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PATHOGENESIS OF TYPE 1 DIABETES (A PUGLIESE AND SJ RICHARDSON, SECTION EDITORS)

How, When, and Where Do Human β-Cells Regenerate?

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Abstract

Purpose of Review Pancreatic β-cells play a critical role in whole-body glucose homeostasis by regulating the release of insulin in response to minute by minute alterations in metabolic demand. As such, β-cells are staunchly resilient but there are circumstances where they can become functionally compromised or physically lost due to pathophysiological changes which culminate in overt hyperglycemia and diabetes.

Recent Findings In humans, β-cell mass appears to be largely defined in the postnatal period and this early replicative and generative phase is followed by a refractory state which persists throughout life. Despite this, efforts to identify physiological and pharmacological factors which might re-initiate β-cell replication (or cause the replenishment of β-cells by neogenesis or transdifferentiation) are beginning to bear fruit.

Summary Controlled manipulation of β-cell mass in humans still represents a holy grail for therapeutic intervention in diabetes, but progress is being made which may lead to ultimate success.

Keywords Proliferation · Diabetes · Transdifferentiation · Islets of Langerhans · β-Cell mass · Ki67

Introduction: Background and Rationale

The β-cell plays a unique role in human physiology being the only cell capable of elaborating a hormone (insulin) which can lower blood glucose by promoting the uptake and metabolism of sugar in peripheral tissues. As a result, physiological glucose homeostasis becomes dysregulated when β-cell function is compromised, or the cells are lost, leading to diabetes mellitus. In normal human physiology, β-cell growth and maturation occur in parallel with fetal development meaning that, during the final trimester of pregnancy, human fetal β-cells are glucose-responsive and become involved intimately in the control of fetal growth [1, 2]. Thus, fetal insulin is correspondingly increased and macrosomia results under conditions when maternal glucose is elevated. Conversely, low birth weight is often associated with reduced fetal insulin secretion [2]. Such scenarios are not evident in most rodent models, where fetal β-cells remain functionally immature until early in postnatal period.

It is clear from this that the growth and development of β-cells represent critical aspects of early life in humans and it is believed that the cells may arise and develop either by neogenesis from stem cell precursors or by replication of existing cells [3]. β-cell replication has been studied most extensively in early postnatal life and much less is known about the precise timing of fetal β-cell proliferation. Indeed, the rate of β-cell mitosis is high in the immediate postnatal period but then declines progressively, and increasingly quickly, to reach a stable but still low rate by about the 2nd year of life [4]. Consequently, the number of β-cells found in children during their early years is probably close to the full complement that is then retained for the remainder of life. Studies of lipofuscin accumulation coupled with mathematical modeling of cell turnover in

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adult β-cells have argued that the cells are very long-lived with minimal rates of division [5].

There is, though, at least one notable exception to this general rule, which applies to women during pregnancy. Over the course of pregnancy, insulin sensitivity declines and must be compensated by an increase in β-cell mass to provide sufficient insulin to meet the increased metabolic demand [6]. In rodents, there is firm evidence that increased β-cell proliferation provides the mechanism by which this requirement is fulfilled while, in humans, the evidence remains equivocal [7]. In rodents, lactogenic hormones such as prolactin promote β-cell proliferation via STAT5-dependent mechanisms, but such responses seem less effective in human islet cells despite the expression of relevant signaling components. It remains unclear whether different factors and/or molecular pathways are involved in promoting β-cell proliferation during pregnancy in humans. Nevertheless, there seems little doubt that β-cell numbers are increased in pregnant women although, in the few studies where this aspect has been addressed, rates of proliferation of existing β-cells appear relatively stable [6]. Thus, the increase may derive mainly from enhanced neogenesis rather than from increased proliferation. Indeed, this paradigm may represent the more general situation in humans where physiologically relevant increases in β-cell proliferation are rarely detected beyond the earliest years of life.

If this is true, then two factors can be considered as critical in determining the number of β-cells present in the human pancreas at different stages of life: (1) the absolute number of β-cells generated during late fetal and early postnatal development and (2) the net rate of β-cell loss which can occur over time. The total number of mature β-cells varies dramatically among individuals [8]. As a result, there is no consensus as to the "typical" β-cell mass at baseline in humans and it is probable that this encompasses a very broad continuum. Consequently, some individuals will have a much greater β-cell reserve than others and this will influence the outcomes to events that place an additional workload on these cells (e.g., in response to obesity-induced insulin resistance). It will also affect the rate at which whole-body glucose homeostasis declines when β-cells are lost, as may happen in either type 1 (T1D) or type 2 diabetes (T2D).

