

## REVIEW

# Targeting β-cell dedifferentiation and transdifferentiation: opportunities and challenges

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## Abstract

The most distinctive pathological characteristics of diabetes mellitus induced by various stressors or immune-mediated injuries are reductions of pancreatic islet β-cell populations and activity. Existing treatment strategies cannot slow disease progression; consequently, research to genetically engineer β-cell mimetics through bi-directional plasticity is ongoing. The current consensus implicates β-cell dedifferentiation as the primary etiology of reduced β-cell mass and activity. This review aims to summarize the etiology and proposed mechanisms of β-cell dedifferentiation and to explore the possibility that there might be a time interval from the onset of β-cell dysfunction caused by dedifferentiation to the development of diabetes, which may offer a therapeutic window to reduce β-cell injury and to stabilize functionality. In addition, to investigate β-cell plasticity, we review strategies for β-cell regeneration utilizing genetic programming, small molecules, cytokines, and bioengineering to transdifferentiate other cell types into β-cells; the development of biomimetic acellular constructs to generate fully functional β-cell-mimetics. However, the maturation of regenerated β-cells is currently limited. Further studies are needed to develop simple and efficient reprogramming methods for assembling perfectly functional β-cells. Future investigations are necessary to transform diabetes into a potentially curable disease.

### Key Words

- ▶ dedifferentiation
- ▶ transdifferentiation
- ▶ β-cell
- ▶ diabetes

*Endocrine Connections*  
(2021) **10**, R213–R228

## Introduction

Diabetes mellitus (DM) is an epidemic chronic disease characterized by impaired glucose homeostasis, leading to hyperglycemia and multiple complications such as cardiopathy, neuropathy, nephropathy, and retinopathy. The prevalence of DM has increased dramatically in recent decades and is projected to rise to 642 million people by 2040 (1), with profound impacts on quality of life, demands for health services, and economic costs (2). There are two common syndromes, type 1 (T1D) and type 2 diabetes (T2D), characterized by an absolute or relative deficiency of β-cells, respectively (3). A previously held belief stipulated that the fate of fully differentiated β-cells is fixed and that gradual β-cell death due to glucotoxicity was

a final outcome of DM (4). Treatment ultimately depended on supplemental insulin and islet transplantation, which alleviated disease severity by reducing or normalizing glycemic levels without curing DM (4). Long-term insulin treatment carries risks of hypoglycemic episodes, weight gain, and an increased incidence of cancer (5). The effectiveness of islet transplantation is limited by a shortage of donor islets and immune rejection (6). Therefore, new therapeutic strategies are urgently needed to prevent and treat this highly prevalent metabolic disorder.

The study of β-cell maturation and physiology demonstrates the heterogeneity and plasticity of mature β-cell phenotypes and function (7). The three main β-cell

phenotypes in diabetes are dedifferentiated, senescent, and transdifferentiated types (8). Glucotoxicity leads to  $\beta$ -cell dedifferentiation during hyperglycemia and reduces the expression of  $\beta$ -cell enrichment genes such as key transcription factors and genes that encode insulin; glucose metabolism, protein processing, and secretory pathways; as well as upregulation of genes that are suppressed or expressed at low levels in normal  $\beta$ -cells, including forbidden and progenitor cell genes (9). Under various stress conditions, mature  $\beta$ -cells may lose their differentiated phenotypes and return to a less differentiated or even a progenitor cell state.  $\beta$ -cell dedifferentiation is a potential adaptive mechanism to escape cell death during physiologic stress (10). Exploring the mechanisms of dedifferentiation may inform new strategies for the reversal of dedifferentiation and the restoration of  $\beta$ -cell functionality. We focus on the possible mechanisms of hyperglycemia-induced dedifferentiation that may result from the cascade of metabolic, oxidative, and endoplasmic reticulum stresses; and on epigenetic changes due to chronic stress. We also review current antidiabetic strategies and potential future research directions that may identify a time window for alleviating the  $\beta$ -cell stress response; thus preserving  $\beta$ -cell mass, regaining cell maturation, improving cellular function, and delaying disease progression.

Current research is focused on methods to increase  $\beta$ -cell numbers, maturity, function, and post-transplantation survival in addition to protecting existing  $\beta$ -cells, thus providing potential breakthroughs in the treatment of DM (11).  $\beta$ -Cell deficiency could be reversed by promoting cellular replication and redifferentiation during the early stages of DM (12). However, the application of these methods has been limited due to low  $\beta$ -cell proliferation rates, instability, and high heterogeneity. Transdifferentiation (13), defined as the phenotype switch between different cell types, obviates the shortcomings of the aforementioned methods and provides a safe and efficient approach to regeneration. The focus herein is on the eventual differentiation of non- $\beta$ -cells into a  $\beta$ -cell phenotype. At present, most studies focus on the transdifferentiation of pancreatic non- $\beta$ -cells; hepatic and biliary cells; gastrointestinal cells into  $\beta$ -cells through genetic programming; cytokines; and small molecules. These cells comprise the leading candidates because of their common endodermal origin with  $\beta$ -cells, abundant populations, and high conversion efficiency. The development of bionic technology offers an expanding range of options for  $\beta$ -cell regeneration.

