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Pancreatic β-Cell Development and Regeneration

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The pancreatic β-cells are essential for regulating glucose homeostasis through the coordinated release of the insulin hormone. Dysfunction of the highly specialized β-cells results in diabetes mellitus, a growing global health epidemic. In this review, we describe the development and function of β-cells the emerging concept of heterogeneity within insulin-producing cells, and the potential of other cell types to assume β-cell functionality via transdifferentiation. We also discuss emerging routes to design cells with minimal β-cell properties and human stem cell differentiation efforts that carry the promise to restore normoglycemia in patients suffering from diabetes.

THE β-CELL

Pancreatic β-cells are vital to the maintenance of systemic glucose homeostasis. In response to elevated blood glucose levels, β-cells secrete the peptide hormone insulin, which stimulates glucose uptake by peripheral tissues such as skeletal muscle, liver, and adipose tissue. Glucose-stimulated insulin secretion (GSIS) occurs when β-cells sense and absorb glucose from the bloodstream through specific glucose transporters. Internalized glucose is metabolized, largely within the mitochondria, to generate ATP. This increase in the intracellular ATP/ADP ratio induces closure of K_{ATP}-sensitive channels and plasma membrane depolarization, causing Ca^{2+} channels along the cell surface to open. The resulting influx of Ca^{2+} ions induces exocytosis of insulin granules from the β-cell interior (Fig. 1). Tight regulation of insulin secretion is critical; constitutive insulin secretion results in chronic low blood glucose, or hypoglycemia, which poses a significant immediate health risk. In contrast, perturbation of β-cell function and diminished insulin secretion results in diabetes and its associated long-term complications.

According to the WHO report (2021), diabetes is an evolving disease affecting 422 million people worldwide and causing 1.6 million deaths annually, with the prevalence rate increasing steadily over the past few decades majorly in middle- and low-income countries. The most prevalent and well-characterized forms of diabetes are type 1 diabetes and type 2 diabetes, which account for nearly all diagnosed cases; however, there exist multiple additional subtypes, including the monogenetic mature onset diabetes of the young (MODY) and transient gestational diabetes (Ahlqvist et al. 2020). Type 1 diabetes is an autoimmune disease caused by the destruction of β-cells by ac-

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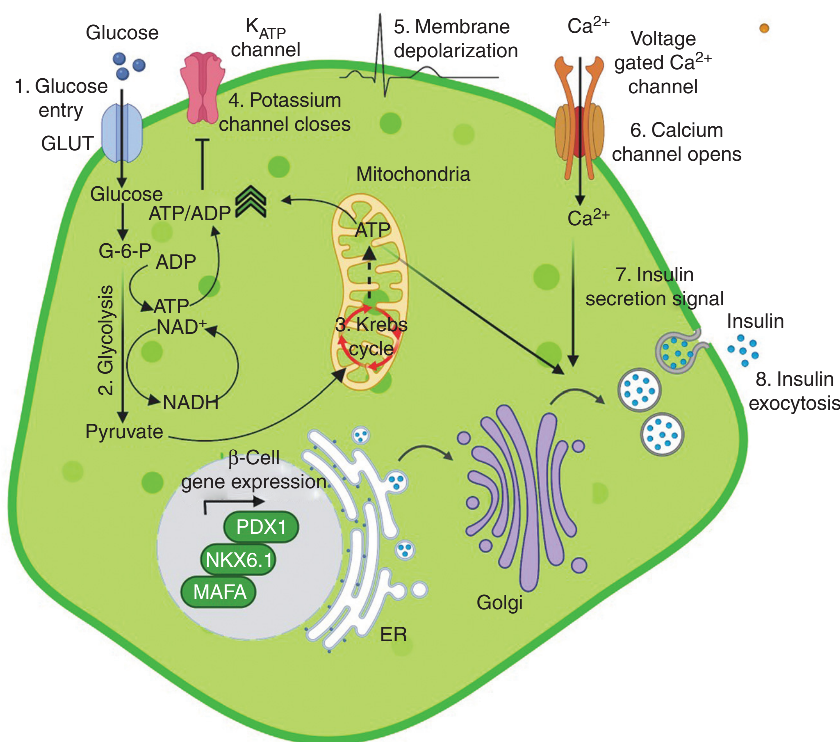

Figure 1. Schematic of key steps of insulin secretion mechanism in mature β-cells with emphasis on key steps. (1) Rise in extracellular glucose concentration leads to its uptake via the glucose transporter GLUT2 (GLUT1 in human β-cells), which is phosphorylated by glucokinase, to (2) glucose-6-phosphate (G-6-P) entering the glycolysis pathway to generate pyruvate, ATP, and NADH. Pyruvate is channeled to mitochondria to undergo metabolism by the Krebs cycle and subsequently generating more ATP (3), which together with ATP generated from glycolysis, increases the ATP/ADP ratio. This causes closure of ATP-dependent K^+ (K_{ATP}) channels (4), leading to reduced K^+ efflux, plasma membrane depolarization (5), and opening of voltage-dependent Ca^{2+} channels (6). This results in Ca^{2+} influx and rise in cytosolic free Ca^{2+} concentration $[Ca^{2+}]$, which acts as a triggering signal for insulin secretion (7). High levels of ATP further facilitate insulin vesicle transport and docking on the plasma membrane and subsequent release into the cytosol (8). (ER) Endoplasmic reticulum.

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tivated immune cells. This wholesale elimination of β-cells results in a near-complete lack of circulating insulin, necessitating treatment with exogenous insulin or a whole-pancreas or islet transplant. Type 2 diabetes, however, is driven by two primary factors: an acquired resistance to insulin by the peripheral tissues responsible for glucose uptake and β-cell dysfunction leading to an inability to produce and secrete sufficient insulin. This review will explore various aspects of β-cell biology: physiology, development, and intra- and intercellular interactions. We will also delve into the concepts of β-cell heterogeneity and plasticity. Finally, we will touch upon modern strategies to generate β-cells in vitro and the novel, emergent technologies designed to improve and protect β-cells.