The reduction of functional β-cell mass which leads to hyperglycemia in both T1D and T2D is due to targeted deletion of existing β-cells or, perhaps, by transdifferentiation of mature β-cells, which causes a loss of β-cell identity (i.e., the expression of typical β-cell genes such as insulin or pancreatic and duodenal homeobox 1 (*Pdx-1*)), as well as a reduced capacity to secrete insulin in response to glucose [9–11]. In T1D, loss of β-cells is the most prevalent mechanism (at least in younger children) and occurs by an immune-mediated process in which islets are infiltrated by specific immune cell subsets that promote selective β-cell death [12]. In this scenario, it might be imagined that a compensatory drive to promote β-cell proliferation would be heightened. Some investigators have failed to find any evidence of increased β-cell proliferation in pancreas samples collected from T1D patients [13], although these investigations involved largely patients with diabetes either in a well-advanced stage or beyond the immediate post-diagnosis period. By contrast, others have used autopsy samples recovered from individuals much closer to the diagnosis of T1D and reported a tenfold increase in β-cell proliferation [14, 15]. These data importantly imply that human β-cells are not entirely refractory to pro-proliferative stimuli and suggest that targeted induction of proliferation might be an attainable goal. Increased β-cell proliferation was detected using two independent markers of mitosis (i.e., Ki67 and Mcm-2) and positively correlated with the extent of islet inflammation. Indeed, β-cell mitosis was rarely seen in the uninflamed islets of individuals with T1D [14]. Furthermore, expansion of β-cell mass driven by soluble factors secreted by T cells consequently to islet infiltration has been reported in animal models of T1D [16]. This may imply that molecules released during the autoimmune attack are able to initiate cell cycle entry in both rodent and human β-cells and it is important that these are identified and characterized as they may offer therapeutic potential.

Irrespective of this evidence, it remains clear that human β-cells are extremely resistant to factors promoting their re-entry into the cell cycle. An obvious explanation for this might be that their complement of cyclins, associated kinases, and other regulatory molecules becomes depleted as they mature such that they lack the capacity to replicate. In a series of elegant studies, Stewart and colleagues have addressed this and shown that this explanation is unlikely [17, 18]. Rather, the authors report that, in human β-cells, many of the critical proteins regulating mitosis are displaced from the nucleus to occupy a primarily cytosolic localization. Thus, it may be the subcellular disposition of these molecules rather than their absolute expression which determines the refractory status of β-cells. However, in exploring this situation further by analysis of islets retained in pancreas sections in situ, it was noted that the subcellular distribution of cyclins and related molecules may be influenced significantly by the handling of tissue [19]. In pancreas sections that had been fixed during lengthy autopsy procedures, cyclins were either completely lost or located mainly in the cytosol of β-cells, consistent with observations made in isolated islets. By contrast, where pancreas samples had been processed and fixed more rapidly after death, proteins belonging to the cell cycle machineries tended to adopt a nuclear localization. Thus, it remains unclear whether the changes in the cellular distribution of key cell cycle regulators in comparison with rodents are either a genuine characteristic of human β-cells or are a consequence of islet isolation procedures and

sample manipulations during the post mortem period. A related point that is debated is the possibility of underestimating the rate of turnover of human β-cells, when using Ki67 labeling measuring cell proliferation. Long-term storage of human pancreases obtained from autopsies or organ donors has been suggested to underlie a decline in Ki67 staining, leading to the erroneous assumption that human β-cells are resistant to division [20, 21•]. Nevertheless, it seems clear that human β-cells express the requisite machinery to induce proliferation if appropriate stimuli are encountered.

Therapeutic Effects of β-Cell Division

As noted above, a characteristic feature that is common to both T1D and T2D is the progressive loss of functional β-cell mass leading to poor glycemic control. Therefore, one of the challenging strategies has been to consider compensating for the lack of insulin, improve glucose homeostasis, and treat diabetes by expanding the population of functional β-cells. To increase the number of functional β-cells in patients with diabetes, insulin-producing cells can be generated either from non-pancreatic somatic cells (e.g., hepatocytes or intestinal cells), pancreatic exocrine cells (e.g., acinar and ductal cells), or pancreatic islet cells (e.g., α-cells), by inducing cell identity switches termed transdifferentiation. The cell types that can be used as a source for generating new β-cells, including the mechanisms that regulate such transdifferentiation processes, are listed in Table 1 and represented in Fig. 1. An alternative approach considered by many groups is the identification of molecules capable of stimulating self-replication of pre-existing human β-cells.

Generation of β-Cells from Non-pancreatic Somatic Cells

Transdifferentiation generally involves the conversion of one differentiated cell type into another mature type of cell, via an intermediate with a dual-phenotype or a dedifferentiation step (Fig. 1, Table 1). Hepatocytes and gastrointestinal cells represent a viable endogenous source for generating insulin-producing cells, since both cell types are derived from the primitive foregut endoderm and share early developmental stages. Many studies have established robust approaches to obtain β-like cells from hepatocytes by overexpression of pancreatic transcription factors that are important for β-cell lineage, such as *Pdx-1* [22, 23] and/or neuronal differentiation 1 (*NeuroD1*; [24]), neurogenin 3 (*Ngn-3*; [25]), and MAF BZIP transcription factor A/B (*MafA/B*; [26]) in murine hepatocytes by adenoviral delivery, leading to an increase in the expression of bioactive insulin and restoration of normoglycemia in multiple diabetic animal models. Importantly for clinical translation, several groups were able to engineer either fetal [27, 28] or adult [29] human liver cells overexpressing *PDX-1* with supplementation of soluble factors, resulting in the activation of insulin promoter and the resolution of the diabetic phenotype after transplantation into streptozotocin (STZ)-treated mice.