Benefiting from excellent reviews of this field, we focus on the state of knowledge of  $\beta$ -cell dedifferentiation and

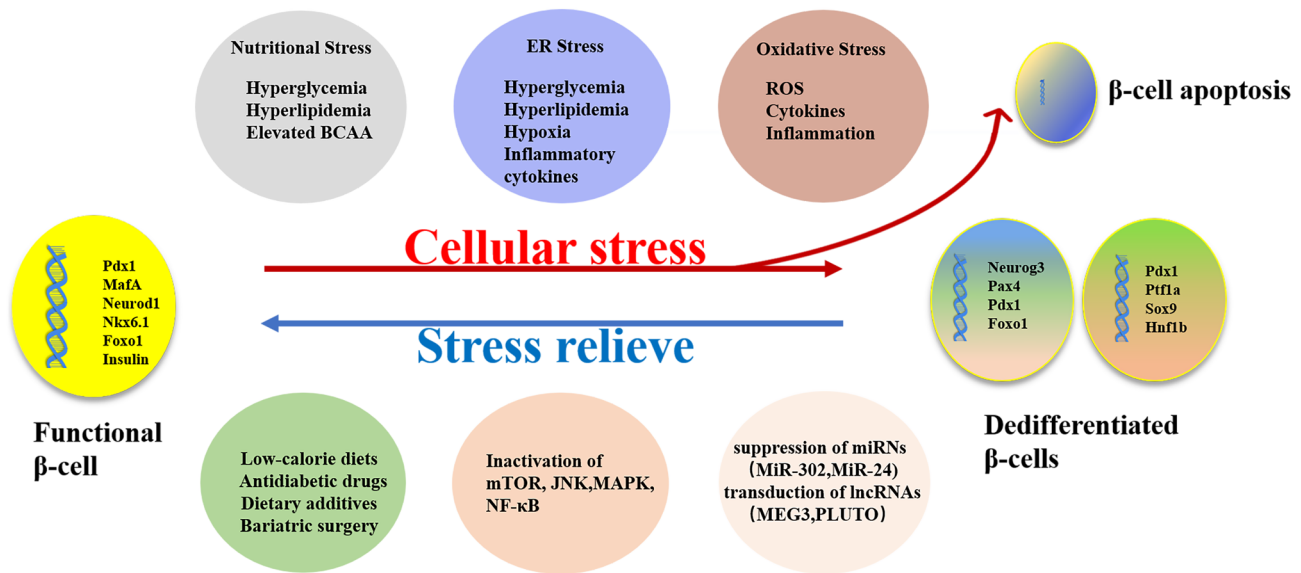
transdifferentiation, as well as the highlights of exciting new research. Optimized protocols to augment functional mature  $\beta$ -cells will guide future precision medicine studies of improved treatment strategies for patients with DM and may even result in potential cures.

## $\beta$ -Cell dedifferentiation

Stress response is an adaptation to environmental changes. Moderate stress responses can induce effective adaptation strategies to improve survival, while an excessive stress response will cause stress injury, leading to the onset and development of a variety of organic and psychological diseases, including DM. A growing body of evidence has shown that the onset and development of DM is closely related to metabolic, oxidative, and endoplasmic reticulum stresses and to epigenetic changes caused by chronic stress. Both T1D and T2D present with a loss of  $\beta$ -cell mass and identity that ultimately impair insulin secretion. The current view is that  $\beta$ -cell loss during the development of DM may be related to  $\beta$ -cell dedifferentiation rather than apoptosis, because the relatively low rate of apoptosis may not fully explain the loss of  $\beta$ -cell mass.  $\beta$ -cell dedifferentiation is characterized by decreased expression of specific genes that maintain the characteristics and function of mature  $\beta$ -cells and by the increased expression of endocrine precursor cell genes and additional genes that are expressed at low levels in normal  $\beta$ -cells. In this section, we discuss the various stresses and mechanisms that trigger dedifferentiation, as well as the currently available methods to inhibit  $\beta$ -cell dedifferentiation (Figs 1 and 2).