HISTOLOGY AND ARCHITECTURE OF THE PANCREATIC ISLET

The pancreas includes two distinct functional compartments. The exocrine pancreas, which accounts for the vast majority of the pancreas, includes the acinar and ductal cells responsible for producing, secreting, and transporting digestive enzymes (Gardner and Jackson 1977; Kern 1993; Williams 2010; Reichert and Rustgi

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2011). The endocrine pancreas refers to the scattered clusters of endocrine cells, vasculature, and neurons, otherwise known as islets of Langerhans, which produce and secrete various peptide hormones (Logothetopoulos et al. 1965; Orci and Unger 1975). The cell-type and functional diversity present within the islet has resulted in their conceptualization as "microorgans." Insulin-secreting β-cells are the predominant endocrine cell type present in the islet; others include α- and δ-cells, which produce and secrete glucagon and somatostatin, respectively (Lazarow 1957; Da Silva Xavier 2018). Islets also possess a small subpopulation of rare endocrine cells known as pancreatic polypeptide (PP) cells and epsilon cells, which secrete ghrelin (Prado et al. 2004). The cell types present within the islet are similar across mammalian species. It is, however, important to note the existence of species-specific differences in islet architecture, particularly relating to cellular proportions and spatial distribution. Given the prevalence of rodent models of pancreatic biology, comparison studies have predominantly focused on differences and commonalities between human and rodent islets (Cabrera et al. 2006; Kim et al. 2009; Steiner et al. 2010; Bonner-Weir et al. 2015; Dolenšek et al. 2015; Tsuchitani et al. 2016). Immunohistochemical analysis of human and rodent islets has revealed that rodent islets are composed of a clustered "core" of β-cells (~80%–90%) surrounded by a "mantle" of α-, δ-, and PP cells at the islet periphery. Human islets, however, possess approximately equivalent numbers of β- and non-β-endocrine cells intermingled within the islet proper, although they also demonstrate a notable degree of islet heterogeneity (Dybala and Hara 2019). These discrepancies must be considered when attempting to extrapolate human β-cell biology from rodent studies.

INTRA-ISLET COMMUNICATION

Disruption of islet architecture in vitro significantly diminishes β-cell function (Dunbar and Walsh 1982; Pipeleers et al. 1982; Weir et al. 1984; Hopcroft et al. 1985). Islet structure lends itself to extensive intercellular communication: signaling between β-cells and neighboring cells may occur through hormones from neighboring α- and δ-cells, angiogenic factors from endothelial cells, neurotransmitters from resident autonomic nerves, and physical cell–cell contact.

Islet Hormones

The hormones produced by α-, β-, and δ-cells are primarily designated for endocrine signaling via secretion into the circulatory system and transport to distant organ systems. However, these hormones also serve as paracrine signaling molecules, facilitating critical intercellular cross talk and intra-islet regulation. This is apparent in the intricate regulatory patterns of glucagon, insulin, and somatostatin. Glucagon regulates glucose homeostasis both globally and within the islet (Tellez et al. 2020). During periods of hypoglycemia, α-cells release glucagon to stimulate hepatic glycogenolysis and gluconeogenesis, thereby raising blood glucose levels (Gromada et al. 2007). However, glucagon has also been shown to promote insulin secretion through interaction with the glucagon receptor (GcgR) or the GLP-1 receptor (GLP-1R) at the β-cell surface (Kieffer et al. 1996; Moens et al. 1998; Huypens et al. 2000; Svendsen et al. 2018). Given the function of insulin as a negative regulator of glucagon, this suggests that glucagon secretion promotes its own negative feedback loop to prevent glucagon-induced hyperglycemia. Conversely, somatostatin is both stimulated by and inhibitory of glucagon and insulin secretion (Koerker et al. 1974; Taborsky et al. 1978; Hauge-Evans et al. 2009), thus providing an additional layer of control to ensure tight regulation of physiological glucose levels.

Vasculature and Nerves

The islet is heavily vascularized, reflecting its endocrine function, nutrient requirements, and robust oxygen demand (Bonner-Weir and Orci 1982; Ballian and Brunicardi 2007; Nyman et al. 2008). Limited vascularization greatly hinders human islet transplants through significant β-cell death and graft failure (Brissova et al. 2004, 2005; Zhang et al. 2004). Type 2 diabetic

islets further display perturbed capillary morphology and density (Brissova et al. 2014). Overexpression of VEGF-A in rodent β-cells increases vascularization, disrupts islet morphogenesis, reshapes endothelial ultrastructure, and drastically alters β-cell proliferation (Cai et al. 2012; Brissova et al. 2014). Islet vascularization is also inextricably linked to innervation: islet-associated endothelial cells produce proneuronal migration molecules, with intra-islet capillaries acting as physical scaffolding for the ingrowth of autonomic nerves (Reinert et al. 2014). Importantly, establishment of islet architecture depends upon interaction between β-cells and local sympathetic nerves; depletion of sympathetic innervation disrupts rodent islet cytoarchitecture and diminishes GSIS (Borden et al. 2013).

Physical Cell–Cell Contact

Physical endocrine cell–cell contact is critical to intra-islet cross talk. These interactions are mediated by a wide array of proteins at the cell surface, including gap junction proteins, cell-adhesion molecules (CAMs), and receptor tyrosine kinases (RTKs).

Gap junctions are formed via the tubular amalgamation of connexin proteins and act as intercellular channels. Particular attention has been devoted to connexin 36 (Cx36), which is preferentially expressed in both rodent and human β-cells (Serre-Beinier et al. 2000, 2009). An increase in gap junctions between neighboring β-cells has been shown to occur during both functional maturation and insulin secretion (Carvalho et al. 2010). Subsequent studies have demonstrated that Cx36 gap junctions, specifically, regulate the synchronization of Ca^{2+} oscillations that occur during GSIS (Calabrese et al. 2003; Ravier et al. 2005; Benninger et al. 2011).