Similarly, insulin expression can be induced in gastrointestinal cells via transient transgenic expression of *Pdx-1*, *MafA*, and *Ngn-3* in vivo [30] (Fig. 1, Table 1). Moreover, downregulation of forkhead box O1 (*FoxO1*) expression in murine enteroendocrine progenitors [32] and human gut organoids [33] increased insulin production, suggesting a new mechanism in the approach to create β-like cells. Interestingly enough, Suzuki et al. demonstrated that the inactive full-length form of glucagon-like peptide 1 (GLP-1) mediated

Table 1 List of somatic cell types as a potential source for generating new β-cells

Cell type	Treatment/modification	Species	Reference
Hepatocytes	Overexpression of *Pdx1*, *NeuroD1*, *Ngn-3*, *MafA/B*	Mouse	[22–26]
Adult and fetal hepatocytes	Overexpression of *PDX-1*	Human	[27–29]
Enterocytes	Overexpression of *Pdx1*, *Ngn-3*, *MafA* - GLP1 treatment	Mouse	[30, 31]
Enteroendocrine progenitors	Downregulation of *FoxO1*	Mouse	[32]
Enterocytes	Downregulation of *FOXO1* - GLP1 treatment	Human	[31, 33]
Acinar cells	Overexpression of *Pdx1*, *Ngn-3*, *MafA* - treatment with cytokines, EGF or CNTF	Mouse	[34–37]
	Treatment with BMP-7	Human	[38]
Ductal cells	Transduction of Pdx1	Rat	[39]
	Treatment with cytokines	Mouse/human	[40]
α-cells	Overexpression of *Pax4* - downregulation of *Arx* and *Dnmt1* - treatment with alloxan, PDL, or acinar damage	Mouse	[41–46]
	Treatment with GABA, artemisinins	Mouse/human	[47, 48•]
	Overexpression of *PDX-1* and *MAFA*	Human	[49, 50]

