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Mechanistic insights and approaches for beta cell regeneration

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Diabetes is characterized by variable loss of insulin-producing beta cells, and new regenerative approaches to increasing the functional beta cell mass of patients hold promise for reversing disease progression. In this Review, we summarize recent chemical biology breakthroughs advancing our knowledge of beta cell regeneration. We present current chemical-based tools, sensors and mechanistic insights into pathways that can be targeted to enhance beta cell regeneration in model organisms. We group the pathways according to the cellular processes they affect, that is, proliferation, conversion of other mature cell types to beta cells and beta cell differentiation from progenitor-like populations. We also suggest assays for assessing the functionality of the regenerated beta cells. Although regeneration processes differ between animal models, such as zebrafish, mice and pigs, regenerative mechanisms identified in any one animal model may be translatable to humans. Overall, chemical biology-based approaches in beta cell regeneration give hope that specific molecular pathways can be targeted to enhance beta cell regeneration.

Diabetes mellitus is an umbrella term that defines a number of diseases manifesting with an inability to control glucose levels. Rare forms of monogenic diabetes termed maturity-onset diabetes of the young are caused by gene mutations that disrupt the function or development of the insulin-producing beta cells of the pancreas. Type 1 diabetes is an autoimmune disease caused by T cell-mediated destruction of insulin-producing beta cells[1], whereas type 2 diabetes, the most common form of diabetes, is characterized by failure of beta cells to meet increased demands driven by multiorgan insulin resistance. While the primary cause of type 2 diabetes is not beta cell loss, reports indicate a reduction of functional beta cell mass in patients with type 2 diabetes as well[2–4]. Therefore, it is well established that, regardless of disease etiology, loss and/or dysfunction of beta cells is a critical event in diabetes progression.

Current chemical and protein-based approaches for diabetes treatments aim to maintain glucose levels within the physiological range. Administration of insulin analogs is indispensable for patients with type 1 diabetes[5], and metformin is highly used to lower glucose in patients with type 2 diabetes by having pleiotropic effects in gut and liver[6]. This occurs through transient inhibition of mitochondrial function as well as binding presenilin enhancer (PEN2) and activating AMP-activated protein kinase (AMPK) in the liver[7]. Additional drug classes, including glucagon-like peptide 1 (GLP-1) analogs, both improve glucose homeostasis and reduce body weight[8,9]. However, patients receiving these therapies often experience various side effects.

Stimulating endogenous beta cell regeneration is an alternative strategy that aims to restore the beta cell population through proliferation, differentiation or reprogramming of cells from different origins (Fig. 1) and may overcome the challenges of current approaches. In this Review, we will focus on in vitro and in vivo approaches by the chemical biology field that advanced our understanding of endogenous beta cell regeneration. We will summarize these efforts with a focus on targetable pathways and the mechanism of action of potent small molecules that can potentially be exploited for future therapeutic development. For exhaustive reviews of the literature on either beta cell regeneration or chemical biology aspects of beta cell biology, we refer to other sources[10,11]. Additionally, we will present the recent advances of chemical probes and sensors used for assessing the functionality of

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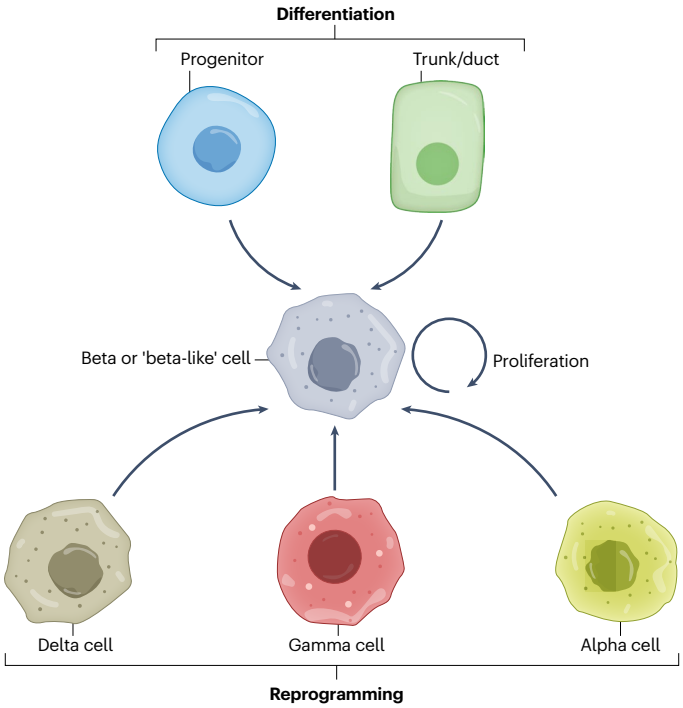

Fig. 1 | Possible cellular origins of beta cell (re)generation within the pancreas. Beta cells can increase in number by proliferation, differentiation from progenitors, trunk or duct and transdifferentiation from several cell types, including glucagon-secreting alpha cells, somatostatin-secreting delta cells and pancreatic polypeptide-secreting gamma cells, which can reprogram to beta-like cells depending on the injury or diabetic model, age and specific stimuli.

regenerated beta cells. Such sensors also have great potential for future chemical screening approaches both in vitro and in vivo.

Cellular mechanisms of beta cell (re)generation

Chemical inducers of beta cell proliferation

It is well documented that the proliferation rate of beta cells peaks shortly after birth and remains marginal for the remainder of the adult life as observed in model organisms and humans[12,13]. Yet, in mouse models of beta cell injury, proliferation of the remaining beta cells can be the major driver of endogenous beta cell regeneration[14–16]. High-content in vitro and in vivo chemical screens have been used to identify small molecules that can be developed as drug candidates to stimulate beta cell proliferation in humans. The most effective, single chemical inducer of beta cell proliferation to date came from a high-content in vitro chemical screen using hepatocarcinoma cells (HepG2). Harmine, a small-molecule inhibitor of the dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A), was found to be a driver of beta cell proliferation as measured through MYC transcriptional activity[17]. Moreover, DYRK1A inhibition has been independently validated by several research groups as a targetable pathway to promote beta cell proliferation with additional compounds including 5-iodotubercidin (5-IT) and GNF-4877 (refs. 18,19). Elucidation of the detailed molecular mechanisms revealed that DYRK1A inhibition disrupted the DREAM complex, a central transcriptional regulator of quiescence in human beta cells[20]. The DREAM complex is a multiprotein assembly that has a central role in regulating proliferation and changes from a pro-quiescent to a pro-proliferative mode of action. This study demonstrated that, in human beta cells, DYRK1A inhibition converts the DREAM complex from a quiescent to a pro-proliferative mode of action and induces many pro-proliferative genes including *FOXM1* and *CDK1*, which are direct target of this complex[20]. The exact biochemical link between DYRK1A and the DREAM complex in beta cells remains to be seen. Current efforts focus on developing more potent, safe and selective DYRK1A inhibitors that can further increase the levels of beta cell proliferation[21,22].

