Targeting the pancreatic β-cell to treat diabetes

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Abstract | Diabetes is a leading cause of morbidity and mortality worldwide, and predicted to affect over 500 million people by 2030. However, this growing burden of disease has not been met with a comparable expansion in therapeutic options. The appreciation of the pancreatic β-cell as a central player in the pathogenesis of both type 1 and type 2 diabetes has renewed focus on ways to improve glucose homeostasis by preserving, expanding and improving the function of this key cell type. Here, we provide an overview of the latest developments in this field, with an emphasis on the most promising strategies identified to date for treating diabetes by targeting the β-cell.

Genome-wide association studies (GWASs). Studies that examine the common genetic variants across case and control populations, and determine statistical correlation with a disease or phenotypic traits. GWASs can be conducted on complex genetic diseases, such as type 2 diabetes, as well as on traits such as fasting glucose levels or insulin secretion.

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Nearly 350 million people worldwide currently suffer from diabetes. Of these, about 5% of patients have type 1 diabetes, which results in the absolute loss of insulin production, whereas ~95% of patients have type 2 diabetes and suffer from impaired insulin sensitivity as well as problems with insulin secretion. β-cells residing in the islets of Langerhans in the pancreas are responsible for the production of insulin. In fact, islets are composed of several endocrine cell types (including α-, β-, δ-, ε- and pancreatic polypeptide (PP) cells), which each secrete various hormones that are responsible for maintaining glucose homeostasis in both fasting and fed states[1,2]. Thus, the preservation of a balance among these cell types is important to the metabolic status of the organism.

Loss of β-cell number and function underlies much of the pathology of diabetes[3,4]. In type 1 diabetes, the immune system recognizes the β-cell as foreign, probably owing to a combination of genetic and environmental factors[2]. The ensuing autoimmune attack results in destruction of the β-cell population, and patients are therefore dependent on exogenously administered insulin for survival. The insulin resistance that precedes overt type 2 diabetes involves an inability of cells in, for example, liver, muscle and adipose tissue to respond to the normal actions of insulin[5]. To compensate for this resistance, pancreatic β-cells subsequently increase their production of insulin; however, β-cell function continues to decline and insulin production eventually becomes inadequate. Genetic factors are thought to contribute to the ultimate failure of β-cells to further increase insulin secretion[6]. Indeed, genome-wide association studies (GWASs) have pointed to correlations between genetic variants associated with type 2 diabetes and defects in insulin secretion[7–10].

Therefore, in both type 1 and type 2 diabetes, ways to preserve or expand pancreatic β-cell mass and function could be an effective therapeutic approach. However, this strategy is challenging because — unlike the liver — the β-cell is not thought to regenerate in adult humans, and is considered a more quiescent cell type[11]. Indeed, most of the current therapies for diabetes focus on managing the symptoms of the disease rather than replacing or preserving β-cell mass (BOX 1). However, by using small molecules to target key cellular processes, we may be able to achieve the goal of increasing β-cell mass.

Stimulation of human β-cell proliferation may have an impact on the repopulation of the pancreas with insulin-producing cells, but a major challenge is to overcome the dormant state of these cells. An alternative approach is to protect β-cells from apoptosis. Like most secretory cells, β-cells are highly sensitive to ER stress[12], and reducing the metabolically hostile effects of persistent hyperglycaemia could help to preserve β-cell mass. Similarly, rendering the β-cell resistant to autoimmune attack in type 1 diabetes may strengthen early-stage therapies and synergize with treatments aimed at inducing immune tolerance[13]. Another, currently speculative, approach is to reprogramme, or transdifferentiate, other cell types in the digestive system to become insulin-producing cells. Genetic successes in rodents suggest that this approach could be feasible[14,15], but the relevance of these findings to humans remains unclear. Finally, preserving β-cell function to safely increase insulin secretion would clearly be valuable in treating diabetes. Although clinically used sulphonylureas augment insulin release (BOX 1), they act in a glucose-independent manner[16] and so identifying ways to improve insulin secretion in a

Box 1 | **Current therapies for diabetes**

Used in both type 1 and type 2 diabetes

- Insulin analogues: recombinant modifications of insulin with altered timing of onset and duration of action, sharing the same mode of action as endogenous insulin; require subcutaneous injection.
- Pramlintide: an analogue of the peptide hormone amylin, which slows gastric emptying, promotes satiety and inhibits glucagon secretion, preventing post-prandial spikes in blood glucose levels; requires subcutaneous injection before each meal.

Used in type 2 diabetes

- Metformin: a biguanide that reduces hepatic glucose production and increases glucose uptake in the periphery, via incompletely understood mechanisms that are likely to involve the activation of AMP-activated protein kinase (AMPK); administered orally.
- Sulphonylureas: augment insulin release largely independently of ambient glucose levels by binding to the sulphonylurea receptor 1 (SUR1) regulatory subunit of the ATP-sensitive potassium channel in pancreatic β-cells, leading to channel closure and cellular depolarization; administered orally.
- Meglitinides: similarly to sulphonylureas, the 'glinides' bind to the SUR1 regulatory subunit of the ATP-sensitive potassium channel on the β-cell, leading to insulin secretion that is largely independent of ambient glucose levels; their half-life is shorter than sulphonylureas, leading to a lower risk of hypoglycaemia; administered orally.
- Thiazolidinediones (TZDs): bind to peroxisome proliferator-activated receptor-γ (PPARγ), a nuclear receptor that regulates key genes involved in glucose and fat metabolism, resulting in increased insulin sensitivity, along with increased adipogenesis; administered orally.
- Glucagon-like peptide 1 (GLP1) analogues: mimetics of the incretin hormone GLP1 that slow gastric emptying, promote satiety, decrease glucagon secretion and increase insulin secretion in a glucose-dependent manner; require subcutaneous injection.
- Dipeptidyl peptidase 4 (DPP4) inhibitors: block the enzyme responsible for inactivating GLP1, leading to increased levels of the incretin hormone in the circulation; administered orally.
- Alpha-glucosidase inhibitors: block the activity of enzymes in the brush border of the small intestine that break down oligosaccharides, leading to decreased absorption of carbohydrates; administered orally.
- Sodium-dependent glucose cotransporter 2 (SGLT2) inhibitors: decrease the activity of the primary transporter involved in glucose reabsorption in the kidney, leading to increased glucose excretion in urine; administered orally.
- Bromocriptine: a potent agonist of dopamine D_2 and serotonin receptors, with an unclear mechanism of action in improving glycaemic control; administered orally.

glucose-dependent manner is crucial for achieving more precise control of blood glucose levels. Here, we present our current understanding of the molecular mechanisms that control β-cell proliferation, apoptosis, reprogramming and insulin secretion, and discuss recent developments in the identification of potential novel molecular targets and therapeutic approaches to increase β-cell mass and function.