## Nutritional stress

The effects of glucose, lipid, and amino acid metabolism on pancreatic  $\beta$ -cells have received increasing attention (14).  $\beta$ -Cells gradually change from their initial adaptive stage to develop metabolic disorders and apoptosis (15). This process was previously ascribed to glucotoxicity, lipotoxicity, glucolipotoxicity, and metabolic stress; however, these proposed etiologies could not accurately reflect the state changes of  $\beta$ -cells in the processes of mixed-nutrient energy balance and imbalance. At present, nutritional stress is considered the most appropriate terminology (16). Transitory metabolic imbalances stimulate insulin synthesis and release (17). Once the balance is broken, usually in the context of a poor lifestyle, long-term overnutrition, and aging,  $\beta$ -cells confront chronic and persistent insults that are aggravated by the individual's

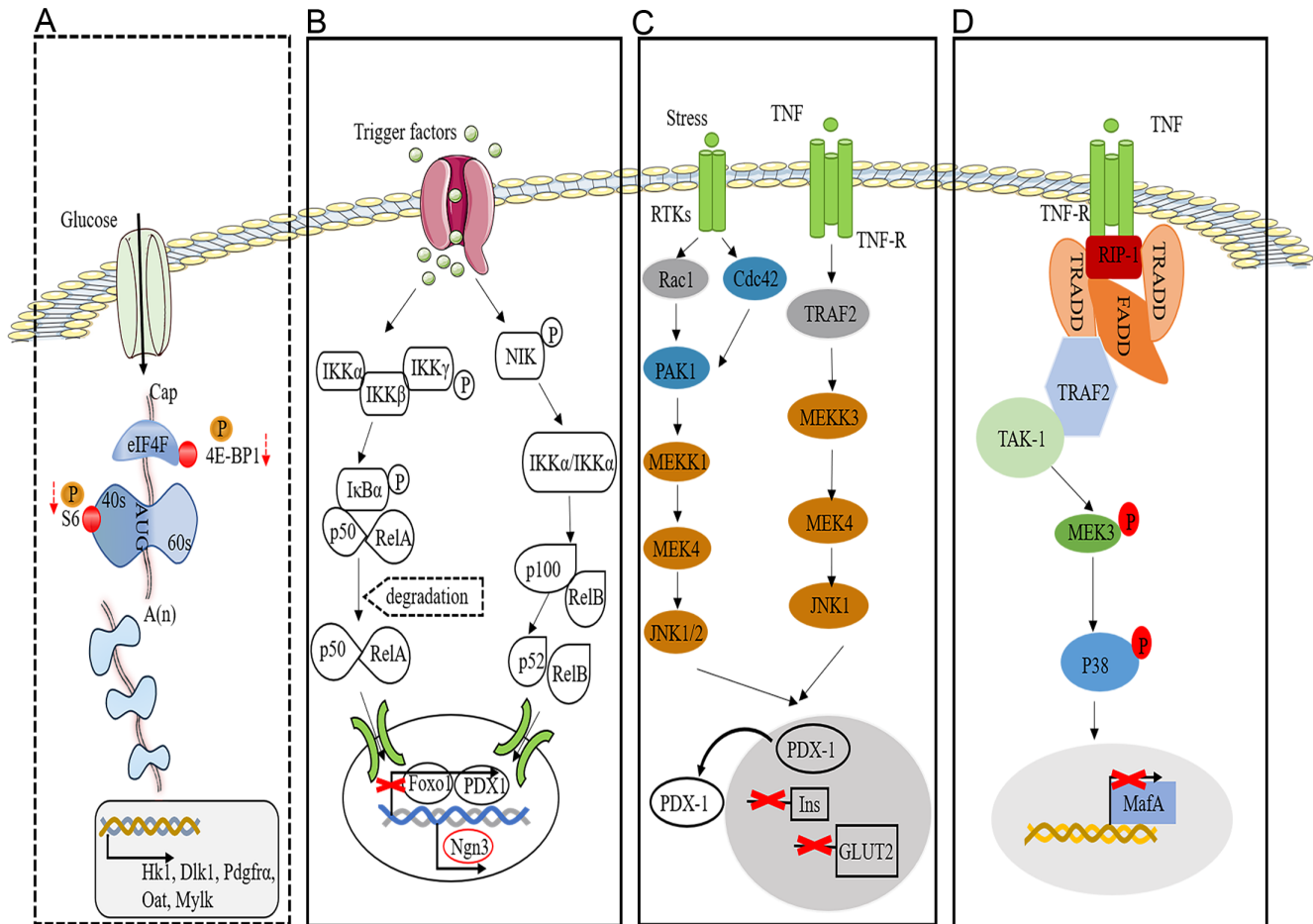


**Figure 1**  
β-Cells lost the mature phenotype under various stressors and dedifferentiated as an adaptive response to avoid apoptosis.

genetic and epigenetic composition (16). Chronic metabolic stress can inhibit transcription factors such as MAF BZIP transcription factor A (*MafA*), pancreatic and duodenal homeobox 1 (*PDX-1*), neuronal differentiation 1 (*NeuroD1*), and insulin gene expression, thus causing β-cell dysfunction and failure, leading to T2DM (18).