The transforming growth factor-β (TGFβ) signaling cascade is also among the most potent molecular pathways to target in regard to beta cell proliferation. Several independent studies showed that TGFβ signaling through the SMAD class of transcriptional regulators can affect mouse beta cell proliferation[23–25]. Moreover, small-molecule inhibitors of TGFβ receptors were shown to promote proliferation of cultured human beta cells and of transplanted human islets in immunocompromised mice[26]. Importantly, a combination of harmine with inhibitors of TGFβ receptors has resulted in the highest percentage of human beta cell proliferation described so far[27]. As these inhibitors often inhibit several TGFβ type I receptors, including the receptors ALK4 (ACVR1B), ALK5 (TGFBR1) and ALK7 (ACVR1C), it is currently unclear which ligand–receptor pairs are important in this context. As TGFβ inhibitors have pleiotropic effects in development and physiology, a targeted, structure-based chemistry approach would be needed to circumvent diverse effects.

Recent high-content in vivo chemical screens for inducers of beta cell proliferation in animal models have provided additional targetable pathways in a physiological context. In this regard, the nitroreductase (NTR)–metronidazole chemical–genetic system in zebrafish has been widely adopted as a way to temporally ablate beta cells. NTR is a bacterial enzyme that converts metronidazole to a cytotoxic product in insulin-producing beta cells, as the expression of NTR is controlled by an insulin promoter, inducing nearly complete beta cell ablation and providing another extensive injury model that can mimic the loss of beta cells in diabetes[28,29]. Using this beta cell ablation system, an in vivo chemical screen of ~7,000 chemicals was performed, identifying 5′-*N*-ethylcarboxamidoadenosine, an adenosine receptor agonist, as a stimulator of beta cell proliferation with translation potential for mouse beta cells[30,31]. The effect was shown to depend on adenosine receptor 2a, as deficiency of this receptor in zebrafish and mice blocked the proliferative phenotype. Furthermore, chemical-based inhibitors of adenosine kinase (ADK), for example, A-134974, can enhance beta cell proliferation in pig, mouse and zebrafish models, and ADK has been recently implicated in a hormone complex of fatty acid-binding protein 4 (FABP4) responsible for increased beta cell mass in mice[31–33]. Mechanistically, the FABP4–ADK interaction was necessary for kinase activity, as ATP generation was more prominent when the complex was intact and was diminished in *Fabp4*-knockout mice[33]. Both ADK inhibition and adenosine receptor agonism converge on ATP metabolic pathway regulation and point it out as important for beta cell proliferation. Although 5-IT was identified as a beta cell mitogen in the context of inhibiting ADK, it was later shown to also inhibit DYRK1A[26,32], and this dual action seems to drive mouse beta cell proliferation[34]. This study demonstrated that 5-IT treatment of mouse islets with a beta cell-specific knockout of *Adk* was still able to induce beta cell proliferation, highlighting the ADK-independent effect of 5-IT, which also acts as a DYRK1A inhibitor[34]. In sum, these data suggest that adenosine signaling and metabolism are an evolutionarily conserved pathway that promotes beta cell proliferation. A new genetically modified version of NTR, NTR 2.0, was lately developed and suggested to have improved efficacy in the ablation process[35]. It would be interesting to see whether the new ablation system can be more efficient for identification of novel small molecules stimulating beta cell proliferation in vivo.

Two additional high-content chemical screens in zebrafish have been performed to specifically identify inducers of beta cell proliferation. These screens used the method of a fluorescent ubiquitylation-based cell cycle indicator as a readout of proliferation[36]. In the first screen, proliferating beta cells were marked by a fluorescently labeled S/G2/M phase marker, geminin, and a role was discovered for retinoic acid, serotonin and glucocorticoids in stimulating beta cell proliferation[37]. In the second screen, geminin was fused to

nanoluciferase to convert the readout of the screen to a luminescence measurement, thereby increasing the throughput. This screen revealed a potent role for a salt-inducible kinase (SIK) inhibitor, HG-9-91-01, in zebrafish and mouse beta cell proliferation through inducing transient unfolded protein response activation[38], although its effect on human beta cell proliferation was modest. We are tempted to speculate that SIKs directly phosphorylate important intermediates of the unfolded protein response and thus modulate the response to endoplasmic reticulum (ER) stress and beta cell proliferation. It should be noted here that high glucose levels in mouse islet cell cultures were important for potent induction of beta cell proliferation. We stress this as an important aspect of in vitro proliferation assays and argue that the culture conditions of islets need to be reported in detail because glucose is also a known stimulant of beta cell proliferation[39]. Intriguingly, the translation potential of these pathways to mammalian systems is documented. Serotonin is a well-known inducer of beta cell proliferation during beta cell expansion in pregnant mice[40]. Furthermore, SIK2 was recently shown to be involved in mouse and human beta cell proliferation[41], indicating that there might be context and/or species differences or a balance between SIKs promoting and inhibiting beta cell proliferation, that is, SIK2 versus SIK1 and SIK3. Overall, an array of small molecules have been identified with potential pro-proliferative beta cell action.