β-cell proliferation

Cell cycle regulation of β-cell mass. Although stimulation of cell division would seem to be among the simpler approaches for increasing β-cell mass, β-cell turnover in healthy adults is very slow, declining rapidly following the first few years after birth[11]. An analysis of islets from organ donors who were between 7 and 66 years of age, however, found β-cells that were positive for proliferation in every sample tested[17]. Additionally, a study of Joslin Diabetes Center Medalists (patients with type 1 diabetes for more than 50 years) showed that all post-mortem pancreata contained some insulin-positive cells[18]. These observations support the hypothesis that human β-cells have a physiological — albeit limited — capacity to proliferate, and give hope to the notion that small-molecule modulation of β-cell-specific proliferation may be possible. However, even if such proliferation were achieved, it would be important to ensure maintenance of β-cell function, as some trade-off between insulin secretion and cell division has been suggested. Furthermore, an uncontrolled increase in cellular proliferation must be avoided at all costs, so as not to exchange one problem (loss of β-cell mass) for another (tumour formation).

The replication of β-cells is controlled by a network of finely tuned cell-cycle regulators[19–21], with expression mirroring the replicative potential of β-cells at each stage of life (FIG. 1). For example, the cell cycle inhibitor p16 (also known as INK4A) is highly expressed in adult islets and is associated with the reduction in β-cell replication observed during ageing[22,23], whereas p27 (a kinase-interacting protein (KIP); also known as a CDK-interacting protein (CIP)) accumulates in terminally differentiated β-cells during embryogenesis and inhibits cell cycle progression[24]. Cyclin D1 and cyclin D2 have a central role in the positive regulation of β-cell proliferation in the human and rodent pancreas[25]; cyclin D3 also has a central role in the human pancreas[26]. A recent comprehensive analysis of G1/S cell cycle components in human β-cells revealed that although all of the crucial components of the cell cycle are present, they generally reside in the cytoplasm[27,28], which is likely to contribute to the refractory nature of human β-cells to proliferate.

Pancreatic β-cell mass
The β-cell population present in the pancreas that, under physiological conditions, results from the balance between growth (from replication, neogenesis and cell size) and cell death (from apoptosis, necrosis and autophagy).

ER stress
A physiological event activated in response to an accumulation of unfolded or misfolded proteins in the lumen of the endoplasmic reticulum (ER). ER stress has two primary aims: to restore normal function of the cell by halting protein translation, and to activate the signalling pathways that lead to an increase in the production of the molecular chaperones involved in protein folding.

Physiological regulation of β-cell mass. Although β-cell mass in adulthood normally remains relatively constant, there are physiological conditions during which an increased metabolic load is compensated for by an increase in β-cell mass. For example, during pregnancy, β-cell mass in both humans and rodents increases in a reversible manner, and in rodents this clearly involves an increase in β-cell replication[29] that coincides with increases in lactogen hormone levels[30]. β-cell replication in pregnant rats appears to correlate well with changes in gene expression within islets, with differentially expressed genes belonging to three main groups: transcriptional regulators, apoptosis pathway members and WNT signalling components, particularly activating transcription factor 3 (*Atf3*) and nuclear protein 1 (*Nupr1*)[31]. Interestingly, the same genes were upregulated *in vitro* after the treatment of non-pregnant islets with prolactin[31], a key lactogenic hormone. ATF3 is known to promote cell proliferation[32], whereas NUPR1 is a downstream target of ATF3 that is expressed in human islets[31], strengthening the connection between lactogens and cell-cycle control.

The neurotransmitter 5-hydroxytryptamine (5-HT; also known as serotonin) also seems to have a prominent role in pregnancy-induced β-cell proliferation, at least in mice[33]. Analysis of the transcriptional differences in islets from pregnant and control mice revealed that the most upregulated genes during pregnancy included two isoforms of tryptophan 5-hydroxylase (TPH), the rate-limiting enzyme in serotonin synthesis[33]: *Tph1* (a 527-fold increase in gene expression) and *Tph2* (a 7-fold increase in gene expression). 5-HT colocalizes with insulin and TPH1 in the β-cells of pregnant mice and is co-secreted with insulin upon glucose stimulation[34]. Interestingly, prolactin induced a 147-fold increase in *Tph1* gene expression in isolated β-cells[33]. Although a recent analysis of pancreatic sections from pregnant human donors did not find that proliferation contributed significantly to the increase in β-cell mass[35], additional studies are needed in this area as the molecular events that occur during pregnancy may point to new therapeutic targets for safely increasing β-cell proliferation.

Several recent studies have focused on circulating factors, particularly from the liver, that are capable of inducing β-cell proliferation. The liver-specific insulin receptor knockout mouse model undergoes significant β-cell hyperplasia[36], and the use of parabiosis (to join the circulatory systems of two mice) demonstrated that a factor secreted from the liver was responsible for this effect[37]. Similarly, parabiosis between young and old mice revealed a circulating factor in young mice that was able to induce β-cell proliferation in old mice[38]. Interestingly, this factor seemed to act in a p16-independent manner, which is an unexpected result given the major role of this cell-cycle inhibitor in ageing. Finally, the discovery of betatrophin[39], a protein that is secreted from the liver in response to chemically induced insulin resistance, demonstrated that mouse β-cell proliferation can increase by 10- to 20-fold over basal levels in response to this factor. These exciting results set the stage for the identification of new factors and mechanism-of-action studies, and may lead to novel targets for therapeutic development.

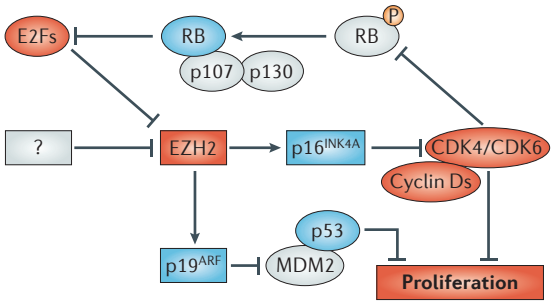

Figure 1 | **Regulation of β-cell proliferation during ageing.** β-cell proliferation declines rapidly in juvenile mice and humans, followed by a further reduction in adulthood. Inhibited components are indicated in red, whereas activated or upregulated proteins are indicated in blue. Levels of the Polycomb protein enhancer of zeste homologue 2 (EZH2) in the β-cell decline during ageing. This effect coincides with a reduction in histone H3 trimethylation at the *INK4A–ARF* locus, which leads to an increase in levels of the cell-cycle inhibitors $p16^{INK4A}$ and $p19^{ARF}$. $p16^{INK4A}$ binds to cyclin-dependent kinase 4 (CDK4) and CDK6, which inhibits their kinase activity on retinoblastoma protein (RB). $p16^{INK4A}$ also prevents the interaction of CDK4 and CDK6 with D-type cyclins, which are required for catalytic activity. The overall effect is hypophosphorylation of RB, which in turn leads to E2F repression and subsequent blockade of EZH2 transcription. Conversely, $p19^{ARF}$ inhibits the ubiquitin ligase activity of MDM2, which stabilizes tumour suppressor p53. Each of these arms of the pathway downstream of EZH2 leads to a decrease in β-cell proliferation during ageing. The factors controlling the levels of EZH2 remain elusive.