In rodent and human pancreatic islets, β-cells induced by high-glucose concentrations exhibited cellular dysfunction, decreased insulin secretion, glucose-stimulated insulin secretion (GSIS), and expression of mature β-cell genes; while expressing progenitor or precursor cell-related genes (19). Elevated glucose levels and the duration of hyperglycemia are the main factors affecting β-cell dedifferentiation (20). Zinc deficiency induced by hyperglycemia may reduce the expression of key β-cell transcription factors *MafA*, paired box 6 (*Pax6*), and NK2 homeobox 2 (*NKX2.2*) and promote β-cell dedifferentiation (21). β-cells (MIN6) cultured in a high-glucose environment exhibited reduced expression of vitamin D receptor (VDR). Vitamin D3 treatment can prevent β-cell dedifferentiation and increase the expression of genes encoding essential transcription factors such as *Pdx1*, *MafA*, and VDR. Notably, the expressions of insulin 1 (*Ins1*) and insulin 2 (*Ins2*) are also increased (22). β-Cell absorbed free fatty acids (FFA) (including triglyceride hydrolyzed by lipoprotein lipase), very low-density lipoprotein, and low-density lipoprotein produced by endocytosis attenuate glucose toxicity (22). Thus, circulating lipid levels appear to influence not only glucose toxicity but also β-cell adaptation to hyperglycemia. Hyperglycemia

and hyperlipidemia interact to impair β-cell function (23, 24). Circulating glucose and lipid levels are elevated prior to the onset of obesity-related T2D, so a reasonable suggestion is that excessive levels of these two nutrients are pathogenic. In the context of overnutrition associated with T2D, disorders of lipid homeostasis associated with hyperlipidemia and hypercholesterolemia, and often elevated plasma FFA, precede the onset of T2D in both human clinical experience and in rodent models. Multiple *in vitro* studies using β-cell lines (INS, MIN6, or HIT cells) or isolated rodent and human islets have shown synergistic toxicity of elevated glucose and FFA on β-cell function and survival (25, 26). A recent 6-year Canadian follow-up study showed a strong negative association between total plasma FFA levels and β-cell function (27). Similarly, another study showed that elevated plasma FFA is strongly associated with decreased β-cell function in children and adults and impaired insulin secretion rather than insulin sensitivity (28). Reduced *in vivo* and *in vitro* GSIS is associated with changes in the glyceride/fatty acid cycle (29, 30, 31, 32, 33, 34). In lipid-treated MIN6 cells, inhibition of *ID1* expression can reduce the expression of islet stress genes and increase insulin secretion. *ID1* is a negative regulator of insulin secretion, and its expression plays a crucial role in the etiology of β-cell dedifferentiation under conditions of glucose intolerance, insulin secretion dysfunction, and increased lipid load, providing a molecular link between chronic lipid-induced damage and β-cell dedifferentiation and dysfunction (35). None of these models demonstrated β-cell apoptosis or reduced cell mass, suggesting that β-cell



**Figure 2**

Mechanisms of β-cell dedifferentiation. (A) Reduced expression of raptor-induced suppression of the mTORC1 signaling pathway increased the expressions of β-cell-specific disallowed genes (e.g. *Hk1*, *Dlk1*, *Pdgfra*, *Oat*, and *Mylk*). (B) Stress is a major contributor to β-cell dedifferentiation via activating NF-κB signaling, which compromises β-cell identity and thus decreases insulin secretion. (C) The JNK pathway is activated under diabetic conditions such as stress and cytokine release, accompanied by *Pdx1* nuclear translocation and suppression of insulin and *GLUT2* gene expression. (D) The p38 MAPK pathway mediates the degradation of endogenous *MafA* during hyperglycemia. The dotted and solid boxes represent signaling pathway inhibition and activation, respectively. 4E-BP1, 4E binding protein 1; eIF4F, eukaryotic initiation factor 4F; Hk1, hexokinase 1; Oat, ornithine aminotransferase; Pdgfra, platelet-derived growth factor receptor α; Mylk, myosin light chain kinase; IKK, IκB kinase; IκB, nuclear factor-kappaB inhibitor alpha; NIK, NF-κB inducing kinase; RTK, receptor tyrosine kinase; TNF, tumor necrosis factor; TRAF2, TNF receptor-associated factor 2; RIP1, receptor-interacting protein1; MEK, MAPK kinase; MEKK, MEK kinase; TRADD, TNFR1-associated death domain-containing protein; FADD, Fas-associated death domain-containing protein; TAK-1, TGF-beta-activating kinase 1.