Apart from small molecules, endogenously circulating peptides and proteins belong to a constantly evolving class of therapeutics for diabetes. Recombinant peptide-based analogs of GLP-1 are being used in clinical practice as diabetes treatments that can stimulate insulin secretion and more recently were used to treat obesity[8]. With regard to studies of beta cell regeneration, streptozotocin (STZ) is a widely used chemical for beta cell ablation in rodents that enters beta cells through glucose transporter 2 (GLUT2) and triggers beta cell apoptosis through DNA damage induction[42]. In the mouse STZ model of beta cell injury, GLP-1 analogs are reported to promote beta cell proliferation, but the effect declines in aging mice[43]. Moreover, beta cell proliferation is induced in human islets transplanted to mice treated with GLP-1, and a synergistic effect has been reported between DYRK1A inhibition and GLP-1 treatment in vitro[44,45]. In the liver-specific insulin receptor knockout mouse model, serpin family B member 1 (SERPINB1) is secreted and circulates following development of liver-specific insulin resistance and induces compensatory beta cell proliferation across species[46]. A recent follow-up study on this class of proteins suggested that pancreatic elastase inhibitors can recapitulate certain beta cell phenotypes observed with SERPINB1 treatment. The effect of the pancreatic elastase inhibitor was partially through the protease-activated receptor 2 (PAR2) pathway and other mechanosignaling pathways[47]. Lastly, a recent antibody-based screen aimed to identify circulating proteins that are present in young mice but not in old mice and that might be involved in beta cell proliferation. This elegant screen uncovered a role for WNT1-inducible signaling pathway protein 1 (WISP1) in beta cell proliferation following STZ-induced beta cell injury through prominent activation of the AKT kinase, an important signaling hub in beta cells[48].

Chemical and protein stimulators of beta cell proliferation are a promising drug class that however has caveats. Unspecific induction of proliferation in off-target tissues is a major concern and can lead to oncogenesis, and, therefore, chemical conjugation strategies are needed to ensure proper delivery of the small molecule to beta cells. Additionally, only a few of the molecular targets involved in beta cell proliferation are convincingly conserved from zebrafish and rodent to human biology (DYRK1A inhibitors, SERPINB1 and TGFβ receptor inhibitors), making the translational gap difficult to overcome and requiring extensive screening approaches using human-based setups[49,50]. Indeed, comparative studies of drug potency using human beta cells are important and should be encouraged; for example, one study concluded that harmine and DYRK1A inhibition were a potent beta cell mitogen, whereas the others had only modest effects[49]. Overall, using in vitro and in vivo chemical biology screens and tools, we now have a better understanding of the mechanisms of beta cell proliferation.

Reprogramming to beta cells

Another cellular mechanism of endogenous beta cell regeneration is reprogramming of other mature cell types to beta-like cells. Genetic studies of reprogramming other cell types to beta cells have generated information on potential druggable targets and the mechanism of action of in vivo reprogramming as a strategy to regenerate beta cells. In mice, the chemical–genetic diphtheria toxin receptor-based model has been proven to be an efficient model to mimic acute beta cell loss. In this model, the insulin promoter drives the expression of diphtheria toxin receptor and the administration of diphtheria toxin leads to the death of more than 99% of beta cells in a specific and temporally controlled manner[51,52]. Using this model, it was demonstrated that glucagon-secreting alpha cells, somatostatin-secreting delta cells and pancreatic polypeptide-secreting gamma cells (also known as PP cells) of the endocrine pancreas can switch fate to beta-like cells and in certain cases reverse mouse hyperglycemia[51–53]. Transdifferentiation to beta cells has been validated in additional model organisms[54–57]. Mechanistically, inhibition of intra-islet insulin, insulin-like growth factor (IGF) and Hedgehog signaling or activation of GLP-1–glucagon signaling[58] can potentiate transdifferentiation of alpha cells to beta-like cells, either by genetic means, peptides or the molecules S961, wortmannin and picropodophyllin[54,57,59]. DNA methyltransferase 1 (DNMT1) and Aristaless-related homeobox (ARX) transcription factor deficiency increases the conversion of alpha to beta-like cells in mice[60], highlighting the epigenetic similarities between these two cell types that facilitate reprogramming[61]. A similar phenotype was observed in a zebrafish model in which only *dnmt1* was knocked out[62]. Furthermore, spontaneous conversion of alpha cells to beta cells was observed in pregnant mice, that is, alpha cells with an $LGR5^{+}$ ductal cell progenitor origin[63], which highlights alpha cell plasticity in states of increased metabolic demand. Overall, these studies suggest that conversion of other cell types is feasible in experimental systems. Alpha-to-beta-like cell reprogramming is the cellular mechanism studied in greater detail and seems to have greater feasibility to succeed given the developmental similarities between alpha and beta cells.

A series of experimental evidence suggests that forced expression of a combination of the beta cell fate-determining transcription factors (including NEUROG3, MAFA, PDX1 or PAX4) in diabetic mouse models can induce reprogramming of alpha, delta or acinar cells into beta cells[64–67]. Indeed, acinar-derived beta cells survived over a year in vivo and reversed hyperglycemic phenotypes[68]. The same combination of transcription factors can induce cell type switches to beta-like cells outside of the pancreas, including in the stomach, intestine and liver[69–72]. A screening platform was set up to increase the efficiency of the conversion process in mouse gut organoids and later translate the findings to human adult gut organoids. Briefly, the screening method was based on mouse gut organoids derived from transgenic mice (RIP:Cre;*ROSA26*^{tdTomato}) that allow recombination and red fluorescence generation when the insulin promoter is activated, indicating beta cell conversion. The authors found that the combined pharmacological inhibition of forkhead box O1 (FOXO1), TGFβ and Notch signaling is more efficient in inducing insulin expression in human gut organoids, and a similar treatment inhibitor combination (FBT10, RepSox and PF-03084014, respectively) could ameliorate diabetes in mice[73]. How this triple-small-molecule treatment could work mechanistically was predicted from previous work on *Foxo1*-knockout mice and endocrine induction in human gut-like organoids[74] as well as from numerous developmental biology studies showing that Notch and TGFβ inhibition promote endocrine induction in the pancreas.