The effects of ageing on β-cell proliferation. β-cell replication decreases with age[11], partly owing to changes in the expression of cell cycle regulators, as described above. In addition, other proteins have important roles in regulating this process and may represent potential targets for reversing the age-associated decline in β-cell proliferation. The loss of replication capacity correlates closely with a reduction in the expression of the gene encoding enhancer of zeste homologue 2 (*EZH2*)[40], which is a histone methyltransferase that is responsible for transcriptional repression via trimethylation of histone H3 on lysine 27 (H3K27me3). EZH2 methylates the *INK4A–ARF* locus, repressing the expression of the cyclin-dependent kinase (CDK) inhibitors p16 and p19 from this region[40] (FIG. 1).

In rodents, methylation of the *Ink4–Arf* locus can also be triggered by β-cell-specific downregulation of the gene phosphatase and tensin homolog (*Pten*)[41], which is a negative regulator of the phosphoinositide 3-kinase (PI3K)–AKT signalling pathway. In β-cells, PI3K–AKT signalling is triggered by mitogenic signals such as insulin-like growth factor 1 (IGF1), insulin and hepatocyte growth factor (HGF)[42–45]. Loss of *Pten* in old mice (more than 3 months old) was associated with a significant increase in β-cell proliferation, whereas deletion of *Pten* in younger mice (1.5 months old) had little effect on the mitotic activity of β-cells[41]. The age-specific effect of *Pten* inhibition on proliferation

suggests that this gene may be a master regulator of β-cell proliferation in adults. Consistent with this hypothesis, islets from *Pten*-null mice exhibited increased AKT phosphorylation, decreased levels of p27 and p16 expression and increased cyclin D1 expression[41]. As expected, *Pten* deletion increased the trimethylation of H3K27me3 at the *INK4A–ARF* locus owing to the induction of *Ezh2* expression[41]. This novel PTEN–EZH2–p16 network may be relevant to humans as well, as the expression of PTEN and p16 is positively correlated[41], opening up new therapeutic possibilities to induce β-cell proliferation.

Small-molecule inducers of β-cell proliferation. Efforts to identify small-molecule inducers of pancreatic β-cell expansion are starting to bear fruit[46]. Three notable compounds reported to date include the diarylamide WS6 (REF. 47), the adenosine kinase inhibitor 5-iodotubercidin (5-IT)[48] and the adenosine receptor agonist 5′-*N*-ethylcarboxamidoadenosine (NECA), which increased β-cell proliferation in zebrafish[49].

WS6 is an analogue of a hit compound (WS1) that was identified in a screen of ~850,000 heterocycles for effects on the proliferation of the mouse β-cell line R7T1 (REF. 47). *In vivo* studies showed that WS6 reduced blood glucose concentrations in hyperglycaemic mice, was associated with ~5% increase in β-cell proliferation after 1 week and caused an overall increase in β-cell mass of 50% after 6 weeks of treatment[47]. WS6 also stimulated the proliferation of ~3% of human β-cells in culture. ERBB3-binding protein 1 (EBP1), a known cell-cycle repressor that binds to histone deacetylases and the retinoblastoma protein[50,51], and inhibitor of NF-κB kinase subunit ε (IKKε), which has a role in regulating the nuclear factor-κB (NF-κB) pathway, were identified as putative targets of WS6 (using a biotinylated analogue of WS6). Although more extensive studies are needed, the identification of WS6 represents one of the few examples of small molecules that are able to induce the proliferation of primary human β-cells *in vitro*.

A small-scale screen (850 bioactive compounds in 96-well plates) of primary rat β-cells identified 5-IT and ABT-702, two well-characterized adenosine kinase inhibitors, as inducers of proliferation, as measured by simultaneous pancreatic and duodenal homeobox protein 1 (PDX1) and Ki67 antigen immunofluorescence[48]. Adenosine kinase has an important role in cellular metabolism and is highly expressed in the liver, kidneys and pancreas[52]. To explore the mechanism of adenosine kinase inhibitor-dependent β-cell replication, the replication rate was measured in islet cultures treated with both 5-IT and a well-characterized replication pathway inhibitor. Both wortmannin and rapamycin suppressed 5-IT-dependent β-cell replication, which suggests that the molecular mechanism by which 5-IT promotes β-cell replication involves the PI3K–mammalian target of rapamycin (mTOR) signalling pathway.

NECA, a nonspecific adenosine receptor agonist that activates G protein-coupled receptor (GPCR) signalling, was identified as an inducer of β-cell proliferation in a screen of ~7,000 small molecules in a zebrafish model of diabetes[49]. Three other small molecules involved in the adenosine signalling pathway were also identified: A-134974 (an adenosine kinase inhibitor), cilostamide and zardaverine. All three small molecules affect adenosine signalling by inhibiting phosphodiesterase 3 (REF. 49). NECA mediated its proliferative effect through the adenosine A_{2A} receptor, which is expressed in β-cells; ablation of this receptor using antisense morpholinos impaired the ability of NECA to induce β-cell proliferation[49]. Together, these results suggest that adenosine signalling may be exploited when designing new therapeutic approaches for the treatment of diabetes. Interestingly, a similar approach was taken in zebrafish to identify small molecules that induce a different phenotype — pancreatic endocrine differentiation[53].

Targeting microRNAs to induce β-cell proliferation. MicroRNAs (miRNAs) are attractive new targets for regulating gene expression and have been proposed to contribute to adaptive changes in β-cell mass during pregnancy[54]. In β-cells, miRNAs regulate the expression of key genes that are important for insulin secretion and β-cell survival by binding to the 3′ untranslated region of target mRNAs, leading to translational inhibition or mRNA degradation[55–59]. During pregnancy, the expression levels of miR-338-3p were downregulated by 17-β estradiol in rat and human islets[54]. The reduction in miR-338-3p was associated with an increase in the proliferation of β-cells, and further studies suggest the involvement of the unconventional estradiol receptor GPER1 (G protein-coupled oestrogen receptor 1; also known as GPR30)[54]. Although the direct targets of miR-338-3p have not yet been identified, this study provides exciting evidence of a role for miRNAs in β-cell proliferation.