CAMs refer to four distinct protein families—integrins, selectins, immunoglobulins, and cadherins—expressed on the cell surface for the purpose of facilitating cell–cell adhesion, signal transduction from the extracellular environment, and downstream intracellular events. Particularly promising have been cadherins, transmembrane glycoproteins that mediate homophilic Ca^{2+}-dependent cell–cell interactions. Loss of E-cadherin has been shown to disrupt gap junction formation, and therefore Ca^{2+} signaling and insulin secretion (Yamagata et al. 2002; Rogers et al. 2007).

Ephrin receptors constitute the largest family of RTKs, unique for their ability to engage in bidirectional signaling. EphA5 receptors and their cognate ephrin-A5 ligands are both expressed in human islets (Konstantinova et al. 2007). Strikingly, loss of forward and reverse signaling between EphA5 and ephrin-A5 resulted in opposite effects: reverse signaling through ephrin-A5 increased insulin secretion during both phases of the glucose response, whereas forward signaling through EphA5 reduced insulin secretion. These effects were attributed to reduced granule fusion events on the β-cell surface, rather than alterations in upstream components of the insulin secretion pathway. Recent studies have found that ablation of the primary cilia located at the β-cell plasma membrane resulted in hyperphosphorylation of the EphA2/3 receptors and a subsequent decrease in glucose tolerance and insulin secretion, similar to that observed upon activation of EphA5 reverse signaling (Volta et al. 2019). The importance of primary cilia to the β-cells has been further bolstered by studies indicating that dysregulation of Hedgehog signaling resulting in part from primary cilia ablation induces diminished glucose tolerance and degradation of β-cell identity (Cervantes et al. 2010; Landsman et al. 2011). Taken together, these findings point to the importance of a wide array of cell-surface proteins and physical cell–cell communication in regulating β-cell function.

ISLET AND β-CELL DEVELOPMENT

β-cell development is regulated in utero by complex interplay between transcription factors and dynamic changes in gene regulatory networks. Postnatal β-cell maturation continues with molecular and metabolic events that enable the β-cell to achieve GSIS. Illuminating the process of β-cell development and maturation is critical to our understanding of β-cell biology and ef-

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forts to counteract the degradation of β-cell identity in addition to mechanisms that occurs during physiological stress and diabetes (Weir and Bonner-Weir 2004; Chakravarthy et al. 2017). Understanding β-cell development and maturation is also vital to the advancement of directed β-cell differentiation protocols, which aim to artificially recapitulate such processes in vitro.

Most of our understanding of β-cell differentiation comes from rodent studies, although recent work has shown that the rodent and human endocrine compartments diverge at certain stages of development (Larsen and Grapin-Botton 2017). In rodents, endocrine differentiation occurs at two stages of embryonic development: the primary transition (E9.0–E12.5), where the main differentiated endocrine cells are α-cells, and secondary transition (E12.5–E15.5), at which point other endocrine cell types, including β-cells, are formed (Pan and Wright 2011; Shih et al. 2013; Nair and Hebrok 2015; Bastidas-Ponce et al. 2017). Human endocrine development is thought to exclusively entail the secondary transition, which gives rise to all hormone-secreting endocrine cells (Jennings et al. 2013); however, it is known that all endocrine cell types arise from a common progenitor pool in both rodent and human. Endocrine development begins with the emergence of endocrine progenitors lowly expressing the transcription factor Neurogenin-3 ($NGN3^{low}$). NGN3 is indispensable for endocrine cell formation; an increase in NGN3 expression generates $NGN3^{high}$ endocrine precursors. From these precursors arises cells positive for the transcription factor Fev, which differentiate to form endocrine cells that express hormones and possess the requisite secretory machinery (Wang et al. 2010; Bechard et al. 2016; Byrnes et al. 2018). At this stage, multiple transcription factors begin to drive the establishment of terminal endocrine cell identities. Expression of ARX favors α-cells; PAX4 favors the formation of β- and δ-cells. Adoption of the β-cell fate additionally depends on the expression of several other transcription factors, such as FOXA2, PDX1, NKX6.1, NEUROD1, NKX2.2, and MNX1 (Arda et al. 2013).

All β-cells express certain genes that are required for the establishment and maintenance of β-cell identity at birth, including *NKX6.1*, *PDX1*, and *NEUROD1*. The initial postnatal maturation phase is largely characterized by the induction of additional β-cell markers and an increase in endocrine cell mass due to the highly, although progressively declining, proliferative nature of immature β-cells (Blum et al. 2012). The Maf family, in particular, is important for the establishment of β-cell identity and functionality (Hang and Stein 2011); in rodents, the switch from MafB to MafA expression heralds β-cell maturation, a clear distinction to the human situation in which *MAFB* expression is retained in mature β-cells (Arda et al. 2016; Cyphert et al. 2019; Russell et al. 2020). During this stage, β-cells further demonstrate an increase in Urocortin3 (UCN3) and SYT4 of the Synaptotagmin (Syt) family, with a concurrent reduction in Neuropeptide Y (NPY) (Blum et al. 2012; van der Meulen et al. 2012; Rodnoi et al. 2017). During the second phase of maturation, β-cells shift from relying predominantly on glycolytic metabolism to favoring aerobic respiration and mitochondrial metabolism, a change that underlies functional maturation (Bonner-Weir et al. 2016). Insulin processing, glucose sensing, and granule exocytosis genes such as *GLUT2*, *GLP-1* receptor, prohormone convertase-1/3, and pyruvate carboxylase are up-regulated (Artner et al. 2007; Nishimura et al. 2015). Intriguingly, the surface markers ST8SIA1 and CD9 additionally delineate four antigenic subgroups of β-cells in human islets upon maturation. These subgroups demonstrate differential expression of genes involved in insulin secretion, such as *GLUT2, PPP1R1A, SUR2,* and *G6PC2*, but similarly express primary β-cell markers (Dorrell et al. 2016). This finding reflects our growing understanding of transcriptional and functional heterogeneity within the β-cell population.