GABA signaling was implicated in alpha-to-beta cell conversion, but these findings were challenged in follow-up studies[75–78]. GABA treatment was shown to induce beta cell proliferation, providing an

explanation for earlier findings[79], and the first clinical trial using a GABA agonist did not report an improvement in glycemic control by increasing insulin in patients with type 1 diabetes but had beneficial effects in preventing hypoglycemic events[80]. No chemical screen has identified new pathways that can potently enhance the reprogramming of other mature pancreatic cell types to insulin-producing beta cells. However, recently, focal adhesion kinase inhibition also led to acinar-to-beta cell conversion[81]. Transcription factors have for a long time been considered as 'undruggable' targets given the nature of their function as DNA-binding proteins. As mentioned, novel small molecules inhibiting FOXO1 expression have been shown to induce beta-like cells in gut organoid systems[82,83]. In this particular case, however, caution needs to be taken, as FOXO1 inhibitors have also been shown to dedifferentiate beta cells to an immature state[84,85]. Thus, several pathways have been identified to stimulate either beta cell proliferation or reprogramming, and those that are conserved across species are probably more important (Fig. 2). However, rigorous examination, on top of molecular profiling, is needed to address the functionality of the transdifferentiated cells using glucose-stimulated insulin secretion assays, as recently demonstrated in one study[59].

Beta cell differentiation

The search for an adult pancreatic progenitor population has generated a wealth of data but also controversy in the beta cell regeneration field. It has been postulated that such progenitors exist in the ductal cell population of the pancreas, as this epithelial cell type gives rise to mature endocrine and ductal cells during embryonic development[86–90]. The contribution of ductal cells giving rise to beta cell regeneration in mice declines rapidly with age and ceases in most mouse models of diabetes[16,88–91]. On the contrary, ductal cells in zebrafish were shown to be a progenitor cell population that can give rise to beta cells throughout the animal lifespan[92]. Notch signaling and the downstream HES1 and HES4 orthologs (*her* genes) actively maintain ductal fate in the smaller ducts of the zebrafish pancreas, and thus the larger ducts (that are Notch independent) have the greatest potential to form beta cells in different stages and through adulthood[92–97].

Three high-content chemical screens in zebrafish have identified new molecular targets for induction of beta cell differentiation[98–100]. A chemical screen combining Notch inhibition with a chemical library of ~2,200 chemicals used beta cell formation in the developing zebrafish pancreas as a readout to identify cyclin-dependent kinase 5 (Cdk5) as a stimulator of beta cell differentiation[98]. This effect could be attributed to the fact that inhibition of Cdk5 blocks translocation of pancreatic and duodenal homeobox 1 (Pdx1) from the nucleus to the cytoplasm[101]. Moreover, CDKs have been shown to phosphorylate and regulate NEUROG3, which is the master regulator of endocrinogenesis in mammals[102,103]. The use of a double-transgenic zebrafish reporter line, tracking Notch signaling together with endocrine progenitor formation by using the *pax6* regulatory element to drive GFP expression, enabled a chemical screen showing that the aldehyde dehydrogenase inhibitor DSF induced beta cell formation by inhibiting retinoic acid synthesis[100]. Development of a double-reporter transgenic zebrafish (*Tg*(*ins:PhiYFP-2a-nsfB, sst2:tagRFP*)) to automate detection of beta cell differentiation was coupled to automated handling of a chemical library comprising 3,348 chemicals. The screen revealed a role for inhibitors of nuclear factor κ light-chain enhancer of activated B cells (NF-κB) in beta cell formation[99], potentially by downregulating the transcription factor Sox9 and thereby the maintenance of the zebrafish pancreatic progenitor status. A recent genetic screen for inducers of beta cell regeneration has implicated one-carbon metabolism, through folate receptor 1 (FOLR1), in beta cell neogenesis in zebrafish and pig pancreas cultures[104], and it has also been shown to increase beta cell mass in mice[105]. Lastly, excessive nutrients lead to increased beta cell mass in zebrafish[106]. Although no beta cell injury takes place in this model, the compensatory beta cell expansion was shown to mainly derive from progenitors in the pancreas, suggesting that different environmental cues may drive the progenitor population toward the beta cell fate[107]. Of note, enzymes with aldehyde dehydrogenase activity that might affect retinoic acid signaling can act as markers of a specialized ductal cell type, the centroacinar cells, which can give rise to beta cells in homeostasis, as shown by mouse lineage-tracing studies[63,108,109]. To this extent, it would be interesting to test the ability of different FDA-approved small-molecule inhibitors of aldehyde dehydrogenase enzymes as inducers of beta cell formation.

The existence of a mammalian progenitor population in the adult pancreas has been a debated topic in the field of beta cell regeneration. Zebrafish are shown to have such a progenitor population within ductal cells throughout life. By leveraging the power of zebrafish chemical screens, it is also feasible to elucidate the molecular targets of newly identified compounds that promote beta cell regeneration. A potent small molecule that came out of a zebrafish chemical screen for inducers of beta cell regeneration, named CID661578, was recently shown to exert its effect by interfering with MAP kinase-interacting kinase 2 (MNK2)[31,110]. This drug target deconvolution approach revealed that selectively stimulating protein synthesis in ductal cells promotes differentiation toward a beta cell fate[110]. Increased protein synthesis has been shown to stimulate differentiation of stem cells in other mouse and human adult tissues, highlighting the potential importance of increased protein synthesis in adult stem cell differentiation for regenerative purposes[111–114]. Overall, understanding the molecular pathways that drive beta cell differentiation in zebrafish is crucial to translate these findings to higher species and ultimately humans. While the zebrafish model system has many advantages, differences between endocrinogenesis in zebrafish and mammalian systems need to be taken into account. For example, zebrafish endocrinogenesis does not rely on Neurog3, which is the master regulator of the process in mammals, but rather the transcription factors Ascl1a and Neurod1 (ref. 115). Moreover, endocrinogenesis is continuous throughout the lifespan of zebrafish (perhaps because the zebrafish grows throughout its whole life), which can make the translation of findings to other adult species in which endocrinogenesis has ceased challenging. Nevertheless, zebrafish has provided valuable insights into beta cell differentiation.