A similar line of research identified miR-7a as being important for controlling β-cell proliferation in both mouse and human islets[60]. miR-7a, along with miR-375, is among the most abundant miRNAs in both rodent and human adult islets[61–63]. Five components of the mTOR signalling pathway (70 kDa ribosomal protein S6 kinase (p70S6K), eukaryotic translation initiation factor 4E (eIF4E), MAPK signal-integrating kinase 1 (MKNK1), MKNK2 and MAPK2-associated protein 1 (MAPKAP1)) were identified as potential targets of miR-7a in mouse and human islets[60]. Specific blockade of miR-7a resulted in the activation of mTOR signalling in dispersed mouse islet cells, with a consequent 2.9-fold increase in β-cell proliferation[60]. Similar results were observed using dispersed human islet cells, with a striking 30-fold increase in Ki67-positive β-cells after the inhibition of miR-7a[60]. These findings identified a novel mechanism for regulating β-cell proliferation mediated by a specific miRNA, opening up a new possibility for increasing β-cell number either *in vivo* or *ex vivo* by targeting key miRNAs.

Protection from β-cell death

Cytokine-induced β-cell apoptosis. An alternative approach for increasing β-cell mass in diabetes is to prevent its loss in the first place. The destruction of β-cells in mouse models of autoimmune diabetes is mediated

by a combination of autoreactive $CD4^+$ and $CD8^+$ T cells, autoantibody-producing B cells and the innate immune system[64,65]. Consistent with studies in animal models, the majority of human loci identified through GWASs of human type 1 diabetes are associated with the immune system[2,66,67]. The cascade of events leading to β-cell apoptosis begins with the migration of macrophages and dendritic cells to pancreatic islets and their subsequent presentation of β-cell-specific antigens, which induces the differentiation of naive T_H0 $CD4^+$ T cells into T helper 1 (T_H1) $CD4^+$ T cells[68–70]. These cells then secrete cytokines (such as interleukin-1β (IL-1β), tumour necrosis factor (TNF) and nitric oxide) that induce the migration of $CD8^+$ cytotoxic T cells to the islets and stimulate β-cells to release chemokines and IL-15, which leads to further immune activation[71,72].

The primary mechanisms of β-cell apoptosis include signalling through FAS ligand (FASL; also known as TNFSF6) on activated $CD8^+$ T cells and FAS receptor on β-cells[73]; secretion of perforin and granzyme B by activated $CD8^+$ T cells[74]; and the secretion of pro-inflammatory cytokines (IL-1β, interferon-γ (IFNγ) and TNF) by immune cells in the islet[75]. FAS activation causes the activation of caspase 8 and leads to mitochondrial membrane permeabilization[76–78]. Perforin induces pore formation in the membrane of β-cells, allowing entry of the protease granzyme B, which activates several caspases. Pro-inflammatory cytokines — the key mediators of β-cell death — initiate the activation of NF-κB and STAT1 (signal transducer and activator of transcription) signalling pathways[79,80] (FIG. 2). IL-1β induces the phosphorylation and activation of TNF receptor-associated factor 6 (TRAF6) and, in turn, activates the IKK complex, which phosphorylates and induces the degradation of inhibitors of NF-κB — the IκBs. Upon release from IκBs, NF-κB translocates to the nucleus and initiates the transcription of apoptosis-inducing genes. IFNγ induces phosphorylation of the Janus kinase (JAK) family, which leads to the recruitment and phosphorylation of STAT1. Upon phosphorylation, STAT1 homodimerizes and migrates to the nucleus to initiate the transcription of apoptosis-inducing genes[81]. TNF also activates the NF-κB pathway by activating the IKK complex through TRAF2 and receptor-interacting serine/threonine protein kinase (RIPK)[82,83]. Substantial crosstalk and synergy exists in cytokine signalling[75,84] (FIG. 2). Hence, combination therapies may be needed to effectively block the harmful cytokine signalling present in β-cells in the diabetic state.

Inhibitors of cytokine-induced β-cell apoptosis. Dysregulated cytokine signalling leading to β-cell apoptosis has been targeted by both genetic and small-molecule approaches (TABLE 1). For example, the cytokine-initiated NF-κB pathway has been genetically targeted by expressing a degradation-resistant NF-κB protein inhibitor (ΔNIκBα) in β-cells, or by the overexpression of sirtuin 1 (*Sirt1*)[85,86]. Additionally, small interfering RNA (siRNA)-mediated knockdown of *Stat1* in INS-1E cells protected them from cytokine-induced apoptosis — an effect consistent with the fact that *Stat1*-knockout mice are protected from immune-mediated diabetes[87].

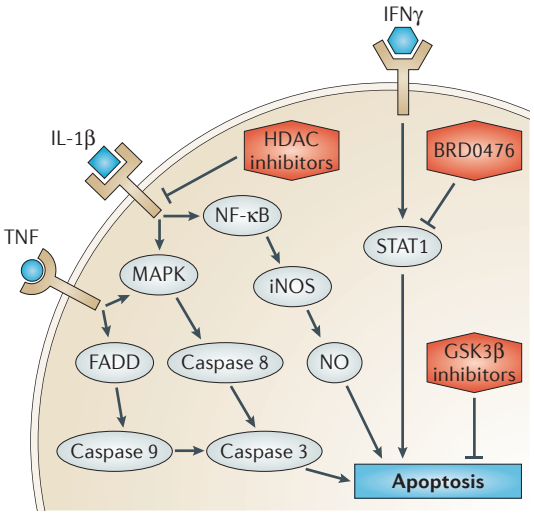

Figure 2 | **Signalling pathways involved in cytokine-induced β-cell apoptosis.** A combination of interleukin-1β (IL-1β), interferon-γ (IFNγ) and tumour necrosis factor (TNF) induce apoptosis in β-cells. IL-1β activates mitogen-activated protein kinase (MAPK) and the nuclear factor-κB (NF-κB) pathways, leading to the activation of inducible nitric oxide synthase (iNOS) and increases in nitric oxide (NO), which ultimately induce cell death. IFNγ largely signals through a Janus kinase (JAK)–signal transducer and activator of transcription (STAT)-mediated signalling pathway, whereas TNF activates FAS-associated death domain protein (FADD) and MAPK pathways, which activate a series of caspase cysteine proteases (first they activate initiator caspases such as caspase 8 and caspase 9, which is followed by the activation of executioner caspases such as caspase 3). Small molecules have been discovered to suppress this process, promoting cell viability and insulin secretion. Histone deacetylase (HDAC) inhibitors inhibit IL-1β-induced signalling through their effects on the NF-κB pathway. Glycogen synthase kinase 3β (GSK3β) inhibitors also suppress apoptosis by inhibiting various cellular processes, including JUN N-terminal kinase (JNK) activation. We have recently reported the discovery of BRD0476, a small-molecule inhibitor of IFNγ-induced STAT1 phosphorylation.