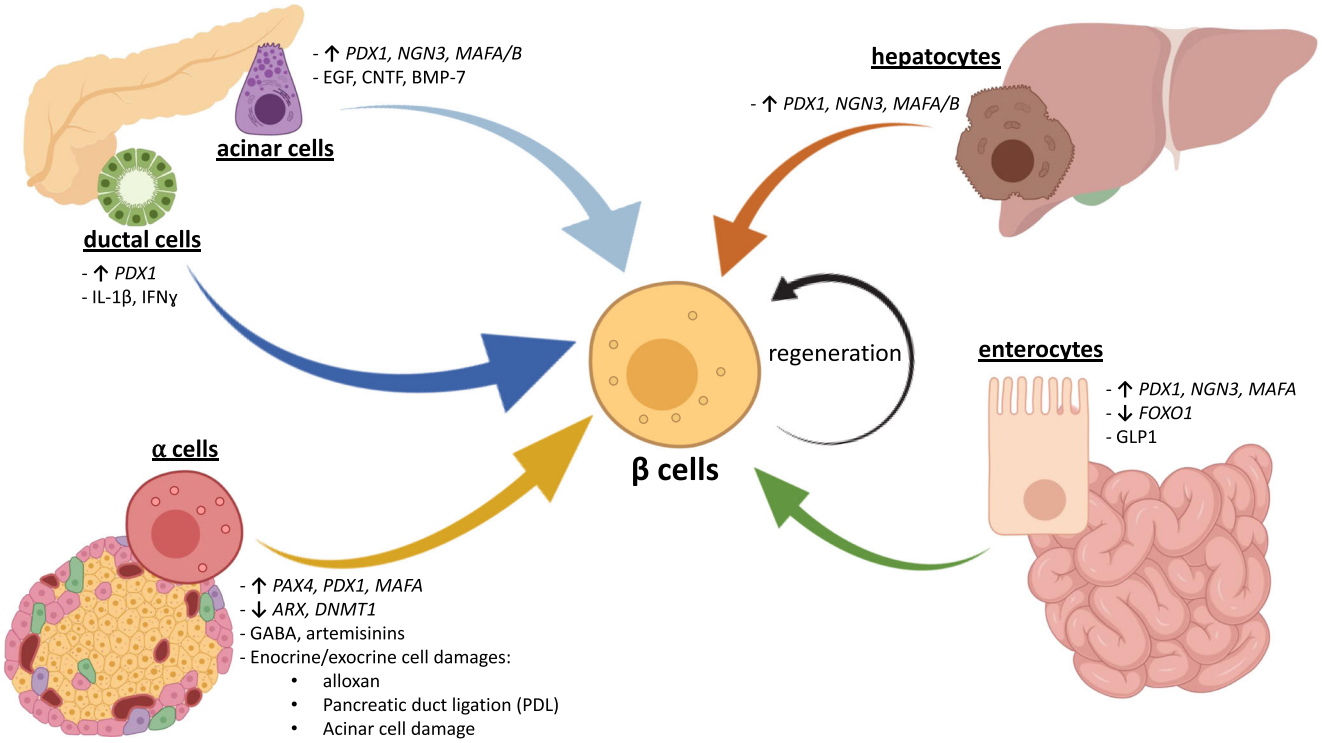

Fig. 1 Generation of human β-like cells by inducing transdifferentiation from adult somatic cells. Representative scheme summarizing the stimuli and/or genetic manipulations that regulate cell-to-cell conversion into human insulin-positive cells with a β-like phenotype. In particular, β-like cells can be generated by hepatocytes (orange arrow), enterocytes (green arrow), α-cells (yellow arrow), ductal cells (dark blue arrow), acinar cells (light blue arrow), or by self-replication of pre-existing β-cells (circular black arrow). Up arrows indicate gene overexpressions; down arrows indicate gene downregulations. *PDX-1*, pancreatic and duodenal homeobox 1; *NGN3*, neurogenin 3; *MAFA/B*, MAF BZIP transcription factor A/B; *FOXO1*, forkhead box O1; GLP1, glucagon-like peptide 1; *PAX4*, paired box 4; *ARX*, aristaless-related homeobox; *DNMT1*, DNA methyltransferase 1; GABA, gamma-aminobutyric acid; PDL, pancreatic duct ligation; IL-1β, interleukin 1β; IFN-γ, interferon-γ; EGF, epidermal growth factor; CNTF, ciliary neurotrophic factor; BMP-7, bone morphogenetic protein 7 (Created with BioRender)

the conversion of rodent and human intestinal epithelial cells into insulin-producing cells by upregulating hepatic nuclear factor 6 (*HNF-6*)–induced expression of Ngn-3 [31]. However, most studies in this field of research are based on observations in rodents and in vitro, proof-of-concept experiments, on human cells. Thus, whether the β-cell reprogramming processes from extra-pancreatic cells can be induced in human patients in vivo with the ultimate goal of increasing β-cell mass and treating diabetes continues to be a significant challenge.

Generation of β-Cells from Exocrine Pancreatic Cells

Acinar cells represent a reasonable source for generating large numbers of β-cells, considering their abundance, close proximity and shared developmental origin with endocrine cells (Fig. 1, Table 1). Acinar-to-β-cell transdifferentiation was not only obtained by overexpressing *Pdx-1*, *Ngn-3*, and *MafA* [34] but also by treatments with cytokines [35] or growth factors [36] as the epidermal growth factor (EGF) and ciliary neurotrophic factor (CNTF) [37] without genetic manipulations in rodent acinar cells in vitro and in vivo. Curiously, a β-like phenotype was induced in acinar cells after treatment with bone morphogenetic protein 7 (BMP-7) in humans [38]. Despite these events having been well described in animal models, translation of the findings to human acinar cells in vitro represents a major task, since in vitro cultured acinar cells display a high tendency to transdifferentiate spontaneously into ductal cells [51, 52]. However, many reports showed similar versatile properties of acinar cells in human pancreases. Single-cell RNA-sequencing and immunohistochemistry experiments had revealed that subpopulations of acinar cells expressed high levels of the transcription factor SRY-Box 9 (*SOX9*), a marker of pancreatic progenitor cells, suggesting the presence of acinar cells in a dedifferentiated stage [53]. The plasticity of human acinar cells in vivo was recently shown by Masini and coworkers, by identifying cells simultaneously expressing insulin and acinar markers within the human pancreases, where they demonstrated a higher prevalence in T2D patients [54]. However, it is worth noting that acinar cell dedifferentiation or genetic reprogramming has the potential to cause adverse effects, including an increased risk of developing tumors such as pancreatic ductal adenocarcinoma [55]. In conclusion, further investigations about the safeness and stability of acinar-to-β reprogramming are necessary to consider

efficiently and safely translating these approaches as therapeutic modalities for patients with diabetes.

During the early stages of pancreas development, ductal cells initiate the transdifferentiation process towards the endocrine lineage in mice, acting as an islet cell progenitor. This process occurs spontaneously in young mice during embryogenesis but not after birth [56]. However, identity transitions from ductal to β-cells were induced in mature cells by activating insulin gene promoter following transduction of PDX-1 protein into rat ductal cells [39]. Furthermore, Valdez et al. reported that pro-inflammatory cytokines increased Ngn-3 expression in murine and human ductal cells and enabled epithelial-mesenchymal transition (EMT), an essential step for initiating differentiation towards endocrine cells, independently of hyperglycemia [40]. Neogenesis of insulin-producing cells from ductal cells has also been reported to occur in humans. Ductal cells obtained from donors with <10 years of age exhibited insulin-positive cells when transplanted under the kidney capsule of nude mice [57]. In addition, Meier and colleagues reported increased levels of insulin-positive ductal cells in obese patients compared with those in non-obese subjects [58]. These findings suggest that duct-to-β transdifferentiation is one of the potential mechanisms by which new β-cells are generated to compensate for systemic insulin resistance due to obesity [59, 60]. Interestingly, there is evidence from in vitro studies that the establishment of persistent enteroviral infection in pancreatic ductal cells may reduce their capacity to differentiate into β-cells [61]. Since enteroviral infection of pancreatic cells has been implicated as a potential underlying cause of T1D [62], this might compromise the ability of β-cells to be replenished in T1D. In addition, insulin-positive ductal cells were observed in transplanted pancreas of T1D patients who received a simultaneous pancreas-kidney transplant (SPK) [63]. Remarkably, Dirice et al. were able to detect ductal cells positive for immature β-cell markers in pregnant women and individuals with T2D, highlighting neogenesis as a compensatory mechanism to expand β-cell mass in physiological and pathophysiological conditions characterized by high insulin demands such as pregnancy or T2D in humans [64].