New human systems for studying beta cell (re) generation

Organoid technology can advance the study of pancreatic progenitors and their contribution to beta cell regeneration. Pancreatic organoids have been successfully generated from mouse and human primary tissue as well stem cell-based differentiation protocols, and their properties have been recently reviewed[116]. These cultures have the advantage of being used for chemical screening approaches to identify new inducers of beta cell differentiation. As a proof of concept, Gonçalves et al.[117] performed a chemical–protein screen that identified the epidermal growth factor (EGF) and fibroblast growth factor (FGF) signaling pathways to be important regulators of human pancreatic progenitor proliferation. Similar screens were performed in stem cell-derived pancreatic progenitors to identify the main pathways involved in forming ductal-like organoids in vitro[118,119]. Advances in screening and phenotyping organoids derived from other organs are likely to pave the way for automated chemical screening using pancreatic organoids[120]. Apart from pancreatic organoid cultures, a recent paper reported the use of a human pancreatic slice system to study beta cell differentiation. This work validated the role of bone morphogenetic protein 7 (BMP7) in inducing beta-like cell signatures in human slice culture, suggesting that this culture system could be exploited for chemical screening or validation of mechanisms of action for compounds[121].

One of the biggest challenges in the field is the reliance on scarce cadaveric donor islets for translating the findings to human beta cell biology. A recently developed islet resource aims to overcome some of these challenges for basic and translational research by making a

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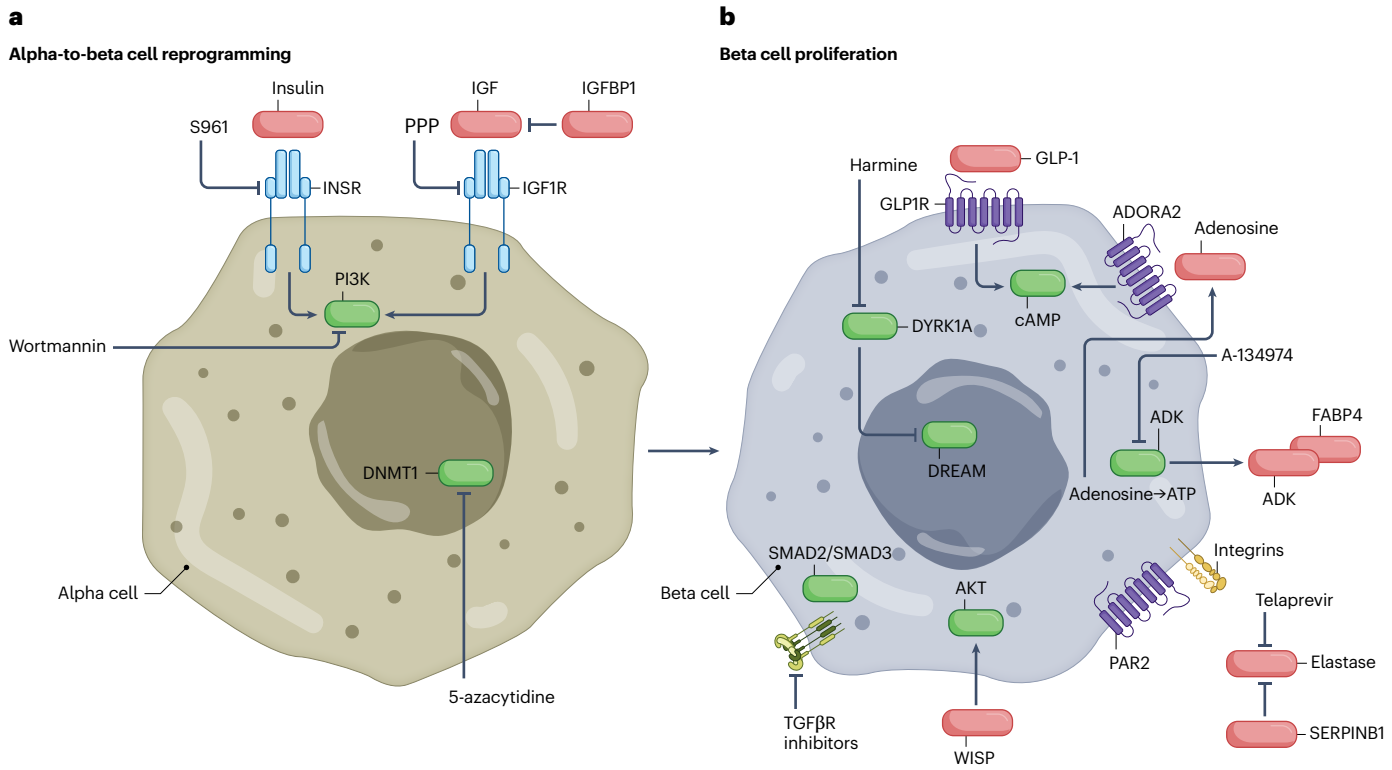

Fig. 2 | Signaling pathways affecting alpha-to-beta cell reprogramming and beta cell proliferation. a, Inhibitors of important signaling pathways in alpha cells stimulating alpha-to-beta cell reprogramming include inhibition of the insulin receptor (INSR), the IGF receptor (IGF1R) and downstream phosphoinositide 3-kinases (PI3K). Additionally, inhibition of DNMT1 alters DNA methylation and enables more efficient reprogramming. IGFBP1, IGF-binding protein 1; PPP, picropodophyllin. **b**, Beta cell proliferation can be stimulated by DYRK1A inhibitors, such as harmine, which disrupt the nuclear DREAM complex. Activation of the GLP-1 receptor (GLP1R) and inhibition of TGFβ receptors (TGFβR), retaining SMAD2 and SMAD3 in the cytoplasm, both stimulate proliferation and synergize with DYRK1A inhibitors. Adenosine receptor (ADORA2) stimulation and ADK inhibition increase beta cell proliferation and can intersect by export of adenosine. ADK can also be secreted and interact with FABP4 to form a hormonal complex increasing beta cell mass. The elastase inhibitor SERPINB1 and small molecules such as telaprevir can act via the mechanosensitive PAR2 and integrin receptors to stimulate beta cell proliferation. WISP can increase beta cell proliferation by activating AKT.

wealth of human islet data available to the research community[122]. The human fetal-derived EndoC-β beta cell line was the first reliable tool to study beta cell proliferation and insulin secretion and is amenable to chemical screens[123,124]. Recently, a superior human beta cell line was generated, EndoC-βH5, which better resembles beta cell biology when compared with EndoC-βH1 (ref. 125). Future studies will highlight its utility for large-scale chemical screens for inducers of beta cell proliferation. Stem cell-derived beta cells are also a potential source to study human beta cell biology. However, despite all the recent breakthroughs to generate mature beta cells (extensively reviewed in ref. 126), these cells fail to recapitulate certain hallmarks of beta cell biology. More targeted differentiation screens for human pancreas biology are needed, such as the small-scale chemical screen that identified factors improving the functionality of these preparations, including the omission of ALK5 inhibition and subsequently the addition of the antiproliferative aurora kinase inhibitor ZM447439 (ref. 127). Overall, new state-of-the-art human culture preparations constitute exciting platforms for the chemical biology field to advance the mechanisms behind and promote human beta cell regeneration and functionality.

