 weeks. Most recently, the Deng laboratory developed a method to reprogram human somatic cells into pluripotency by using a combination of small-molecule compounds rather than genetic manipulation.[113] The human chemically induced PSC-derived islets (hCiPSC-islets) generated by this protocol contain insulin granules as well as α- and δ-like cells, and they display similar features to human islets in gene expression and biphasic insulin secretion. Implantation of hCiPSC-islets into the abdominal rectus sheath of diabetic monkeys restores endogenous insulin secretion and glycemic stability.[114] Currently, several stem cell-based therapies for diabetes have already entered clinical trials. One notable trial (No. NCT04786262) was conducted by Vertex. Pharmaceuticals (Boston, USA), involving the transplantation of stem cell-derived islets (VX-880) into T1D patients. VX-880 can improve glycemic control and reduce the requirements of exogenous insulin in all recipients.

Although there has been tremendous progress with the generation of hPSCs *in vitro*, the generated β-like cells do not recapitulate primary β-cells in some aspects, such as calcium response and mitochondrial metabolism,[115] and the amount of glucose-stimulated insulin secretion in hPSC-derived β-cells is lower than that of their primary counterparts.[111] Additionally, the reprograming protocols focus on eliminating polyhormonal cells that are generated *in vitro*, but a subset of polyhormonal cells naturally occur in human islets and particularly in fetal islets.[116]

Conclusions and Perspectives

Different diabetic therapeutics aim to replenish β-cell mass and hence restore β-cell function. However, the existence of heterogeneous β-cell subpopulations poses many challenges in terms of designing therapeutics or strategies for modulating this disease. Recent single-cell analyses support the presence of both mature and immature β-cell populations,[117] and SIX2 and SIX3 have been identified as crucial transcription factors in human β-cell maturation by enhancing insulin content and secretion. In some studies, β-cells are even divided into four distinct populations according to the expression of the cell surface markers CD9 and ST8SIA1,[118] and $CD9^+$ cells show less mature β-cell phenotypes with low levels of NKX6.1, MAFA, and C-peptide. In addition, β-cell heterogeneity is also present at epigenetic levels, and two major subtypes with high and low H3K27me3 expression have been identified.[119] Importantly, β-cell heterogeneity could be altered in diabetes or under some stress conditions.[117,118] For example, among three β-cell clusters that vary in proliferative potential, the percentage of highly proliferative

cells is significantly decreased in the presence of T2D.[117] β-cells also display dynamic heterogeneity characterized by low or high levels of insulin or UPR in a sequential order under stress conditions,[118] which allows β-cells to adapt to various insults such as oxidative and ER stress.

Pancreatic β-cell performance in response to glucose alterations is dependent on islet organization and cell composition,[111] raising questions regarding the use of purified β-cells *vs*. aggregates of islet cells for transplantation therapy. Veres *et al*[120] found that hPSC-derived pancreatic cells contained all main islet endocrine cell types including β-like cells, α-like cells, a small set of δ-like cells, and intestinal enterochromaffin-like cells. They sorted β-like cells for CD49a/ITGA1, a cell-surface marker found in β-cells, to remove enterochromaffin-like cells that are off targets generated in stem cell differentiation; the remaining cells are indeed reaggregated into islet organoid structures showing glucose responsiveness. Yoshihara *et al*[121] employ multicellular spheroids that mimic the cellular architecture and cell-type diversity of islets to generate insulin-positive as well as glucose-responsive and immune-evasive human islet-like organoids from iPSCs. Transplantation of the β-like cells generated from this protocol into diabetic immune-competent mice rapidly re-establishes glucose homeostasis.[121] Balboa *et al*[115] recently produced hPSC-derived islets that comprise ~40%, ~45%, and ~4% of β-, α-, and δ-cells, respectively. These islet-like clusters have similar glucose or incretin hormone responsiveness to primary human islets.

After transplantation, stem cell-derived β-like cells are attacked and destroyed by the recipient's immune system. Immunosuppressants are commonly used to suppress the immune response, resulting in serious negative adverse effects. In place of immunosuppressive agents, various approaches have been optimized and reported to circumvent immune rejection, such as islet encapsulation, immune cloaking, and the generation of localized immunoprotective niches. Although many challenges remain unanswered, we believe that β-cell regenerative therapies will represent a viable cure for diabetes in the not-too-distant future with the recognition of mechanisms responsible for β-cell development and mature endocrine cell plasticity, remarkable advances in improved protocols to generate exogenous β-cells from stem cells, and single-cell studies.

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Conflicts of interest

None.

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