Generation of β-Cells from Endocrine Pancreatic Cells

Other efforts in parallel have focused on targeting α-cells as a pool for expanding β-cell mass (Fig. 1, Table 1). The rationale for this approach is based on several studies which suggest that β-cells originate from α-cells during islet embryogenesis in mice [65–68]. Furthermore, the close lineage relationships and the large overlap of transcriptomes between α and β-cells, in addition to the apparent high resistance of α-cells to metabolic stressors, represent some of the advantages for considering α-to-β transdifferentiation as an approach with therapeutic potential. However, other reports caution that differentiation of α-to-β-cells with the consequent depletion of α-cells and the resultant lower circulating glucagon levels could increase the risks of hypoglycemic events [69]. Notwithstanding these concerns, studies in rodents showed that loss of α-cells did not affect overall health or lifespan and glucagon signaling was still preserved by a small number of α-cells [41, 70].

To further explore the use of α-cells as a practical tool to expand β-cells and treat diabetes, Collombat and colleagues reported that ectopic overexpression of Paired Box 4 (*Pax4*) mediates the differentiation of murine progenitor endocrine cells into β-cells after generating an intermediate α-like cell stage [41]. Independent studies have confirmed that upregulation of *Pax4*, induced by inhibition of aristaless-related homeobox (*Arx*), converted α-cells into insulin-producing cells [42]. In addition, downregulation of *Arx* in combination with the silencing of DNA methyltransferase 1 (*Dnmt1*) gene expression, specifically in α-cells, promoted rapid changes in the whole transcriptome towards a phenotype typical of native β-cells [43]. Some parallels in this context have been reported in T1D in humans. For example, α-cells have been shown to express reduced levels of *DNMT1* and *ARX* genes in T1D patients [43]. Moreover, transdifferentiation α-to-β is induced in mice after almost complete β-cell depletion using β-cell toxins [44] in combination with pancreatic duct ligation (PDL) [45] or acinar tissue damages [46].

Recently, gamma-aminobutyric acid (GABA) signaling has been a matter of much attention, after the discovery of its role in mediating α-to-β transdifferentiation. In particular, in vivo treatment with GABA caused downregulation of *Arx* specifically in α-cells, generating cells expressing *Ngn-3* and insulin with a high proliferation rate, able to restore euglycemia in diabetic mice [47]. Independently, Li et al. reported similar results by treating human islets with artemisinins, a group of antimalarial drugs, able to modulate gephyrin, a binding protein of the GABA receptor [48•]. However, Ackermann and colleagues failed to reproduce these findings, using a tamoxifen-inducible Glucagon-Cre YFP mouse model to track α-cell fate upon GABA or artenusate treatments [71•]. Whether the protocols used in the two studies play a role in the differences in the outcome is unclear [72], and further investigations are necessary to better define the role of GABA in inducing α-to-β transition. Finally, gene therapy approaches have been touted as a more efficient way to convert α-cells into β-like cells. In particular, adenovirus delivery of transgenic constructs carrying *PDX-1* and *MAFA* genes into α-cells via the pancreatic duct has been shown to expand the β-cell population and to counteract diabetes in STZ-treated and NOD mice [49]. α-to-β identity switches were shown to be induced also in human islets upon *PDX-1-MAFA* combined overexpression. Additionally, these genetically derived β-like cells were able to ameliorate glucose intolerance in diabetic immunodeficient mice and

enabled maintenance of euglycemia for 6 months after transplantation, highlighting the potential therapeutic properties of such applications [50].

A Role for Endogenous/Exogenous Factors That Can Stimulate Human β-Cell Proliferation

As we have explained, it is generally accepted that adult human β-cells have a very low capacity to divide. Nevertheless, it is also evident that β-cells have an intrinsic flexibility to adapt to environmental changes which require expansion of insulin-producing cells in mammals. This flexibility has been mostly studied in rodent β-cells. For example, several studies have shown that β-cell mass is maintained by duplication of pre-existing β-cells, rather than differentiation from pancreatic progenitors or other cell types [73–75]. In this context, there has been considerable effort to characterize the molecular signaling pathways that can regulate or trigger β-cell replication (accurately reviewed in [76–79]). A challenge in translating the findings in rodents to human β-cells is largely due to a dissimilarity of the pathways that regulate cell replication between the two species [80–82]. An attractive unbiased approach that has garnered attention is the use of high-throughput screenings to identify small molecules or to identify endogenous factors capable of specifically activating the proliferative machinery in human β-cells (Fig. 2).

The versatility and ability to optimize high-throughput screening (HTS) assays have provided an important platform in this field. Among the initial HTS studies, focused on the identification of compounds to activate β-cell proliferation, was the discovery of diarylurea WS1, which could induce proliferation of R7T1 cells, a quiescent rat β-cell line [83]. The analogue WS6, a diarylamide, was also able to stimulate proliferation in human β-cells [83], as well as α-cells [84], mainly by repressing the function of the inhibitor of nuclear factor kappa-B kinase subunit epsilon (IKK-ε), a negative regulator of the nuclear factor kappa-B (NFκB), and by regulating the cell replication suppressor ErbB3-binding protein 1 (EBP1), thereby stimulating E2F1-mediated cell proliferation (Fig. 2) [83]. Wang et al. found harmine, a plant-derived alkaloid drug, as an inducer of the *MYC* promoter and cell proliferation in HepG2 cells using a HTS. The authors confirmed that mitogenic pathways were also induced in cultured and transplanted human β-cells [85••]. The major target of harmine is the dual-specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A), which regulates phosphorylation and activity of the nuclear factor of activated T cell (NFAT). NFAT is usually localized in the cytosol in a phosphorylated state, but when DYRK1A function is inhibited by harmine, the phosphorylation levels are reduced and it translocates into the nucleus to promote its mitogenic function [85••]. Recently, another DYRK1A inhibitor, 5-iodotubercidin (5-IT), was also identified from an HTS using dissociated human islet cells [86••, 87]. The discovery that 5-IT is able to induce β-cell regeneration also in transplanted human islets provides another example of targeting the DYRK1A-NFAT axis to expand β-cell mass with implications for therapeutics in patients with diabetes (Fig. 2).