dysfunction, rather than cell death, promotes the onset of DM (36). The effects of glucose and lipids on β-cells in different experiments were discriminating. Another study suggested that β-cell dedifferentiation could be induced in lipotoxic conditions with or without hyperglycemia (37). This may be related to the composition and quantity of FFA. Nutritional stress in β-cells may result not only from glucose and lipids but also from amino acids, especially branched-chain amino acids (BCAA), including leucine, isoleucine, and valine. These three amino acids, as well as tyrosine and phenylalanine, were increased while glycine was decreased in obese hyperinsulinemic patients (14, 38, 39). Elevated plasma levels of BCAA and aromatic

amino acids (tyrosine and tryptophan) are associated with obesity, insulin resistance, and susceptibility to T2D. The β-cell dysfunction induced by disordered amino acid metabolism is possibly mediated through the continued activation of the mammalian target of rapamycin (mTOR) signaling and consequent mitochondrial dysfunction (40).

An increasing body of evidence suggests that reduced nutritional stress can improve β-cell function (37). Low-calorie diets (41), hypoglycemic drugs (42), dietary additives (43), or bariatric surgery (44, 45) have led to diabetic remission and/or improved insulin secretion in a significant proportion of T2D patients. We suggest that in

most cases, these benefits are derived from the reversal of pancreatic β-cells dedifferentiation (37, 46).

### Endoplasmic reticulum stress

Endoplasmic reticulum (ER) stress is caused primarily by reactive oxygen species (ROS) accumulation, toxic substances, and genetic mutations. Stressors usually originate from changes in the cellular internal environment and include protein misfolding, aggregation of misfolded proteins, calcium homeostasis disorders, and other ER dysfunctions (47). In prediabetes, complexes of misfolded proteins accumulate in the ER of β-cells, suggesting that the disruption of ER balance is an early event in the development of T2D (48). There is solid evidence that ER stress contributes to β-cell dysfunction in both T1D and T2D (49, 50, 51). In the context of DM, chronic ER stressors such as hyperglycemia, hyperlipidemia, hypoxia, and pro-inflammatory cytokines (TNF-α, IL-1) lead to the gradual loss of β-cell-specific transcription factors, including *Pdx1*, *MafA*, and forkhead box O1 (*FoxO1*), and the acquisition of endocrine progenitor cell markers such as neurogenin3 (*Ngn3*) and octamer-binding transcription factor 4 (*Oct4*) (52, 53, 54). In this setting, ER homeostasis will collapse and initiate the unfolded protein response (UPR). The UPR is a protective mechanism that maintains the balance between synthesis and degradation; supports correct folding and function; sustains protein homeostasis, or proteostasis (55). Many factors in pancreatic β-cells can disrupt the UPR balance to trigger ER stress. These include genetic mutations, cytokines, infections, excess nutrients, islet amyloid polypeptide (IAPP), and insulin resistance (IR) and can result in DM (50, 56, 57, 58). This disruption of the adaptive UPR promotes diabetic progression and β-cell dedifferentiation (59). Combinatorial signals from the three core components of the UPR (protein kinase R-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6)) initially trigger transcriptional programs that upregulate genes encoding many of the aforementioned ER-resident protein-folding machines that play important roles in insulin biosynthesis (60, 61). Consequently, further research is needed to clarify which steps of the ER stress process are easily targeted, because activation of key proteins of the UPR may be beneficial in the treatment of T2D (62). In fact, some of the therapeutic effects of drugs currently used to treat DM may stem from their ability to regulate ER stress, target protein folding, and modulate the UPR signaling pathway. These processes may provide

targets for future drug candidates such as chemical chaperone molecules 4-phenylbutyric acid and taurine-deoxycholic acid to promote correct protein folding and cellular function (63, 64).

### Oxidative stress

Oxidative stress and metabolic disorders are considered the two most important pathogenic factors of insulin secretion disorder (65). The development of T2D results from a variety of cellular changes associated with oxidative stress and impaired redox signaling, usually caused by lipotoxicity and glucotoxicity due to continued overfeeding (66). Oxidative stress is not only closely related to lipotoxicity and glucotoxicity but is also associated with inflammation, ER stress, hypoxia, and mitochondrial damage in the promotion of β-cell dedifferentiation (66, 67, 68). Oxidative stress impairs pancreatic β-cell maintenance and function (69), which is widely believed to be associated with the onset of DM and diabetic complications. The consequent increase of oxidized biological components leads directly not only to pathologies such as inhibited insulin secretion but also to the induction of new cellular responses, namely programmed cell death, i.e., apoptosis. These phenomena occur in parallel after cumulative oxidative stress reaches a certain threshold (66). In T1D, ROS promote autoimmune responses, cytokine release, and inflammation-induced impairment of β-cell function (70). Drews *et al.* suggested that oxidative stress-mediated loss of cellular function (e.g. impaired secretion and increased insulin resistance) plays an important role in the pathogenesis of both T1D and T2D (36). Oxidative stress-related β-cell dysfunction induces cytoplasmic transposition and inactivation of transcription factors *MafA*, *NKX6.1*, and *Pdx1* in the islets of T2D patients (54, 71). The expression of oxidative stress-related genes increased the release of pro-inflammatory cytokines and upregulated the expressions of pancreatic progenitor cell-specific transcription factors such as SRY-Box transcription factor 4 (*SOX4*), SRY-Box transcription factor 9 (*SOX9*), inhibitor of DNA binding (*ID2*), and vimentin in islet cells cultured for 3 days. These findings indicated that cells dedifferentiated under oxidative stress *in vitro* (72). Oxidative stress leads to dephosphorylation and inactivation of these pathways by stimulating the activity of phosphatases such as protein tyrosine phosphatase 1B (PTP1B) and SH2-containing tyrosine-protein phosphatase, thus inhibiting insulin effect (73). Free radicals not only have the aforesaid direct effects but can also indirectly activate various intracellular signaling