FUNCTIONAL HETEROGENEITY OF β-CELLS

β-cells exhibit heterogeneous properties both within the individual islets and between islets localized to different areas of the pancreas (Dorrell et al. 2016). Single-cell transcriptomic and

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proteomic data, as well as experimental studies, suggest that this heterogeneity encompasses both gene expression and β-cell function, including processes such as glucose sensing, intracellular calcium signaling, metabolism, insulin biosynthesis, and insulin secretion (Pipeleers et al. 1994; van der Meulen et al. 2017; Mawla and Huising 2019). From these differences arise a diverse, hierarchical network of β-cells, all contributing to the maintenance of glucose homeostasis (Johnston et al. 2016; Salem et al. 2019).

Recent evidence from rodents indicates the presence of a subpopulation of β-cells known as "hub cells," which demonstrate increased expression of glucokinase and glucose oxidation genes, hyperpolarized mitochondria, reduced PDX1 and NKX6.1, and higher metabolic activity despite fewer insulin granules. These cells have been proposed to make connections with, and control the activity of, other "follower" β-cells (Johnston et al. 2016; Xin et al. 2016; Salem et al. 2019). Although these cells have not yet been identified in human islets, the identification and characterization of hub cells reveals a novel mechanism for concerted insulin secretion across β-cells within rodent islets. These hub cells constitute 1%–10% of islet surface, display many electrically coupled cellular connections, and exhibit accelerated and sustained Ca^{2+} signaling upon glucose stimulation, which precedes that of the "follower" cells. Hub-cell inactivation by photoablation performed in zebra fish islets disrupted the synchronization of Ca^{2+} signaling across the islet, whereas similar perturbation had no effect in follower cells. Hub cells may therefore serve as "pacemakers" to synchronize glucose-stimulated Ca^{2+} signaling and insulin granule exocytosis across networks of follower cells throughout the islet. The difference in the glucose responsiveness between the hub and follower cells may be due to varying levels of glucose uptake, unknown mechanisms of Ca^{2+} signal synchronization, or changes in islet architecture upon isolation. Interestingly, hub cells seem to largely escape immune destruction in type 1 diabetes (Liu and Hebrok 2017; Oram et al. 2019).

Emerging from these studies is the concept that the function of individual β-cells is determined by intercellular and intracellular signaling events, with a subset of β-cells more extensively involved in the coordination of insulin secretion necessary for ensuring that the islet functions as an integrated unit, despite the diversity of cell types that comprise it. Loss of these interactions has been shown to perturb glucose stimulation threshold and the kinetics of insulin secretion; furthermore, altered distribution of islet cell populations has been implicated as a hallmark of obesity, aging, and type 2 diabetes (Dorrell et al. 2016). However, several questions regarding β-cell diversity remain unanswered, including whether they arise from subtle differences in developmental programs or settle into subpopulations at later stages of maturation, where they are localized in the 3D islet architecture, how that localization relates to their function, and, finally, whether subpopulations that are fluent with cells within one population can assume properties of the others (Liu and Hebrok 2017).

PANCREAS REGENERATION

Defining and replicating the regenerative properties of the pancreas has been a tantalizing goal. The different cellular components of the pancreas, exocrine and endocrine, display different regenerative potentials (Zhou and Melton 2018). Furthermore, regenerative capacity is linked to the particular damage inflicted upon the organ.

Regeneration of Exocrine Pancreas

Acinar cell regeneration has been studied in detail upon treatment with caerulein, a human cholecystokinin analog, that results in pancreatitis-like phenotypes and a transient dedifferentiation of acinar cells toward a duct-like identity. Short-term caerulein treatment appears to be completely reversible within several days (Jensen et al. 2005; Morris et al. 2010). The regenerative process involves transient phases of inflammation, metaplasia, and redifferentiation. Two distinct modes of regeneration have been proposed to occur in models of pancreatitis (Murtaugh and Keefe 2015): the generation of

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new acinar cells from preexisting acinar cells (Desai et al. 2007; Strobel et al. 2007; Blaine et al. 2010) or the redifferentiation of degranulated and duct-like acinar cells toward their normal functional state. The study of acinar redifferentiation has led to the identification of proinflammatory and developmentally regulated genes that guide this process. Deletion of key components of the Hedgehog, Notch, and Wnt pathways as well as NR5A2 and PTF1A transcription factors from acinar cells severely disrupts exocrine regeneration (David et al. 2005; Fendrich et al. 2008; Siveke et al. 2008; Morris et al. 2010; Stanger and Hebrok 2013; Von Figura et al. 2014; Hoang et al. 2016). Of note, the regenerative process is highjacked in the presence of oncogenic Kras, the predominant oncogene in pancreatic ductal adenocarcinoma (Morris et al. 2010).

Regeneration of the Endocrine Pancreas

Extensive research has been conducted to determine the extent to which endocrine cells within the pancreas can regenerate upon injury. Early islet regeneration studies relied on rodent injury models, including pancreatectomy, pancreatic duct ligation, and chemical ablation of β-cells with toxins streptozotocin (STZ) or alloxan. In particular, pancreatic duct ligation has been used extensively to demonstrate regenerative potential of acinar and insulin-producing β-cells in rodents (Lehv and Fitzgerald 1968; Bonner Weir et al. 1983). With regard to β-cell regeneration, early studies implicated pancreatic duct cells as progenitors for endocrine cells (Bonner-Weir et al. 1993). Follow-up studies, however, supported the notion that existing β-cells, and not pancreatic duct cells, serve as progenitors for regenerating β-cells after pancreatic duct ligation (Solar et al. 2009).