Other pathways explored to regulate β-cell division include inhibitors of TGF-β. The signals transduced intracellularly by TGF-β receptors results in the activation of SMAD proteins that translocate into the nucleus to modulate gene expression, together with the stimulation of non-canonical effectors such as ERK, p38, JNK, and AKT [88, 89]. Recently, Dhawan et al. observed that the compound SB431542, a TGF-β signaling inhibitor, promotes β-cell proliferation in human islets in vitro and in vivo by repressing the expression of $p16^{INK4a}$, an inhibitor of cell cycle machinery, induced by the activation of SMAD3 (Fig. 2) [90]. Interestingly, proliferation levels of human β-cell can be further increased by up to 5–8% of the total β-cell population by the combined pharmacological blockade of DYRK1A activity and TGF-β pathway. The simultaneous treatment of human islets with harmine and a TGF-β signaling inhibitor resulted in the activation of cyclins and cyclin-dependent kinases, on the one hand, and inhibition of key cell cycle regulators, on the other, in a synergistic fashion [91••].

Insulin/IGF-1 receptors and proteins in their signaling pathways are known to induce proliferation in virtually all mammalian cells by activating the PI3K-AKT signaling pathway and subsequent activation of MAPK signaling, and through inhibition of GSK3β activity (Fig. 2). GSK3β has been characterized as an inhibitor of replication in both rodent β-cells [92–94] and human islets [95]. Thus, the identification of GSK3β inhibitors as stimulators of human β-cell proliferation has been exploited for potential clinical therapeutics. In particular, two small molecules synthetized from a pyrazine scaffold, GNF7156 and GNF4877, were identified as GSK3β inhibitors and potent regulators of β-cell proliferation in cultured and engrafted human islets [96]. Although the application of chemical compounds to target stimulators/repressors of cell division selectively in human β-cells has been demonstrated to be efficient and accurate, it continues to raise concerns about the safety and the possibility of off-target, undesired effects when administered systemically in patients with diabetes. One approach to circumvent the side effects of exogenous compounds is the identification of endogenous factors that can expand β-cell mass in physiological conditions.

In this context, pregnancy has been widely studied as a physiological state characterized by a compensatory increase in β-cells in response to the high insulin demand. As mentioned earlier, the dramatic increase in rodent β-cell proliferation and mass during pregnancy was linked to the activation of FoxM1, menin, and serotonin pathways mediated by