Early approaches aimed at targeting cytokine signalling with small molecules involved the use of natural products (for example, flavonoids) or plant extracts (for example, *Artemisia capillaris*, St John's wort and *Radix asari*)[88–91]. However, the bioactive components were generic antioxidants or anti-inflammatory agents that were not specific to β-cells, and effects observed were modest. Multiple attempts have been made to target components of IL-1β signalling. For example, cell-permeable peptides targeting the activation of JUN N-terminal kinase (JNK) blocked the deleterious effects of IL-1β in the mouse βTC-3 β-cell line[92]. Similarly, the recombinant IL-1 receptor antagonist anakinra is effective in non-obese diabetic (NOD) and streptozotocin (STZ)-treated models of type 1 diabetes; this protein also inhibits human β-cell apoptosis induced by high levels of

glucose[93,94]. Despite these promising findings, anakinra did not improve β-cell function in clinical studies of type 1 diabetes[95]. However, anakinra was effective in improving β-cell function in patients with type 2 diabetes, leading to improved levels of glycated haemoglobin. These results hint at the potentially greater importance of IL-1β signalling in type 2 diabetes[96–98].

Recent efforts have also focused on histone deacetylases (HDACs) as important regulators of cytokine-induced β-cell apoptosis (TABLE 1). The broad-spectrum HDAC inhibitors suberoylanilide hydroxamic acid (SAHA; also known as vorinostat) and trichostatin A (TSA) block cytokine-induced β-cell apoptosis *in vitro*, possibly by interfering with IL-1β and NF-κB signalling[99] (FIG. 2). TSA and the inhibitor givinostat (originally known as ITF-2357) had protective effects on the development of diabetes in the NOD mouse model[100,101]. Chemical and genetic knockdown studies indicate that HDAC1 and HDAC3 are key targets[102,103]; knockdown of *Hdac3* in cell culture reduces the transcription of inflammatory genes by reducing the binding of NF-κB to DNA.

Our own studies show that inhibition of HDAC3 also protects β-cells *in vitro* and *in vivo* from apoptosis induced by glucose and free fatty acids (B.K.W. and M. Lundh, unpublished observations); therefore, the development of isoform-selective HDAC inhibitors will be important for validating these targets. Our laboratory has attempted to systematically organize and catalyse the discovery of small-molecule suppressors of cytokine-induced β-cell apoptosis by developing a suite of cell-based assays for high-throughput screening. We found that targeting glycogen synthase kinase 3β (GSK3β) with alsterpaullone suppressed cytokine-induced β-cell apoptosis[104]. Furthermore, larger-scale screening identified BRD0476, a novel compound with protective effects *in vitro*[105,106]. Targeting β-cell apoptosis alone, however, may not be sufficient to protect β-cell mass. A combination of immune suppression and β-cell protection may be required for a comprehensive approach to treating diabetes.

Glucolipotoxicity-induced β-cell apoptosis. In addition to inflammatory cytokines, high levels of glucose and free fatty acids promote β-cell apoptosis — or glucolipotoxicity — by inducing ER stress, reactive oxygen species (ROS) formation and oxidative stress[107,108]. Each of these areas of cell biology has been reviewed extensively in the literature[107,109–111]. Glucolipotoxicity impairs the ability of the ER to fold proteins; chemical chaperones, which alleviate ER stress by assisting with protein folding, are being investigated as potential therapeutic agents[112]. Several other classes of drug targets are being used or investigated to block glucolipotoxicity-induced β-cell dysfunction and apoptosis. For example, GSK3β inhibitors also protect β-cells from glucolipotoxicity[113] and are effective at alleviating hyperglycaemia in Zucker diabetic fatty rats[114,115]. Metformin, a first-line drug for type 2 diabetes, may also protect human β-cells from glucolipotoxicity *in vitro*[116]. Other antidiabetic drugs, including rosiglitazone and incretin mimetics, seem to have beneficial effects on β-cell function in type 2 diabetes, although the evidence for this is not conclusive[117–121]. Of course, compounds that directly decrease glucose levels may have a similar effect on β-cell survival, and so it is also possible that each of these drugs may be acting in a β-cell-independent fashion. Again, targeting β-cell apoptosis may require adjunct therapies, such as those directed towards the immune system; new compounds that promote β-cell survival will enable this question to be answered.

β-cell reprogramming

Another promising cell-based therapeutic approach for increasing β-cell mass involves directed differentiation from stem cells (reviewed in REF. 122). High-throughput screening to identify compounds that promote differentiation led to the discovery of indolactam V, a protein kinase C (PKC) activator, which induced the differentiation of embryonic stem cells to PDX1-positive pancreatic progenitor cells[123]. More recently, insulin-producing cells have been generated from bone-marrow-derived stem cells[124] and mesenchymal stem cells[125]. In addition, whole-organism screening in zebrafish led to the discovery that disulphiram or mycophenolic acid could induce β-cell differentiation from precursor cells by inhibiting retinoic acid synthesis or by inhibiting cellular GTP levels, respectively[53].

Ultimately, the pioneering methods of induced pluripotent stem cell (iPSC) generation[126] may enable the medical community to leverage a cell resource derived directly from the patient. Indeed, the recent success in generating iPSCs using a small-molecule cocktail of six compounds[127] has the potential to further accelerate this process. In most of these cases, however, complete differentiation to a β-cell state was not achieved *in vitro*; rather, transplantation into mice was required for full maturation[128]. Viacyte, a company based in San Diego, has developed a system for generating human pancreatic progenitors from embryonic stem cells intended for subcutaneous transplantation[129]. Concerns remain regarding the immunogenicity of these cells and whether such cells will result in sustained insulin production in humans. Despite these questions, ongoing efforts should lead to the filing of an investigational new drug in the next few years, and will answer the question of whether these less mature cells can undergo complete differentiation to β-cells in humans.

The promise of transdifferentiation therapy. Perhaps more speculative, but also potentially more exciting, is whether other cell types in the organism can be reprogrammed, or transdifferentiated, to an insulin-producing β-cell-like state[130]. Reports of spontaneous *in vitro* reprogramming of human islet cells[131], as well as global analyses of genetic and chromatin differences in α- and β-cells[132–134], point to the remarkable plasticity of cell states within the pancreas. These findings have corroborated attempts to genetically manipulate cell fates to generate insulin-producing cells (FIG. 3), and provide promise that small-molecule methods to reprogramme cells into a β-cell-like state may be possible.

Oxidative stress
The effect of an imbalance between the production of reactive oxygen species and the ability of biological systems to detoxify the reactive intermediates or to repair the resulting damage.

Glucolipotoxicity
Elevated levels of glucose and fatty acids that contribute to β-cell dysfunction and death.

Zucker diabetic fatty rats
A rat model of type 2 diabetes containing a mutation of the leptin receptor.