Pancreatic β-cell ablation using STZ, a chemical toxin that structurally mimics glucose and is imported into the β-cells through GLUT2 receptors (Szkudelski 2001; Graham et al. 2011), or alloxan, a selective inhibitor of glucokinase, have been extensively used to ablate rodent β-cells (Lenzen 2008). Depending on drug dosage, the entire β-cell mass can be partially or nearly completely ablated in a few days. Extensive studies have found no convincing evidence for extensive β-cell regeneration in adult animals following chemical ablation (Rankin and Kushner 2009; Tschen et al. 2009). However, β-cells in young rodents have high proliferative capacity that declines rapidly with age (Byrne et al. 1995; Montanya et al. 2000; Teta et al. 2005). β-cell replacement has been observed in young animals, but the source of these new β-cells after injury was identified as replication from preexisting β-cells (Dor et al. 2004). In humans, removal of 50% of the pancreas leads to insulin-dependent diabetes (Kumar et al. 2008; Menge et al. 2008). Although juvenile human pancreas offers some regeneration after pancreatectomy, this capacity rapidly declines with age and appears completely absent in adult humans (Berrocal et al. 2005; Menge et al. 2008; Rankin and Kushner 2009).

More recently, transdifferentiation of α- or δ-cells into β-cells has been reported upon diphtheria toxin-based β-cell ablation in mice (Thorel et al. 2010; Chera et al. 2014). The molecular mechanism of this conversion between islet cell types has yet to be uncovered, and the extent to which new β-cells form from their endocrine relatives appears to be rather small. Furthermore, replicative capacity of adult human β-cells is still a matter of debate; however, islet hyperplasia has been reported during pregnancy, stimulated by placental lactogen and prolactin hormones (Kalousova et al. 2010; Kim et al. 2010; Ohara-Imaizumi et al. 2013). High-fat diet-induced obesity and insulin resistance condition also leads to increase in β-cell mass (Rieck and Kaestner 2010; Mezza and Kulkarni 2014; Wang et al. 2015a,b; Saunders and Powers 2016). The molecular pathways that drive these increases in β-cell mass in obesity and insulin resistance have yet to be fully elucidated. Studies using insulinoma samples have implicated the dual-specificity tyrosine-regulated kinase-1a (DYRK1A) and its inhibitor Harmine (Wang et al. 2014) as novel regulators of human β-cell proliferation. Considering that Harmine elicits proliferative responses in other cells, combining it with glucagon-like peptide receptor agonists allows for more specifically targeting β-cells to

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induce proliferation (Ackeifi et al. 2020). Additional studies will be necessary to further refine safe stimulation of endogenous human β-cell expansion.

β-CELL DEDIFFERENTIATION, REDIFFERENTIATION, AND *TRANS*-DIFFERENTIATION

In addition to the presence of defined β-cell subpopulations, emerging evidence indicates the propensity for insulin-producing cells to lose or regain their differentiation state.

Dedifferentiation

Dedifferentiation may be defined as the loss of the mature β-cell transcriptional profile (Puri et al. 2015) and resulting functional decline. Unlike many other terminally differentiated cell types, β-cells are plastic and may undergo dedifferentiation under certain pathophysiological conditions, such as chronic hyperglycemia, obesity, and aging (Kayali et al. 2007; Dhawan et al. 2011; Puri et al. 2013; John et al. 2018). Maintaining the β-cell identity requires the induction and repression of genes characteristic of mature and immature β-cells, respectively. Dedifferentiation entails the down-regulation of canonical β-cell maturity genes, such as *PDX1, NKX6.1, MAFA, MAFB*, and *GLUT2*, as well as genes involved in mitochondrial metabolism and insulin biosynthesis and secretion. Conversely, dedifferentiated β-cells exhibit increased levels of "disallowed" genes expressed in immature β- and endocrine progenitor cells, including *NGN3, LDHA, MCT4*, and *SOX9*. These transcriptional changes are accompanied by a loss in β-cell mass, which is attributable specifically to β-cell dedifferentiation, rather than increased apoptosis (Talchai et al. 2012).

Single-cell RNA sequencing of human islets has revealed that islet cells can dedifferentiate ex vivo; furthermore, β-cells from type 2 diabetic patients have been found to adopt the gene expression profile of endocrine progenitors, which is suggestive of dedifferentiation. (Talchai et al. 2012; Guo et al. 2013a; Brereton et al. 2014; Wang et al. 2014; Cinti et al. 2016; Jacobson and Liu 2016; Dirice et al. 2019). Although the precise mechanism of β-cells dedifferentiation remains unknown, potential players include hypoxia, oxidative stress and reactive oxygen species (ROS) accumulation, and inflammation-induced endoplasmic reticulum (ER) stress (Sherry et al. 2006; Puri et al. 2009, 2013; Hasnain et al. 2014; Nordmann et al. 2017).

Redifferentiation

Prior to the advent of stem cell–derived β-cells, one potential strategy for overcoming the shortage of cadaveric donor islets was in vitro expansion of human β-cells (Bar et al. 2012). However, this process resulted in β-cell dedifferentiation and epithelial-to-mesenchymal transition, likely due to the loss of islet architecture and the induction of nonphysiological proliferation (Puri et al. 2018). Subsequent research demonstrated that human β-cells could be partially redifferentiated in vitro postexpansion through a variety of methods, including inhibition of the Notch signaling pathway, overexpression of miR-375, or culture within specialized 3D hydrogel systems (Bar et al. 2012; Nathan et al. 2015; Aloy-Reverté et al. 2018). Despite this, the use of human β-cells expanded in vitro soon lost appeal as a therapeutic option due to issues with efficiency, propensity for repeated dedifferentiation, and lack of evidence of functionality (Baeyens et al. 2018; Brovkina and Dashinimaev 2020).

Trans-Differentiation of β-Cells from α- and Exocrine Cells

β-cell *trans*-differentiation, or the direct interconversion between β-cells and other cell types, is thought to represent a potential therapeutic strategy for diabetes (Bouwens et al. 2013; Aguayo-Mazzucato and Bonner-Weir 2018). However, efforts to take advantage of this phenomenon have been hampered by a number of scientific and logistical concerns, including the lack of a clear understanding of pancreatic plasticity, insufficient characterization of *trans*-differentiated cells, and difficulty of inducing

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cellular *trans*-differentiation with spatial and temporal precision.