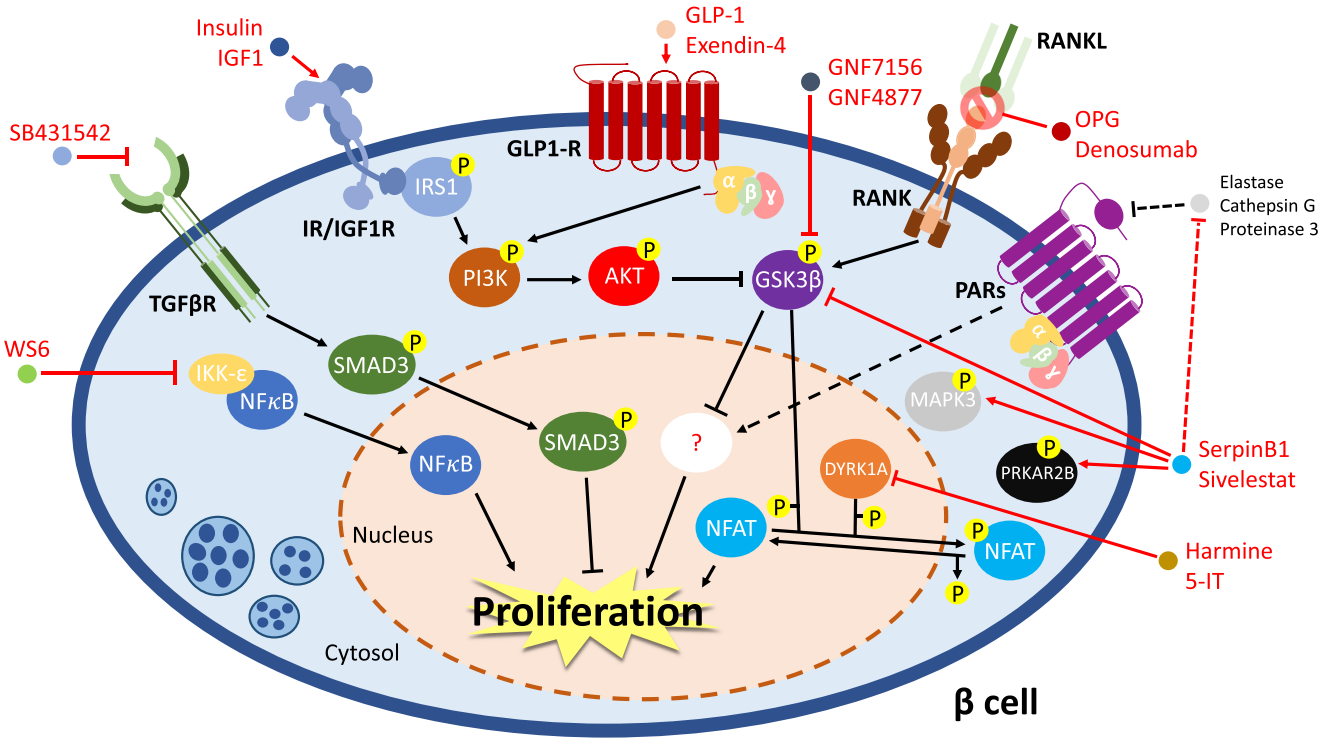

Fig. 2 Molecular mechanism(s) regulating human β-cell proliferation. The nuclear factor kappa-B (NFκB) is retained in the cytosol and its function is repressed by inhibitor of nuclear factor kappa-B kinase subunit epsilon (IKK-ε). WS6 blocks IKK-ε inhibition on NFκB, which can translocate into the nucleus and promote cell growth. TGF-β pathway impacts β-cell proliferation via the activation of SMAD3. SB431542, a TGF-βR inhibitor, promotes cell growth by preventing SMAD3 activation. Insulin receptor (IR)/insulin-like growth factor (IGF1R) and glucagon-like peptide 1 receptor (GLP-1R) signaling pathways trigger β-cell regeneration via modulating PI3K-AKT axis, resulting in the inhibition of glycogen synthase kinase-3β (GSK3β) activity. In addition, inactivation of GSK3β is also obtained by treatments with GNF7156 and GNF4877, GSK3β inhibitors, or osteoprotegerin (OPG) or denosumab. In particular, OPG and denosumab act as mimics of receptor activator of nuclear factor kappa-B ligand (RANKL), preventing its interaction with receptor activator of nuclear factor kappa-B (RANK) and avoiding the activation of the extrinsic apoptotic pathways. Moreover, the hepatokine SerpinB1 and the elastase inhibitor sivelestat stimulate human β-cell proliferation increasing the phosphorylation levels of mitogen-activated protein kinase 3 (MAPK3), protein kinase cAMP-dependent type II regulatory subunit beta (PRKAR2B) and GSK3β, likely following inhibition of proteases as elastase, cathepsin G, or proteinase 3. These effects might involve the protease-activated receptor (PARs) signaling, but such a hypothesis requires further investigations (dotted lines and arrows). The dual-specificity tyrosine-regulated kinase-1a (DYRK1A) represses β-cell proliferation by phosphorylating and retaining into the cytosol the nuclear factor of activated T cells (NFAT). The inhibition of DYRK1A, using small molecules as harmine or 5-iodotubercidin (5-IT), results in the decrease of the phosphorylation state of NFAT, which translocate into the nucleus and activate the mitogenic pathways in human β-cells

prolactin (PRL) and placental lactogen (PL) [97–101]. While such a mechanism has not been confirmed in humans, osteoprotegerin (OPG), one of the upregulated proteins in mice during pregnancy [102], is expressed in human β-cells in response to cytokine treatment [103]. OPG is a cytokine receptor belonging to the superfamily of tumor necrosis factor (TNF) receptors and was initially discovered as a soluble decoy receptor for the receptor activator of nuclear factor kappa-B ligand (RANKL) and the TNF-related apoptosis-inducing ligand (TRAIL) [104]. When bound by OPG, RANKL and TRAIL are prevented from interacting with their cognate receptors, receptor activator of nuclear factor kappa-B (RANK), and death receptor (DR), respectively, and are unable to induce cell death programs. Of relevance to human β-cells, OPG blocked RANKL/TRAIL-mediated apoptotic pathways by preventing phosphorylation of p38 MAPK to protect β-cells from cytokine-induced death [103]. OPG or denosumab, an FDA-approved monoclonal antibody which mimics the functions of OPG, promoted human β-cell proliferation by inhibiting RANKL-RANK association and stimulating phosphorylation and inactivation of GSK3β (Fig. 2) [105].

As mentioned in the previous paragraphs, the differences in the molecular pathways that control β-cell proliferation between mice and humans are one of the challenges in translating animal research into the clinic. A case in point is the incretin hormone GLP-1. GLP-1, or its analogue exendin-4, is well-known potentiator of glucose-stimulated insulin secretion in both rodent and human islets [106]. The incretins have also been reported to induce replication of rodent β-cells by regulating multiple signaling pathways [107]. For example, GLP-1 or exendin-4 activates the GLP-1 receptor (GLP-1R), a G

protein–coupled receptor, to stimulate the PI3K-AKT cascade, the cAMP-PKA-CREB axis, and PKCζ in a non-canonical fashion (Fig. 2) [108]. Furthermore, exendin-4 activates proliferative processes via the mTORC1 signaling [109]. GLP-1 has also been reported to upregulate the cell cycle proteins including cyclin D1 and cyclin A2, in combination with the degradation of the cell cycle inhibitor p27 [110, 111]. While these data generated much excitement in the field, none of these findings have been replicated in human islet/β-cells to date, revealing either the poor similarity of GLP-1 signaling effectors among different mammalian species or the lack of a precise protocol. One notable exception is the ability of exendin-4 treatment to stimulate β-cell proliferation in transplanted human islets isolated from young donors (~ 18 years old) [112]. Whether GLP-1 and its agonists can be therapeutically adapted for treating younger patients with diabetes awaits further investigation.