pathways such as mTOR, nuclear factor-kappa b (Nf-κB), p38 mitogen-activated protein kinases (p38 MAPK), stress-activated protein kinase/c-Jun NH (2)-terminal kinase (JNK/SAPK), hexosamine pathways, protein kinase C (PKC), and advanced glycation end product/receptor for AGE (AGE/RAGE) interaction (74). The inactivation of mTOR signaling reduced β-cell mass and insulin secretion. The mTOR complex 1(mTORC1) was suppressed under oxidative stress, suggesting that the mTOR pathway has a potential role in β-cell dedifferentiation under oxidative stress (75, 76). p38 inhibition can reduce β-cell apoptosis and protect cellular function (77). Administration of antioxidants to β-cells may restore free radical scavenging potential and reduce oxidative stress, thereby increasing *PDX-1*, *Ins-1*, *ngn3*, *GLUT*, and *IRS-1* expressions, thus promoting β-cell regeneration and subsequent pancreatic insulin release (78). Oxidative stress in the setting of diabetes activates the JNK pathway, which impairs insulin signaling, thereby increasing *IRS1* serine phosphorylation and decreasing both *IRS1* tyrosine phosphorylation and *IRS1*-associated PI3K activity. JNK activation reduces the phosphorylation of *FoxO1*, which in turn inhibits the expression of the insulin transcription factor *Pdx1*, thus lowering insulin levels and ultimately impairing β-cell function (79, 80). Activation of the p38 MAPK and JNK signaling pathways contributes to β-cell dysfunction in the pathogenesis of T2DM (81).

Commonly used antidiabetic drugs include metformin, thiazolidinediones, α-glucosidase inhibitors, insulin, glucagon-like peptide-1 (GLP-1) receptor agonists, dipeptidyl peptidase-4 inhibitors, and sodium-glucose cotransporter type 2 inhibitors (SGLT2is); which may enhance the regulation of adaptive responses to multiple stressors. Metformin has direct and indirect antioxidant and anti-inflammatory properties, inhibits the PERK/CHOP signaling pathway, reduces the ER stress response, and also prevents lipotoxic β-cell apoptosis (82, 83). Pioglitazone attenuates β-cell oxidative stress, inflammation, and ER stress by inhibiting Nf-κB activation (84). Rosiglitazone prevents oxidative stress by regulating Nf-κB activity through a PPARα-dependent mechanism (85). Exendin-4 augments cellular defenses by inducing the ER chaperone BIP and the anti-apoptotic protein JUNB (86), and also prevents β-cell dysfunction and apoptosis by inhibiting the activation of JNK and p38 MAPK signaling (87, 88). SGLT2i attenuates the oxidative stress mediated by AGE-RAGE (89). Drugs that are currently used to treat diabetes exhibit excellent performance in stabilizing glycemic levels; however, the morbidity caused by diabetic complications

obliges the development of novel alternatives and the creation of new preventive protocols for patients at high risk of insulin resistance (24, 63).

The discovery of safe and effective antioxidants could yield novel therapeutic options for the treatment of DM. Significantly, vitamin E, contained in nuts, reduces cellular oxidation by reacting with lipid radicals produced in the lipid peroxidation chain. Vitamin C, obtained from green leafy vegetables and fruits, acts synergistically with vitamin E to quench ROS through antioxidant activity (90). Several herbal derivatives (i.e. curcumin, cinnamon, garlic, and resveratrol) may have potential roles in maintaining β-cell function and inhibiting oxidative injury through their antioxidant properties (90).