α- to β-Cell

Interconversion between α- and β-cells has generated particular excitement due to their derivation from the same lineage, localization within the islet, overlap in a number of endocrine and secretory genes, and relative abundance in type 1 diabetic pancreata. Given that these cell types originate from a common endocrine progenitor cell, two theories regarding the broad mechanism of *trans*-differentiation have evolved: first, that glucagon-positive α-cells, at least in humans, become insulin-positive β-cells through an intermediate, double hormone-positive stage; and second, that α-cells revert back into endocrine progenitors, which then proceed to adopt the β-cell phenotype (van der Meulen and Huising 2015). *Trans*-differentiation has been shown to occur between pancreatic α- and β-cells upon damage to the pancreas and ablation of endogenous β-cells, as well as through various genetic manipulations. Depletion of rodent β-cells through acute treatment with diphtheria toxin, as well as pancreatic ductal ligation combined with alloxan-induced β-cell ablation, both result in β-cell neogenesis from α-cells, as confirmed by lineage tracing (Chung et al. 2010; Thorel et al. 2010). Genetically engineered mouse models have revealed that ectopic overexpression of the early β-cell transcription factor PAX4, or knockout of the α-cell transcription factor ARX, have each been shown to promote α- to β-cell *trans*-differentiation (Collombat et al. 2010; Courtney et al. 2013). Promising research has shown that endogenous rodent α-cells can be reprogrammed into β-cells and used to cure autoimmune diabetes through viral gene therapy and overexpression of PDX1 and MAFA (Xiao et al. 2018). Furthermore, Tang and colleagues recently demonstrated that reversal of diabetic autoimmunity through induction of haploidentical mixed chimerism, followed by administration of gastrin and epidermal growth factor (EGF), can result in rodent β-cell neogenesis via α- to β-cell *trans*-differentiation (Tang et al. 2020). This *trans*-differentiation, combined with reactivation of insulin-low dysfunctional β-cells, was able to achieve normoglycemia in mice models of type 1 diabetes.

Exocrine to β-Cell

Pancreatic exocrine cells have demonstrated a degree of plasticity and the ability to adopt β-cell characteristics under certain conditions. Early experiments demonstrated that rodent pancreatic exocrine cells could be isolated and cultured in vitro with leukemia inhibitory factor (LIF) and EGF to generate insulin-producing cells (Baeyens et al. 2005); reprogramming of endogenous rodent exocrine cells in vivo was later achieved through the adenoviral delivery and induction of one or more key β-cell transcription factors NGN3, PDX1, and MAFA (Zhou et al. 2008; Miyazaki et al. 2021). These and subsequent studies have found that pancreatic acinar, not ductal cells, were the predominant targets for exocrine- to β-cell *trans*-differentiation in both experimental models and type 1 diabetic patients (Masini et al. 2017). Recent research demonstrating that culturing human acinar and ductal cells together in 3D cultures known as "pancreatospheres" results in acinar, but not ductal cells; adopting a progenitor-like transcriptional profile may offer a potential explanation for the discrepancy in *trans*-differentiation capacity between these two cell types (Baldan et al. 2019). Whereas ductal cells have been shown to potentially possess some measure of *trans*-differentiation potential (Yatoh et al. 2007), the increased efficacy of acinar- to β-cell *trans*-differentiation and surplus of acinar cells within the human pancreas have resulted in ductal cells largely being sidelined as potential *trans*-differentiation targets.

Hepatocyte to β-Cell

Research efforts have additionally focused on hepatocytes as a potential source for β-cell *trans*-differentiation. Hepatocytes can be harvested directly from type 1 diabetic patients, thereby bypassing the issue of immune rejection; accordingly, these cells represent particu-

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larly appealing prospects for expansion and *trans*-differentiation. Ectopic expression of PDX1 in both rodent liver in vivo and human hepatocytes in vitro results in up-regulation of critical β-cell markers, such as the transcription factors NKX6.1 and NKX2.2, as well as genes critical for insulin biosynthesis and processing, such as *INS1*, *INS2*, and *PC1/3* (Ferber et al. 2000; Sapir et al. 2005). The efficiency of this process was found to be improved treatment with the GLP-1R agonist extendin 4 or the growth factor PGDF (Aviv et al. 2009; Chang et al. 2016). However, there remain significant hurdles to the production of mature β-cells from hepatocytes, including the use of viral vectors for delivery and induction of ectopic PDX1, the propensity for transdifferentiated cells to express pancreatic progenitor or exocrine markers, and relatively low GSIS. Furthermore, the capacity for *trans*-differentiation has been found to be restricted to a small population of *trans*-differentiation-competent hepatocytes enriched for Wnt signaling (Cohen et al. 2018).

GENERATION OF HUMAN STEM CELL–DERIVED β-CELLS IN VITRO

Transplantation of cadaveric human islets has long been used as a treatment strategy for type 1 diabetes. Despite its success, this procedure comes with numerous challenges, including a limited supply of donor islets, engraftment failure, and immune rejection. The last several decades have therefore seen significant efforts to establish protocols for generation of functional β-cells from human pluripotent stem cells (hPSCs) for the dual purposes of replacing cadaveric islets and serving as platforms to interrogate β-cell biology in human cells. The majority of this work was performed using human embryonic stem cells (hESCs); however, the use of induced pluripotent stem cells (iPSCs), derived from adult somatic cells, has become increasingly common.

Initial attempts to generate insulin-producing cells relied on the spontaneous differentiation of hESCs enriched for Nestin, an intermediate filament protein expressed in neural stem cells and pancreatic progenitors (Hunziker and Stein 2000; Assady et al. 2001). However, the cells resulting from these protocols proved incapable of insulin biosynthesis (Rajagopal et al. 2003) Attention therefore turned toward the establishment of directed differentiation protocols based on known principles of rodent pancreatic development. Selective introduction of growth factors, agonists, and small-molecule inhibitors facilitated the stepwise progression of hESCs through definitive endoderm, to pancreatic and endocrine progenitors, ultimately resulting in the generation of insulin-producing β-cells (D'Amour et al. 2005; Guo et al. 2013b; Rezania et al. 2013, 2014; Pagliuca et al. 2014; Russ et al. 2015). The cells yielded by these protocols largely resembled immature β-cells, as characterized by their inability to engage in GSIS.