Pancreas-selective delivery of drugs

Unbiased approaches coupled with genetically targeted animal models have generated a wealth of information of new pathways that can be exploited to stimulate beta cell proliferation and/or differentiation. Yet, most of these pathways manifest with either unwanted proliferation of other cell types or the proliferation or differentiation threshold achieved is low and not enough to regenerate the original beta cell mass. Selective delivery of potent small molecules to beta cells is a strategy that can be used to eliminate the specificity issues of drugs (Fig. 3). GLP-1 has successfully been used to guide antisense oligonucleotides of *Ddit3* (also known as *Chop*)[128] and estrogen[129] to beta cells, increasing beta cell functionality and reducing ER stress. However, the GLP-1 receptor is also expressed in other tissues (for example, hypothalamus and lung), limiting the specific delivery of drugs conjugated to GLP-1. Taking advantage of the high zinc concentration observed in beta cells, a new zinc-chelating system was recently designed for specifically delivering small molecules to beta cells[130,131]. Adding to the complexity of this discourse, new human beta cell states and subtypes have been described owing to the rapid expansion of single-cell methodologies. Two beta cell subtypes with a transcriptomic signature of proliferation have already been described in the literature and are characterized by the absence of the Flattop protein ($FLTP^-$ beta cells) in mice[132] and the presence of the leukemia-inducible factor (LIF^+ beta cells) in human islets[133]. Designing targeted chemical approaches for new small molecules to modulate the function of proteins within specific beta cell subpopulations could be a fruitful approach to generate inducers of beta cell regeneration with better safety profiles. Antibody-mediated delivery also holds future promise, and the nucleoside triphosphate diphosphohydrolase 3 (NTPDase3)-targeting antibody has been recently discovered as a beta cell-specific reagent that could be used to deliver drugs to beta cells, but no conjugation to small molecules has been reported as of yet[134]. Development of reagents and approaches to ensure targeted delivery of small molecules to beta cells is ongoing and much anticipated.

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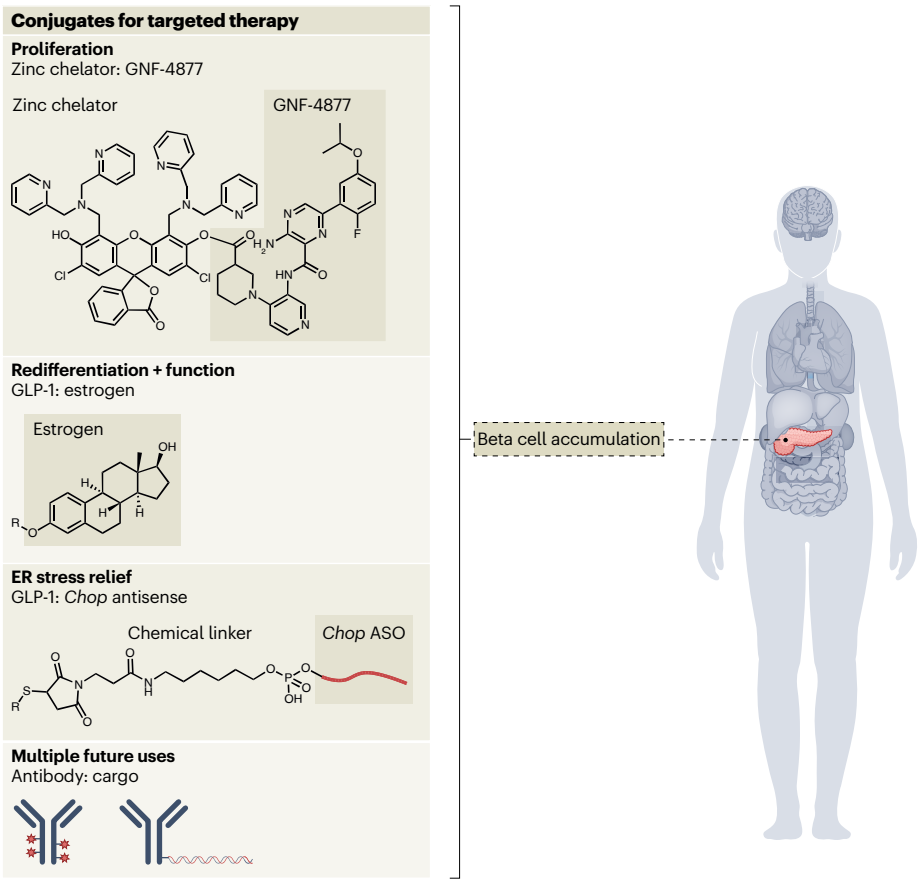

Fig. 3 | Targeted delivery of drugs stimulating different mechanisms of beta cell regeneration. Several concepts have been developed to accumulate drug candidates in the pancreatic compartment, including conjugates with GLP-1 or zinc-chelating groups. For example, the zinc chelator GNF-4877 (called ZnPD5) prodrug was cleverly designed such that hydrolysis only occurs when zinc is bound to the chelate, releasing active cargo. Antibody conjugates have been shown to accumulate in islets, for example, by targeting the beta cell-specific protein NTPDase3. These types of conjugates can stimulate beta cell proliferation, redifferentiation, function or survival (by reducing ER stress) with limited systemic adverse effects. ASO, antisense oligonucleotide.

Hallmarks and sensors for examining beta cell functionality

Beta cell functionality can be assessed through the use of chemical (or chemical-based) sensors and probes and can be incorporated into screening platforms to not only assess beta cell (re)generation but also maturity or maturation. Investigating the expression level of lineage-determining molecular markers, including transcription factors and insulin, has been routinely applied in single-cell studies to classify the beta cell maturation level[135,136] but is insufficient to evaluate function. Mature beta cells need to respond to increases in ambient glucose by secreting insulin, which involves various physiological changes such as transport and metabolism of the sugar, electrical activity and an increase in the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_c$), which triggers exocytosis of insulin secretory granules[137]. Many of these changes can be assessed through the use of chemical (or chemical-based) sensors and probes.