In addition, the interaction of multiple cellular stressors induces epigenetic changes that can disrupt cellular function and trigger β-cell dedifferentiation. DNA methylation, histone modification, and noncoding RNA (ncRNA)-mediated gene regulation are examples of epigenetic mechanisms (91). A role of ncRNAs in epigenetic inheritance has been suggested recently (91). The function of ncRNAs, including miRNAs and long noncoding RNAs (lncRNAs), in β-cell dedifferentiation, has attracted increasing attention (91). lncRNA is abundant in β-cells and plays a vital role in differentiation (92). β-cell-specific lncRNAs interact with transcription factors to orchestrate transcription networks. lncRNAs could represent therapeutic targets to mitigate β-cell dysfunction. *MiR-24* may trigger β-cell dedifferentiation (93). Ectopic *MiR-24* expression in Min6 cells and primary islets increases *Ngn3* and *SOX9* expression and also inhibits its direct target *Ire1α*, which consequently reduces *XBPI* and *ATF4* expressions. *MiR-302* upregulation simultaneously suppresses the expression of several β-cell identity genes such as *NeuroD1*, peroxisome proliferator-activated receptor α (*PPARα*), and lysine acetyltransferase 2B (*Kat2B*), suggesting a role of *MiR-302* as a therapeutic target to prevent β-cell dedifferentiation (94). Defects in β-cell-specific lncRNAs cause DM in humans (95). MEG3, an lncRNA associated with normal β-cell function, acts as a unique controller to decrease *MafA* and *Pdx-1* expressions. A crucial mechanistic role of *PLUTO* in preventing β-cell dedifferentiation is highly likely (9). *PLUTO* promotes *Pdx1* expression by facilitating binding between the *Pdx1* promoter and its enhancer cluster (95). Novel lncRNAs will probably be characterized in the near future to further elucidate their regulation of pancreatic development and β-cell function (96). The role of lncRNAs in β-cell dedifferentiation is a fascinating research topic that deserves further exploration.

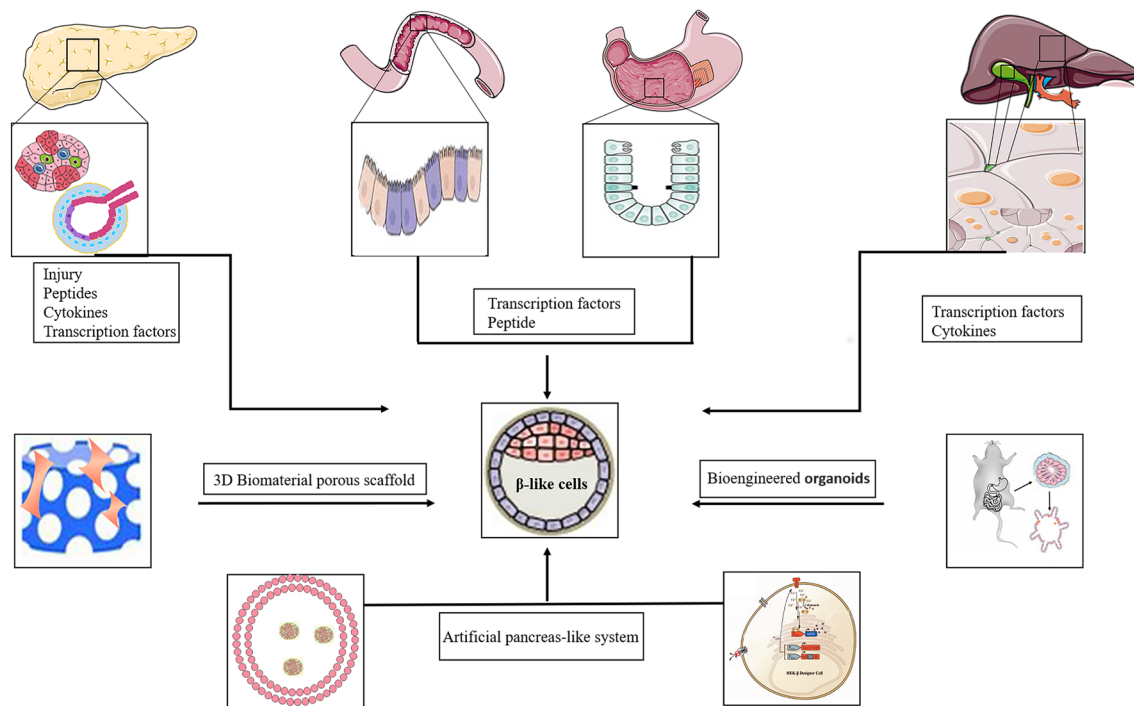
Although existing therapies improve glycemic control effectively, the increasing prevalence of serious diabetic complications suggests that targeting existing β-cell populations is inadequate. The exploitation of new resources to produce β-like cells may generate solutions to replenish depleted β-cells. Transdifferentiation offers a promising option.