Recent work has led to the development of novel protocols for the purpose of driving further maturation of these immature β-cells, thereby allowing them to better recapitulate those found in human islets. Isolation, purification, and reaggregation of immature β-cells has been found to produce enriched clusters of insulin-positive β-cells that strongly resemble human islets in terms of transcriptional profile, dynamic GSIS, Ca^{2+} signaling, mitochondrial metabolism, and glucose regulation upon transplantation into diabetic mice (Nair et al. 2019). Further strategies for improving hPSC-derived β-cells have focused on modulation of signaling pathways such as transforming growth factor β (TGF-β), metabolic augmentation, and purification of distinct endodermal progenitor populations prior to differentiation (Velazco-Cruz et al. 2019; Davis et al. 2020; Mahaddalkar et al. 2020).

Despite significant improvements in the production and transplantation of hPSC-derived β-cells, autoimmune rejection continues to pose a threat to their engraftment and survival. Immune rejection may be avoided by generating β-cells from patient-specific iPSCs, but such an approach is prohibitively difficult to scale and economically unviable. Under consideration are biobanks containing a limited number of iPSC lines with human leukocyte antigen (HLA) types that match the majority of poten-

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tial recipients within specific ethnic populations (Nakatsuji et al. 2008; Gourraud et al. 2012; Taylor et al. 2012). Unfortunately, this strategy fails to ameliorate concerns regarding autoimmune rejection in patients with type 1 diabetes. For that reason, gene-editing strategies for immunomodulation of hPSC-derived β-cells are currently under active investigation. One such method, referred to as "immune cloaking," entails removing the MHC class I genes (HLA) that control presentation of auto- and allo-antigens to provoke an immune response (Fig. 2; van der Torren et al. 2017; Deuse et al. 2019; Han et al. 2019). Deletion of all HLA proteins could further confer total immune protection; it was recently shown that abolishment of HLA-A, HLA-B, and partial HLA-C expression through deletion of β-2-microglobulin, paired with continued expression of HLA E/G, conferred hypo-immunogenicity and allowed hPSC-derived β-cells to evade T-cell attack (Xu et al. 2019). Similar immune evasion strategies include T-cell inactivation and subsequent prevention of β-cell loss via β-cell-specific overexpression of PDL1-CTLA4Ig molecules (El Khatib et al. 2015). Importantly, reducing immunogenicity of transplanted hPSC-derived β-cells also increases the probability of neoplastic growths and pathogenic infections. This issue may be tackled through the use of genetic "kill switches," as discussed below.

ADVANCES IN β-CELL TECHNOLOGIES

Research is progressively focusing on the generation of stem cell–derived β-cells as a viable large-scale treatment option for type 1 diabetes. Despite significant advancements in β-cell differentiation protocols, the requirement for multiple complex media formulations and the proven establishment of heterogeneous end-stage cell populations continues to render the process both challenging and inconsistent. Furthermore, the propensity for variability across differentiations, threat of autoimmune graft rejection, and safety risks inherent to stem cell

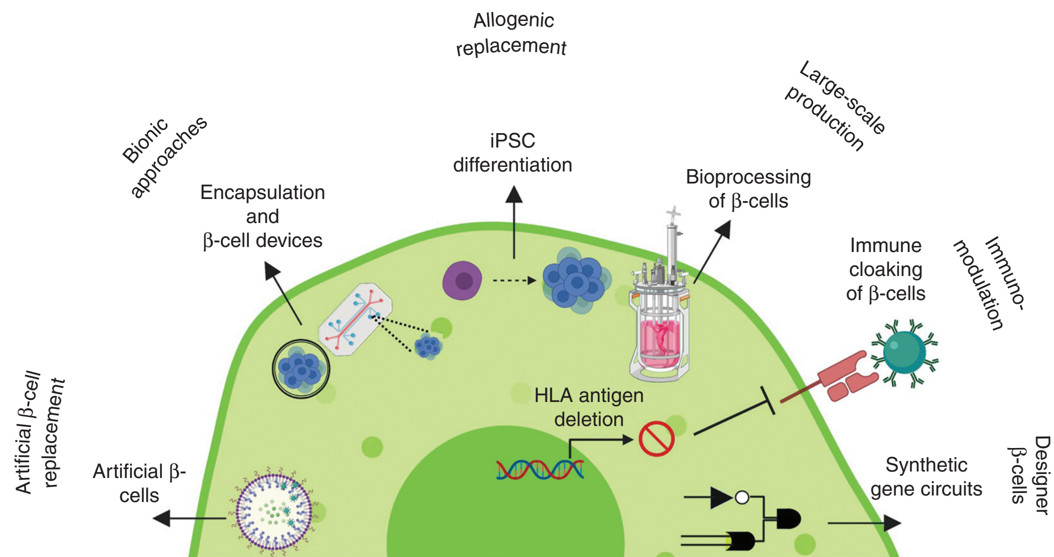

Figure 2. Advances in β-cell therapy. (1) Designer β-cells with synthetic gene circuits for precise control of developmental, metabolic, and/or signaling pathways. (2) Immunomodulation approaches to generate β-cells invisible to the host immune system. (3) Bioprocessing technologies for scalable manufacture of β-cells. (4) Use of patient-specific iPS cells for generation of β-cells for allogenic transplantation. (5) Encapsulation and scaffold-based β-cell devices for delivery, immune suppression, and drug delivery. (6) Recapitulating the minimal β-cell machinery for insulin release with artificial β-cells. (HLA) Human leukocyte antigen.

transplantation pose significant hurdles to their use as a therapeutic strategy. To address these concerns, multiple synthetic biology and technological approaches, including the creation of synthetic gene networks for lineage control, genetic “kill switches” to bolster transplant safety, completely artificial β-cells, and β-cell encapsulation devices are currently under development.