Induced pluripotent stem cell
(iPSC). A type of pluripotent stem cell that can be generated directly from adult cells. Efforts to generate these cells have ranged from genetic to chemical, and combinations of both.

Table 1 | **Current and potential strategies for targeting β-cells**

Strategies	Target	Agents (companies)	Current development phase
Insulin secretion	SUR1	Sulphonylureas (Pfizer, Roche, Sanofi-Aventis, Boehringher Ingelheim)	Approved
	GPR40	TAK-875 (Takeda)	Phase III
	KCNJ11	Sulphonylureas	Approved
	PPARγ	Thiazolidinediones (GlaxoSmithKline, Takeda, Daiichi Sankyo)	Suspended
Incretin effects	DPP4	Gliptins (Merck, Novartis, Boehringher Ingelheim, LG Life Sciences, Takeda)	Approved and in Phase III
	GPR120 (REF. 174)	TUG891, tool compounds	Discovery
Insulin secretion, proliferation	GCK	AMG-151 (Array Biopharma), PF-04937319 (Pfizer)	Phase II
Incretin effects, proliferation	GLP1	GLP1 analogues (Amylin Pharmaceuticals, Novo Nordisk)	Approved
	GPR119	MBX-2982 (CymaBay Therapeutics)	Phase II
Proliferation	CCND1 (REF. 26)	N/A	Therapeutic target
	CDK6 (REF. 26)	N/A	Therapeutic target
Proliferation, protection from apoptosis	GSK3β[175]	N/A	Preclinical
Protection from apoptosis	HDAC1	Nonselective inhibitors such as vorinostat (Merck)	Approved for other indications
	HDAC3	Nonselective inhibitors such as vorinostat (Merck)	Approved for other indications
	IL-1β	IL-1 receptor antagonist anakinra (Amgen)	Approved for other indications
	JNK[176]	Tool compounds	Therapeutic target
	NF-κB[85]	HDAC inhibitors may act through this pathway	Approved for other indications
Reprogramming of acinar cells[15]	MAFA	N/A	Therapeutic target
	NGN3	N/A	Therapeutic target
	PDX1	N/A	Therapeutic target
Reprogramming of α-cells	PAX4 (REF. 14)	N/A	Therapeutic target

CCND1, cyclin D1; CDK6, cyclin-dependent kinase 6; DPP4, dipeptidyl peptidase 4; GCK, glucokinase; GLP1, glucagon-like peptide 1; GPR40, free fatty acid receptor 1; GPR119, G protein-coupled receptor 119; GSK3β, glycogen synthase kinase 3β; HDAC1, histone deacetylase 1; IL-1β, interleukin-1β; JNK, JUN N-terminal kinase; KCNJ11, potassium inwardly rectifying channel, subfamily J, member 11; N/A, not available; NF-κB, nuclear factor-κB; NGN3, neurogenin 3; PAX4, paired box 4; PDX1, pancreatic and duodenal homeobox protein 1; PPARγ, peroxisome proliferator-activated receptor-γ; SUR1, sulphonylurea receptor 1.

A natural place to start with β-cell transdifferentiation is the α-cell. A fellow hormone-secreting cell type, the developmental lineage of the α-cell is almost identical to that of the β-cell, and detailed research has delved into the developmental relationship between these two cell types[135]. Landmark studies described the generation of β-cells in mice by forcing the expression of the transcription factor paired box 4 (*Pax4*) in α-cells[14,136] (FIG. 3). Ectopic expression of PAX4 using a glucagon-driven Cre–LoxP system resulted in the development of enlarged pancreatic islets composed primarily of β-cells[14]. Notably, the hypoglucagonaemia caused by this system induced neurogenin 3 (NGN3)-dependent α-cell neogenesis from the ductal tissue; these cells, in turn, expressed *Pax4* and transdifferentiated into β-cells, further increasing islet size[14]. A small-molecule inducer of *Pax4* expression may, therefore, provide greater temporal control of this process.

Other transcription factors, such as PDX1, are capable of reprogramming endocrine progenitor cells towards a β-cell fate by stimulating their cellular transdifferentiation from α-cells[137]. A model of extreme β-cell loss in mice, induced through selective ablation of these cells by diphtheria toxin, followed by careful lineage tracing of β-cell regeneration, also provided evidence that mouse α-cells can be converted to β-cells[138]. Such studies bolster the notion that cell plasticity could be leveraged using small molecules to manipulate cell fate. Whether these observations can be translated to human biology remains to be seen.

Pancreatic duct ligation has been used in mice to cause loss of β-cell mass and then study the ensuing β-cell regeneration. Important research has recently shown that this technique results in the regeneration of NGN3-positive cells in pancreatic ducts[139]. These multipotent progenitor cells give rise to all cell types of the islet, and these reports added reprogramming to the potential repertoire of β-cell regeneration. Unlike α-cells, cultured human ductal cells can be successfully induced to express a subset of endocrine-specific genes through *Ngn3* overexpression[140] or by treatment with activin A and exendin 4 in the presence of high glucose levels[141]. However, the origin of new β-cells is by no means resolved, leading to questions about the feasibility of reprogramming duct cells.

Cellular transdifferentiation
The conversion of one terminally differentiated cell state to another. This process can also be thought of as a subset of cellular reprogramming, because cells can be reprogrammed by dedifferentiation followed by directed differentiation to a new cell state.