Beta cell metabolism

Cell metabolism can be monitored at a population level using Seahorse extracellular flux assays, which measure oxygen consumption and extracellular acidification rates as a proxy for oxidative phosphorylation and glycolytic rates, respectively. At the single-cell level, the fluorescent properties of native flavine and pyridine nucleotides allow monitoring of flavin adenine dinucleotide ($FADH_2$) and nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) dynamics[138], and fluorescent protein-based sensors such as Perceval(HR) have proven beneficial to study ATP or the ATP/ADP ratio, although sensor signal interpretation is complicated by strong pH dependence[139–141]. Several probes are available that report changes in mitochondrial membrane potential[142–144].

Electrical activity and Ca^{2+} measurements

Direct measurements of electrical activity and the kinetics of specific ion channels require patch-clamp recordings. Recently, such electrophysiological measurements have been combined with single-cell RNA-sequencing analysis, providing improved possibilities to understand functional heterogeneity among individual islet cells[145]. The changes in $[Ca^{2+}]_c$ induced by beta cell electrical activity directly control granule trafficking and fusion and can be used as a proxy measurement for beta cell functionality. Changes in free intracellular calcium $[Ca^{2+}]_i$ are convenient to measure, and a wide array of Ca^{2+} sensors applicable to beta cells are available with different affinities and spectral properties. Low-molecular-weight Ca^{2+} indicator compounds can easily be loaded into cells, while genetically encoded Ca^{2+} sensors offer the advantage to enable expression in specific islet cell populations[146] and also enable recordings of Ca^{2+} signaling patterns in vivo[147]. Glucose-stimulated beta cells exhibit characteristic patterns of synchronized Ca^{2+} oscillations[145], which probably underlie the pulsatile pattern of insulin secretion that characterizes the healthy state of mature beta cells[148,149]. There is heterogeneity in responses among individual cells in terms of Ca^{2+} signals and secretion[150], but whether there are molecularly distinct cell subpopulations with pacemaker properties, referred to as hub cells[150] or leader cells[147], remains debated[151,152].

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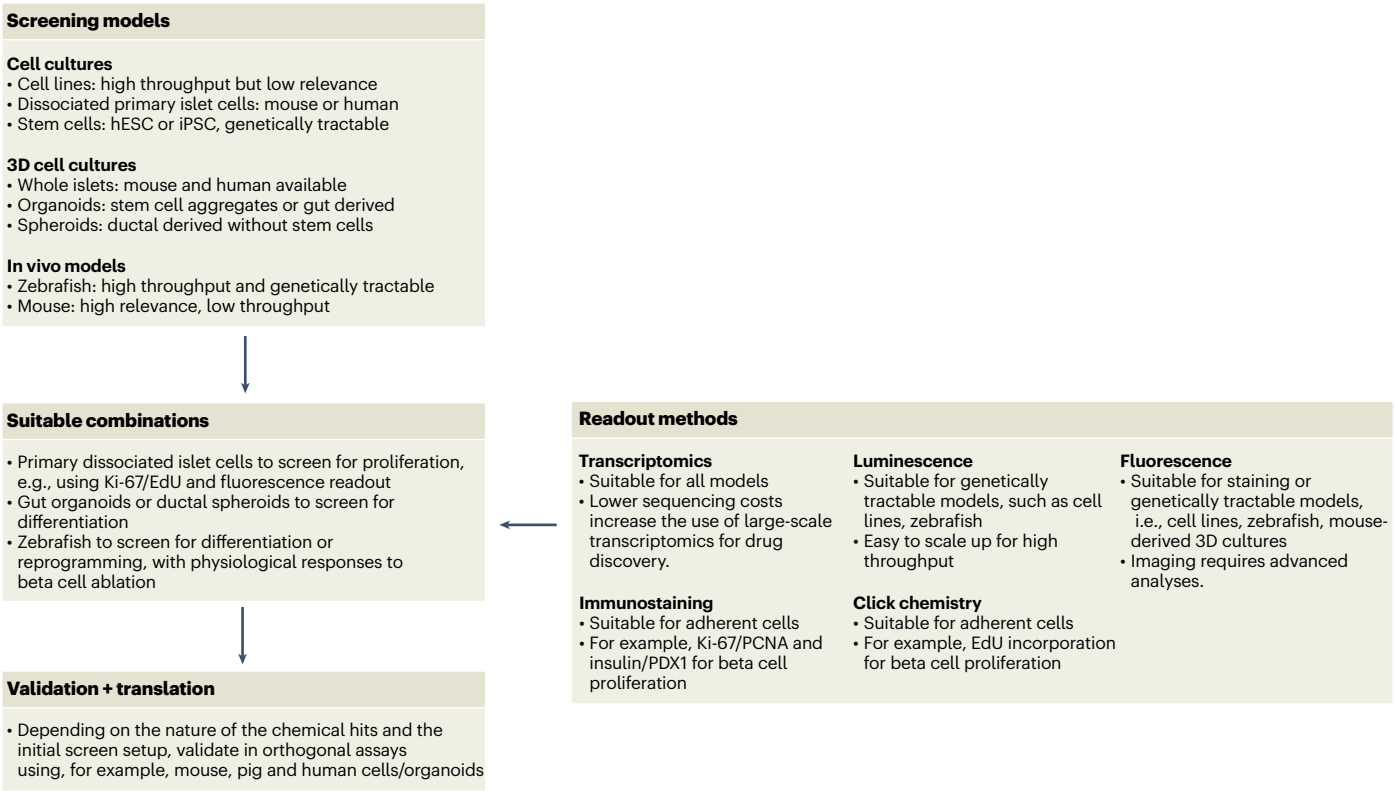

Fig. 4 | Plausible screening pipelines for chemicals stimulating beta cell regeneration. A number of aspects should be taken into account when designing chemical screens for beta cells, including selection of screening models, type of readout and suitable combinations thereof as well as orthogonal assays for validation and translation. EdU, 5-ethynyl-2′-deoxyuridine; hESC, human embryonic stem cell; iPSC, induced pluripotent stem cell; PCNA, proliferating cell nuclear antigen; PDX1, pancreatic and duodenal homeobox 1.