One observation appreciated over several decades is the ability of β-cells to compensate for states of insulin resistance both in the presence and in the absence of diabetes. Thus, obese individuals display a remarkable ability to increase their β-cell mass as a form of compensation to delay the onset of diabetes [113]. The signals that contribute to the expansion of β-cell mass could be multiple and may arise from multiple metabolic organs that are insulin resistant. Studies aimed at understanding inter-organ cross talk have begun to yield some important insights in this area [114]. One such example is the identification of SerpinB1, a hepatokine produced by the insulin-resistant liver involving FoxO1 regulation [115, 116]. The levels of circulating SerpinB1 have been shown to be altered in many models of diabetes both in rodents and humans [115–118]. SerpinB1 belongs to the serine protease inhibitor family and targets elastase, proteinase 3, and cathepsin G, among other proteases (Fig. 2) [119]. The protease inhibitor has been reported to induce a twofold increase in the proliferation in zebrafish, mouse, and human β-cells [115]. Using a phosphoproteomic analysis, El Ouaamari and colleagues observed that SerpinB1 treatment of mouse islets led to an increase in phosphorylation of the protein kinase cAMP-dependent type II regulatory subunit β (PRKAR2B), GSK3, and MAPK3 (Fig. 2). To begin to explore the mechanism of action of SerpinB1, El Ouaamari et al. focused on elastase inhibition and reported that mouse and human islets treated with established chemical elastase inhibitors, such as sivelestat or GW311616A, showed similar results (Fig. 2) [115]. Thus, one pathway that may be modulated by SerpinB1 is elastase inhibition in the islet extracellular environment that leads to the activation of the proliferative pathways within β-cells. Whether SerpinB1 modulates alternative pathways including the inhibition of other proteases and/or the regulation of protease-activated receptors (PARs) requires further research (Fig. 2).

β-Cell Mass and Functional Responses

An important insight which is now gaining widespread acceptance is that, even in patients with long-standing T1D, there is a functional reserve of β-cells. This is a surprising finding which has challenged the long-held dogma that T1D only develops when 80–90% of β-cells are lost. This is probably the case in children who develop T1D within the first 7 years of life but, in those who are older at onset (teens and beyond), β-cell loss is much less extensive and residual β-cells may persist for many years [120]. Indeed, apparently, normal β-cells can still be found in the islets of some of the cohort of Joslin medalists who had lived with T1D for more than 50 years prior to their death [121]. Moreover, analysis of C-peptide levels in patients living with T1D for increasing periods has provoked the surprising conclusion that the rate of β-cell loss is initially exponential but then declines dramatically at about 7 years post-diagnosis such that β-cell mass remains stable thereafter [122•]. This could imply that autoimmunity is halted at this point (which seems inherently unlikely) or that a population of β-cells is retained which, for reasons that remain unexplored, are not visible to the immune system and can persist for long periods.

A key question then arises as to whether these persistent cells are functionally competent or if they are compromised. This issue has not been resolved although it has been reported that many patients are insulin "microsecretors" and that their circulating C-peptide levels increase after a meal [123]. This implies that at least a proportion of the residual β-cells must retain functional competence although it is uncertain whether this is true for the majority. In this context, studies of islets isolated from newly diagnosed patients imply that they may be refractory to stimulation upon initial isolation but that glucose-induced insulin release can be restored during a period of ex vivo culture [124]. Irrespective of the precise number of functional β-cells at any specific point in the disease process, these results suggest two important conclusions. Firstly, that absolute β-cell mass may not equate directly to functional β-cell mass in people with T1D and, secondly, that β-cell functionality can be retained (or restored) in the face of a long-term autoimmune state. This raises the hope that a targeted expansion of endogenous β-cell mass, stimulated by using combinations of the methods and factors outlined above or by new approaches still to be discovered, could become a realistic possibility in the future, even among patients who have lived with T1D for many years. This is an exciting prospect but the challenges to fulfill this potential should not be overlooked. Importantly, for example, the use of targeted agents such as the DYRK1A inhibitor, harmine, is not selective in their actions and causes the expansion of both β and other islet endocrine cells [87]. Similarly, in patients with recent onset T1D where β-cell proliferation is increased in inflamed islets, an expansion of α-cell mass is also detected

[14]. Thus, methods must be established which can both target the β-cells selectively and are sufficiently well regulated that they do not lead to uncontrolled proliferation.

Conclusions

Taken together, the work summarized in this review gives cause for optimism that approaches which enhance endogenous β-cell mass are within reach. The physiological resilience of the cells and their natural longevity may be factors which act in their favor as technologies become available which allow their selective expansion in periods of life when they would normally exist in a post-mitotic state.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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