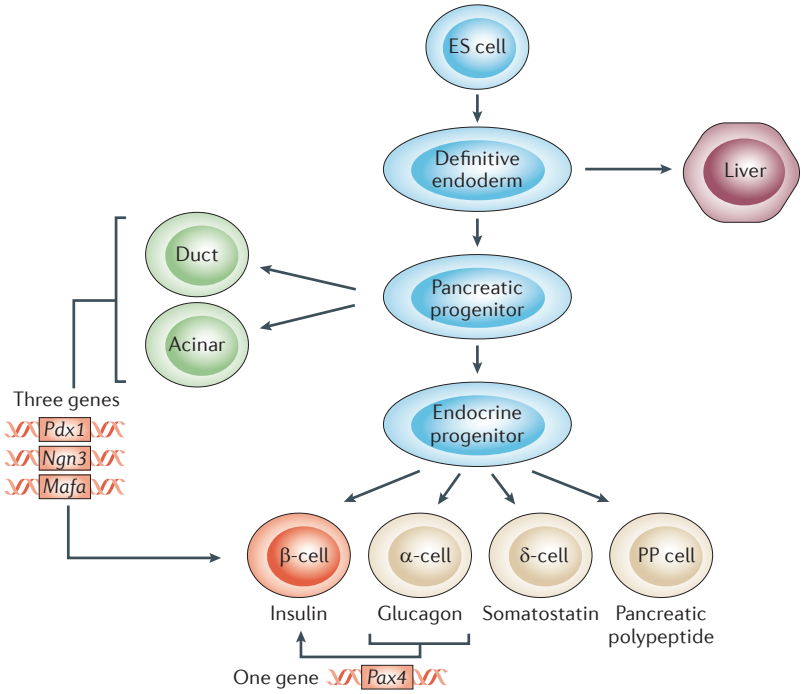

Figure 3 | **Insights into potential β-cell reprogramming targets from the development of pancreatic lineages.** Development of the endocrine pancreas from embryonic stem (ES) cells proceeds through a number of progenitor stages (shown in blue), with other cell types in the gastrointestinal system (shown in maroon) and the exocrine pancreas (shown in green) specified along the way. Regeneration of β-cells (shown in red) in mice from acinar tissue can be achieved by the transduction of three master regulatory transcription factors: pancreatic and duodenal homeobox protein 1 (*Pdx1*), neurogenin 3 (*Ngn3*) and *Mafa*. Transdifferentiation of β-cells from α-cells through the overexpression of one transcription factor, paired box 4 (*Pax4*), has demonstrated the feasibility of this approach for increasing β-cell numbers *in vivo*.

Secretagogues
Stimuli that induce insulin secretion from β-cells. Secretagogues include small molecules, such as the sulphonylurea class of drugs, peptide growth factors and glucose itself.

Glucose-stimulated insulin secretion
The process by which β-cells sense and secrete insulin in response to a glucose challenge. β-cells are uniquely engineered to carry out this role, and this function is considered the gold standard of β-cell identity. Glucose-stimulated insulin secretion involves specialized signalling pathways and secretion machinery. In many cases of partial cellular reprogramming, this function is the one that is not fully achieved.

A general shift in the understanding of β-cell transdifferentiation occurred in 2009 when an important paper reported on the generation of insulin-positive cells in mouse acinar tissue after prolonged expression of *Pdx1*, *Ngn3* and the transcription factor *Mafa*[15] (FIG. 3). This observation was replicated in cell culture[142], but again it is unclear whether this finding can be translated to human cells. Some evidence that this might be achievable in human cells is provided by our own studies, which indicate that prolonged overexpression of these three transcription factors in human PANC-1 ductal adenocarcinoma cells induces the expression of a subset of β-cell-specific genes[177]. It remains unclear whether small molecules can induce true cell-fate conversion, but results generated in mice reflect the promise of this approach.

Promotion of insulin secretion

In conjunction with attempts to increase β-cell mass, approaches to increase β-cell function are also gaining therapeutic traction. Type 2 diabetes is known to be characterized by both impaired insulin function and a failure of insulin secretion to compensate for this defect[143]. Despite intense efforts over the past 30 years to develop new drugs for type 2 diabetes that increase insulin secretion, only two classes of small molecules that directly target the β-cell (that is, sulphonylureas and glinides) are currently approved by the US Food and Drug Administration (FDA) for the treatment of this disease. Both classes target the same protein — the sulphonylurea receptor 1 (SUR1; also known as ABCC8) regulatory subunit of the ATP-sensitive potassium channel[143] (BOX 1). Moreover, these compounds augment insulin secretion largely independently of the ambient glucose concentration, thus increasing the risk of dangerous hypoglycaemia and limiting their widespread use[144,145].

Insulin secretagogues in development. Various currently used drugs for diabetes, such as sulphonylureas, glinides, thiazolidinediones, glucagon-like peptide 1 (GLP1) analogues and dipeptidyl peptidase 4 (DPP4) inhibitors (also called gliptins), ultimately aim to promote insulin secretion. Of these, only sulphonylureas and glinides are small molecules that directly target the β-cell. Notably, sulphonylureas do not act in a glucose-dependent manner[14]. The search for novel, safe, glucose-dependent insulin secretagogues has yielded a few promising candidates that are in various stages of clinical development, including compounds targeting glucokinase and the GPCRs free fatty acid receptor 1 (FFAR1; also known as GPR40), GPR119 and GPR120 (also known as FFAR4) (TABLE 1).

Glucokinase is a key regulator of glucose homeostasis, functioning as the glucose sensor in both pancreatic β-cells and hepatocytes[146]. For over a decade, small-molecule activators of glucokinase have been reported to improve glycaemia in animal models of diabetes by augmenting insulin secretion from the β-cell and enhancing glucose uptake by the liver[147]. Excitement surrounding these beneficial effects led to the rapid development and patenting of over 100 structurally diverse glucokinase activators, each of which allosterically enhances enzyme activity[148]. Although several compounds have progressed to Phase II clinical trials (such as AMG-151 from Array Biopharma and PF-04937319 from Pfizer; see TABLE 1), they have been plagued by worrisome side effects, including hypoglycaemia in clinical trial participants and hepatic steatosis in rodent models, thus clouding the future of this class of potential antidiabetic therapies[149].

GPCRs are attractive as drug targets because they transduce signals that are known to increase glucose-stimulated insulin secretion, they can be modulated by small molecules and they have a track record of successful modulation in type 2 diabetes (for example, GLP1 analogues). The activation of GPR40, which is a receptor for unsaturated medium- and long-chain fatty acids, leads to the elevation of intracellular calcium and increased PKC activity, thus augmenting insulin secretion but only in the presence of glucose[150]. Although the mechanisms of action of GPR119 and GPR120 in β-cells are not entirely clear, the activation of either GPCR seems to have beneficial effects on insulin secretion through stimulation of GLP1 activity[148]. Small molecules targeting several of these nutrient-sensing GPCRs in the β-cell are currently undergoing clinical evaluation[151]. For example, TAK-875, a small-molecule agonist of GPR40 that is being developed by Takeda Pharmaceutical, has entered Phase III clinical trials after demonstrating

profound effects — comparable in magnitude to the sulphonylurea glimepiride — on glucose-stimulated insulin secretion in clinical trial participants, but without the risk of inducing hypoglycaemia[152]. In addition, CymaBay Therapeutics is currently investigating the effects of the GPR119 agonist MBX-2982 in Phase II clinical trials.

Nominating drug targets through human genetics. The tools of human genetics hold great promise in the search for additional drug targets within the β-cell that can be safely modulated to enhance insulin release. Although traditional genetic approaches such as linkage analysis and candidate gene studies are underpowered for detecting variants of modest effect on disease risk, the recent advent of GWASs has transformed the field, leading to the rapid identification of more than 60 genetic loci harbouring variants that are robustly associated with type 2 diabetes at a genome-wide level of significance ($P < 5 \times 10^{-8}$)[9,153–155] (Supplementary information S1 (table)).