Metabolic coupling factors and cyclic AMP

The consensus model that ATP from oxidative phosphorylation controls K_{ATP} activity and oscillations in $[Ca^{2+}]_i$ has recently been challenged in favor of a model in which phosphoenolpyruvate cycling is caused by anaplerotic mitochondrial pyruvate metabolism and ATP generation via pyruvate kinase reaction[153]. Irrespective of the source of ATP causing K_{ATP} channel closure and membrane depolarization, the metabolism of glucose generates additional signals that amplify Ca^{2+}-dependent exocytosis[154]. The presence of a robust metabolic amplification of insulin secretion is characteristic of mature beta cells and easy to demonstrate by detecting the secreted hormone (see below). Mature beta cells are also characterized by the ability to generate cyclic AMP (cAMP), a second messenger with a strong amplifying action on Ca^{2+}-triggered exocytosis[155]. Recordings of beta cell cAMP signals have mainly been made with sensors based on translocation or fluorescence resonance energy transfer[155], but the list of available tools is continuously growing.

Insulin secretion

The signaling evoked by glucose, hormones and neural factors eventually triggers insulin secretion. Mature beta cells are characterized by low insulin secretion below the stimulation threshold for glucose (~6 mM in the mouse and somewhat lower in humans) and at least an order-of-magnitude higher secretion rate at high glucose concentrations[156]. While secretion from intact islets can be detected with immunoassays, optical sensors or electrophysiological techniques are required to monitor secretion with single-cell sensitivity. Capacitance recordings allow the detection of single-granule release and estimation of total exocytosis capacity[157]. Imaging of granules with fluorescent-labeled cargo or membrane proteins has provided important insights into mechanisms of granule recruitment, docking and priming and defects associated with human type 2 diabetes[158]. Extracellular fluorescent markers[159] and zinc probes[150,152] have been used to obtain spatial information on exocytosis. These tools help to distinguish immature and beta-like cells from mature bona fide beta cells on the basis of proper insulin secretion kinetics.

Conclusions and future perspectives

Endogenous beta cell regeneration is an attractive, yet challenging, approach to develop potential new therapies for patients suffering from diabetes. Possible advantages of induced beta cell regeneration over stem cell-derived beta cell implantation could be the likely lower cost as well as avoidance of immunosuppressant regimens, carcinogenicity, rejection and/or risk of insufficient encapsulation of the implant. While there have been many advances in small-molecule development to find compounds that induce beta cell regeneration, certain limitations remain to be overcome before it is a viable treatment option. First, ideal inducers of beta cell proliferation need to be specific and stimulate a sufficient level of proliferation that can prevail over the rate of beta cell depletion. Unfortunately, our current understanding of the hindrance to beta cell proliferation is not sufficient to identify new ways to solve this problem. Second, directed cell reprogramming is limited to low rates and is often dependent on the precise delivery of genes (predominantly encoding transcription factors) to the correct cell type[51,52]. Delivery of the necessary transcription factors to the correct cell type would be the next logical step for this approach to be a viable potential therapeutic. Third, further study in various model organisms and cultures of human tissue and organoids is necessary to explore the full potential of a possible evolutionarily conserved neogenic niche in the adult human pancreas, that is, as there is limited beta cell neogenesis in the mouse models that are commonly used[16].

In this regard, the zebrafish is a suitable model to study neogenesis, and scattered evidence shows that porcine pancreatic ductal cells can differentiate into beta cells in vitro. Fourth, it is important to further understand the heterogeneity of the different pancreatic cell populations and their involvement in homeostasis and disease, which is made possible with recent technological advancements in transcriptional and protein profiling.

Chemical biology can provide answers to some of the above questions. Advances in the field of chemical sensors for various metabolic processes will open new avenues to screen and validate small molecules for diabetes treatment. Establishing an automated pipeline for chemical screens using in vitro organoid systems will also expand the repertoire of human-relevant systems for beta cell regeneration. Furthermore, simultaneous in vivo drug screens for more than one phenotype, for example, using a combination of beta cell regeneration and functionality with the use of Ca^{2+} sensors can speed up the translational drug discovery pipeline. Lastly, it is important that screening efforts not only focusing on identification of novel small molecules expanding functional beta cell mass but also aim to repurpose authority-approved drugs or natural products for the disease. This will be feasible with screening regulatory authority-approved chemical libraries as well as targeted screening approaches of known bioactive chemicals or probes for molecular pathways involved in the processes. Depending on which regenerative mechanism to target, there are several viable combinations of screening models and readouts that should be considered for high throughput and relevance (Fig. 4).

Although insights into how the pancreas develops in vivo (gleaned mainly from studies in mutant mice) have informed current protocols for deriving beta cells from stem cells in vitro, any induction of beta cell regeneration in vivo will ultimately need to meet the bar set by stem cell-derived beta cell therapies in humans. (Re)generation of beta cells from endogenous progenitors also comes with certain challenges. First, we need to address the functionality of the newly formed beta cells using rigorous in vitro and in vivo biochemical characterization, going beyond the traditional molecular profiling performed with omic technologies. Second, we need to assess the vulnerability of the new beta cells to cell death and/or dedifferentiation[85]. In type 1 diabetes, we need to assess the immunogenicity and cell death of the newly formed beta cells. What volume of beta cells needs to be regenerated, and will immunosuppression or engineered immunoevasion be required for newly generated cells to survive? In type 2 diabetes, we also need to assess dedifferentiation, as it is thought to be a larger issue than beta cell death[85]. Answers to these questions are needed before a clinically relevant treatment becomes possible.

In summary, despite the scientific advances in the cellular and molecular mechanisms of beta cell regeneration, there are high requirements for regenerative or replacement options to make an impact, as they need to be safe and outperform current delivery systems of insulin that often give sufficient treatment of diabetes for decades. A more attainable goal is therapy that preserves the remaining beta cell mass in type 1 diabetes, indicated by the recently approved teplizumab and drug candidates in trials (for example, verapamil)[160]. This is encouraging for the future, as a combination of a regenerative therapy with a preserving therapy may reach the goal of becoming disease modifying.

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