Programmable, Synthetic Gene Circuits, and Lineage Control Networks

Synthetic gene circuits are designed to facilitate dynamic gene expression through the coupling of signaling cascades and transcription factor–based gene switches with reversible and differential sensitivity to chemical compounds (Fig. 2). Creating synthetic gene circuits involves assembling multicomponent genetic constructs specifically designed to reprogram cells for the purpose of enacting a lineage specific developmental program and, potentially, enable therapeutic function. These gene circuits create “smarter” cells and allow for the development of gene therapies with increased efficacy, precision, and control. Significant efforts are currently underway to use synthetic gene circuits to create functional, pre-programmed β-cell differentiation protocols that coordinate the timely induction and repression of multiple cell fate regulators at various stages of pancreatic development. Zhou et al. (2008) and Ariyachet et al. (2016) have generated a synthetic lineage-control network with mutually exclusive expression switches for the transcription factors NGN3, PDX1, and MAFA to drive differentiation of pancreatic progenitor cells into β-like cells. Saxena et al. (2016) designed a genetic circuit triggered by the common food additive vanillic acid and characterized by time delay and feedforward amplifier properties. In their system, this allowed for timely and controlled expression and repression of NGN3, PDX1, and MAFA. Such synthetic lineage control networks have an advantage of using the endogenous pathways rather than external signaling inputs as well as more precise feedback.

Genetic Kill Switches

Whereas hPSC-derived β-cells hold great promise for treatment of type 1 diabetes, safety concerns surrounding their introduction to an uncontrolled physiological environment remain. One such risk is the indiscriminate growth of uncommitted progenitors, leading to the formation of teratomas. These concerns necessitate the incorporation of a genetic safety mechanism deemed a “kill” or “suicide” switch that can be activated to selectively eliminate aberrant or malfunctioning transplanted cells (Bonini et al. 2007). Current mechanisms designed to achieve this targeted cellular ablation include the CD20-based complementation system, the ganciclovir-dependent herpes simplex virus thymidine kinase (HSV-TK) system (Jones et al. 2014), and drug-inducible Cas9 suicide gene cassettes (Yagyu et al. 2015). It is important to note that these approaches entail off-target effects and may fall prey to the development of drug resistance. The latter concern may potentially be mitigated by the development of a dual kill switch based on the HSV-TK model and other genes such as the cell-cycle regulator *CDK1* (Kotini et al. 2016; Liang et al. 2018). A microRNA-based system reliant upon microRNAs expressed in undifferentiated cells, such as Let7, is also under further clinical investigation (Qadir et al. 2019).

β-Cell Devices

The use of β-cell encapsulation devices has emerged as a promising delivery mechanism and a potential strategy for overcoming the requirement for immunosuppressants upon β-cell transplantation (Sneddon et al. 2018). Extensive efforts have focused on the generation of ideal approaches to cell encapsulation, including optimization of encapsulation material and topography, transplantation site, configuration to improve vascularization, and immune modulation (Fig. 2). Current strategies include cellular macro-encapsulation in devices made of polymers such as polytetrafluoroethylene or polycaprolactone, or microencapsulation in materials including alginate, polyacrylate, or colla-

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gen. These biocompatible encapsulation devices are designed to provide an ideal environment for optimal β-cell function, sequestering the immune response and providing vascularization and effective nutrient and waste exchange (Scharp and Marchetti 2014). Also under investigation are drug- and cytokine-releasing scaffolds, as well as procedures involving cotransplantation with mesenchymal stem cells (Desai and Shea 2017).

Artificial β-Cells

Artificial β-cells are biomimetic assemblies designed to recapitulate key β-cell functions (Fig. 2). They possess a vesicle superstructure—notably, an outer large vesicle mimicking the plasma membrane paired with small, inner liposomal vesicles loaded with insulin (Chen et al. 2018). These synthetic bodies are equipped with glucose-responsive metabolic systems and pH-sensitive membrane-fusion machinery to facilitate glucose sensing, signal transduction, and insulin release through vesicle fusion. The reversible nature of pH-sensitive polyethylene glycol (PEG) attachment and detachment causes random collision of the small, liposomal insulin vesicles with the outer vesicle plasma membrane, thereby leading to sustained, dynamic insulin release in response to glucose stimulation. The primary advantage of artificial β-cells is that they can either be encapsulated within an injectable gel and transplanted directly or delivered through transcutaneous micro-needle patches, thereby avoiding the requirement for immunosuppressive drugs. Further advancements will likely include improvements in the sensor structure to increase longevity and decrease sensor delay.

SUMMARY

Loss of functional β-cells is the central event leading to type 1 and type 2 diabetes mellitus. According to the World Health Organization (WHO) report, the number of people with diabetes rose from 108 million in 1980 to 422 million in 2014 while its prevalence has been rising more rapidly in low- and middle-income countries leading to premature deaths as well as several other comorbid conditions. Understanding β-cell development and function is key to our efforts in devising strategies in combating diabetes. Over the past decade, research focus on the pathogenesis of diabetes mellitus has shifted from animal models to morphological and functional studies on human islet cells. The tremendous strides made in the fields of pancreatic and β-cell biology discussed in this review—islet architecture and communication, β-cell development, and heterogeneity, and various forms of cellular differentiation—have positioned us to both understand this disease to a greater degree than ever before and develop innovative new strategies, such as in vitro differentiation of hPSCs into β-cells and increasingly advanced β-cell technologies for tackling and circumventing it. These studies have helped us in developing novel therapies to prevent β-cell death or to restore lost β-cell mass in advanced disease. While much remains to be done, through these research efforts we may begin to imagine a society in which diabetes is treated with far more efficacy than previously achieved—and, quite possibly, cured entirely.

COMPETING INTEREST STATEMENT

M.H. is on the SAB of Encellin Inc. and Thymmune Therapeutics Inc., holds stocks in Encellin Inc., Thymmune Therapeutics Inc., and Viacyte Inc., and has received research support from Eli Lilly. He is the co-founder, SAB member of Minutia Inc., and EndoCrine, and holds stocks and options in both companies.

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