Although much work remains to be done to determine the causal variants at each locus and how they impart risk for disease, one striking finding that has emerged is that more variants affect β-cell function than insulin sensitivity (Supplementary information S1 (table))[156,157]. For example, of the 31 risk variants examined in the DIAGRAM+ meta-analysis, 10 were associated with reduced β-cell function (as estimated by HOMA-B), whereas only 3 were associated with reduced insulin sensitivity (as estimated by HOMA-IR)[158]. Risk variants have also been found in or near genes that are known to have key roles in the regulation of insulin secretion: for example, potassium inwardly rectifying channel, subfamily J, member 11 (*KCNJ11*), glucokinase (*GCK*), wolfram syndrome 1 (*WFS1*), hepatocyte nuclear factor 1 alpha (*HNF1A*), *HNF1B* and *HNF4A*[7,8,10]. This increases confidence in the results and suggests that risk variants may provide clues regarding other proteins in the β-cell that regulate insulin secretion.

Zinc transporter 8 (ZNT8; encoded by *SLC30A8*) and CDK5 regulatory subunit-associated protein 1-like 1 (*CDKAL1*) are two genes that had not been studied in much detail before their nomination as type 2 diabetes genes by GWASs, but have since been shown to have key roles in insulin secretion. The *SLC30A8* gene encodes the protein ZNT8, which is the primary zinc transporter in the β-cell and determines the amount of this element stored alongside insulin in secretory granules[159]. Recent studies have demonstrated that zinc that is co-secreted with insulin from β-cells acts to decrease insulin secretion by neighbouring β-cells, and also decreases hepatic clearance of insulin from the portal circulation during first-pass metabolism[160]. As the *SLC30A8* risk variants described to date seem to decrease protein function[161], these findings have sparked intense interest in designing modulators of ZNT8 as possible therapeutics. Similarly, the function of the *CKDAL1* gene was poorly understood before the identification of variants linking it to type 2 diabetes[162–164]. Since this discovery, *CKDAL1* has been shown to encode a methylthiotransferase that is required for proper translation of pro-insulin, catalysing a key modification of lysine tRNA that prevents the misreading of corresponding codons in mRNA transcripts[165]. Drugs targeting this protein may therefore improve the accuracy of protein translation in the β-cell, thereby decreasing ER stress and improving insulin secretion.

As next-generation sequencing technologies continue to advance and more diverse populations are examined, the number of variants linked to type 2 diabetes and related traits will probably continue to grow, providing improved insight into the pathogenesis of the disease as well as the identification of additional candidate targets within the β-cell for the development of safe, glucose-dependent secretagogues.

Nominating drug targets through phenotypic approaches. Insulin secretion is regulated by complex and incompletely understood molecular and biochemical networks within the β-cell and, as such, the selection of appropriate therapeutic targets remains challenging. In this context, phenotypic screening represents an attractive complementary approach for finding novel insulin secretagogues and their cognate proteins in a relatively unbiased manner.

To date, two small-scale, proof-of-principle screens for insulin secretion have been reported. First, a screen of 1,280 known bioactive compounds in INS-1 832/13 cells in a 96-well format identified dopamine receptor antagonists as glucose-dependent insulin secretagogues[166]. More recently, a similar screen of 4,691 compounds in INS-1E cells in a 384-well format recovered known insulin secretagogues and found new compounds that seemed to have glucose-dependent effects[167]. Common to both screens was the use of the insulin enzyme-linked immunosorbent assay (ELISA) as a functional readout, which is challenging to adapt to high-throughput large-scale experiments and thereby limits the number of small molecules that can be effectively screened for activity in β-cells. The cost of these assays is also prohibitive for many academic settings, as suggested by the industry setting of the two screens described.

To identify an alternative to immunoassays for measuring insulin secretion, two groups used fluorescent reporters of insulin gene expression as a functional readout for genetic and chemical screens of β-cell function. Ku *et al.*[168] screened a library of siRNA constructs targeting GPCRs in mouse MIN6 cells, identifying GPR27 (also known as SREB1) as a regulator of insulin secretion. Likewise, Hill *et al.*[169] tested 1,319 extracts from marine invertebrates for insulin and PDX1 expression in MIN6 cells; they identified seven extracts, one of which increased insulin secretion through an unclear mechanism. Although this indirect approach for identifying drug targets and secretagogues holds promise, it remains to be determined whether the expression and secretion of insulin are sufficiently correlated for gene expression assays to provide the sensitivity needed for larger screening campaigns.

Future perspectives

A paradigm shift is underway in the field of diabetes research, with renewed focus on the pancreatic β-cell as a key player in both type 1 and type 2 diabetes, and with increased interest in identifying novel disease-relevant drug targets in this cell type (TABLE 1). The next stage

HOMA-B
Homeostatic model assessment of β-cell function. A method to estimate the steady-state β-cell function of an individual relative to a non-diabetic reference population, based on fasting measurements of plasma glucose and plasma insulin.

HOMA-IR
Homeostatic model assessment of insulin resistance. A method to estimate the steady-state insulin resistance of an individual relative to a non-diabetic reference population, based on fasting measurements of plasma glucose and plasma insulin.

of discovery will require innovative, multidisciplinary approaches to systematically investigate and modulate the biological pathways that regulate β-cell survival, function and proliferation.

Such approaches are not without their challenges, however. Promoting β-cell proliferation, for example, must be exquisitely cell-specific so as to avoid uncontrolled hyperplasia. Similarly, uncontrolled insulin secretion can cause lethal hypoglycaemia. Important measures must therefore be taken, not only to validate these approaches as potential therapeutic strategies for diabetes but also to ensure their safe use in the context of animal models and, ultimately, in clinical trials. Given the technical challenges associated with inducing selective proliferation or transdifferentiation, small-molecule promotion of β-cell survival may provide our first opportunities to test this therapeutic approach in animal models and even in humans.

In some cases, technologies have begun to emerge that will support these efforts. For example, single-cell technologies may now enable the determination of molecular signatures of the proliferation of individual β-cells instead of whole islets, allowing cell type-specific measurements from pools of heterogeneous cell types (for example, whole islets)[170,171]. Likewise, genome engineering tools such as transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR)–Cas9 programmable nucleases[172] now afford the opportunity to delete genes or introduce disease-associated variants in relevant cell types, at user-specified sites, thus greatly accelerating the identification of new drug targets within the β-cell.

In other cases, technical challenges persist. For example, the paucity of reliable human β-cell lines for study remains a key barrier to research, despite recent advances in this area[173]. Similarly, techniques to track β-cell mass *in vivo* in a non-invasive manner are not yet consistent enough for determining the effects of potential mitogens on β-cell mass in animal models. Last, although the number of genetic variants linked to diabetes continues to expand at a rapid pace, the functional annotation of these variants — in particular linking variants to effects on the β-cell — has not kept pace. Advances in these areas will be vital for translating emerging discoveries into the identification of actionable drug targets.

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Competing interests statement
The authors declare competing interests: see Web version for details.

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