### β-Cell transdifferentiation

Cellular transdifferentiation, also known as lineage reprogramming, can be used to regenerate β-cells (97). Since hepatic, gastrointestinal, and pancreatic exocrine cells are derived from common endodermal progenitor cells, the transdifferentiation of developmentally related cells into β-cells can be easily accomplished. Because of identical developmental transcription mechanisms, similar epigenetic landscapes, and unique locations of endogenous cells, only a small portion of the epigenome needs to be rearranged, thus providing an attractive process for cellular reprogramming (98). The efficacy of particular technical strategies and the feasibility of using specific cell types for transdifferentiation are key questions that have been explored in exciting new studies of pancreatic β-cell regeneration (11) (Fig. 3 and Table 1).

### Transdifferentiation of pancreatic non-β-cells to β-cells

Pancreatic non-β-cells, such as α, δ, acinar, and duct cells, share developmental histories and have similar epigenetic profiles. Because they may share common pathways during transdifferentiation into β-cells (97), we summarize their processes here. α-cell to β-cell transdifferentiation was observed upon β-cell loss in mice from puberty to adulthood (13). Near-total *in vivo* ablation of β-cells can be induced by diphtheria toxin (99), pancreatic duct ligation (PDL), and partial pancreatectomy (PPX) (98). PDL and PPX promote β-cell transdifferentiation not only from α-cells but also from duct (100) and acinar cells (101) in murine models. However, cell reprogramming is technically difficult. Current transdifferentiation strategies lack uniform methods. Efficiencies are usually low, and reprogrammed cells may exhibit unstable or immature phenotypes (97). Regulation of transcription factor expression and the use of several drugs offer more promising options. *MafA* and *Pdx1* are essential transcription factors that interact with other factors to regulate transdifferentiation. The ablation of the Aristaless-related homeobox gene (*Arx*) (102) or the overexpression of *PAX4* (103), *MafA*, and *Pdx1* (104) promotes the transdifferentiation of β-cells from α-cells. Transdifferentiation from adult murine pancreatic duct (105)



**Figure 3** Alternative sources of β-cells by transdifferentiation from other cell types.

**Table 1** Promising strategies for pancreatic β-cell regeneration.

Cell types	Instructive strategies	Advantages	Limitations and challenges	Functioning validity (weeks)	Involved mechanisms	Refs
Pancreatic non-β-cells Acinar cells	Injury (PDL, PPX); Transduction of pancreatic transcription factors <i>MafA</i> , <i>PDX1</i> and <i>Ngn3</i> / <i>NeuroD1</i> ; Administration of cytokines EGF, CNTF, nicotinamide, LIF; Peptide (GLP-1)	Similar developmental background to β-cells <i>in vivo</i> ; Large proportion and flexible plasticity; Share quantity of transcription factors; Corresponding metabolic mechanism and hormone secretion	Low conversion rate; Weak proliferative capacity and stability; Immature morphology and biological function; The reprogramming efficiency with or without gene manipulation need to be improved	2	PI3K/Akt pathways; MAPK/STAT3 signaling pathways; Erk1/2 signaling pathways	(101, 111, 112, 113)
Duct cells	Injury (PDL, PPX); Ectopic overexpression of <i>MafA</i> , <i>PDX1</i> and <i>Ngn3</i> / <i>NeuroD1</i> , <i>Pax6</i> needed for human ductal reprogramming; Administration of cytokines TGFα, DNA methyltransferase inhibitor; Peptide (GLP-1, gastrin)			5–12		(100, 107, 114, 115)
α and δ cells	Almost complete ablation of β-cells (PDL, PPX, diphtheria toxin); Overexpression of <i>Pdx1</i> , <i>PAX4</i> , <i>MafA</i> ; Suppression of <i>Arx</i> ; Peptides (GLP-1, GABA, artemisinin)			4–16		(98, 99, 102, 103, 104, 108, 109, 110)
Liver cells	Ectopically overexpressing <i>PDX1</i> and <i>NeuroD1</i> ; Down-regulating the expression of <i>HNF1α</i> and <i>HNF4α</i> ; Specific factors (GLP-1R, Notch inhibitors, TGF-β inhibitors)	Conveniently accessible; Sufficient source and cultivate easily; Great regeneration and conversion ability; Share common characteristics including responsiveness to glucose, and mass of specific transcription factors	Efficient viral transfection strategies accompanied with safety concerns; Exploring cytokine or chemical induced safe and effective transition is prospective	4–8	Wnt signaling pathway	(119, 120, 121)
Biliary cells	Transduction of pancreatic transcription factors <i>Pdx1</i> , <i>NeuroD1</i> , <i>Ngn3</i> , <i>MafA</i> or <i>Pdx1/VP16</i> ; Excision of <i>Hes1</i>	Conveniently accessible	Limited source of cells; Transient transformation; Expand cell source and optimize reprogramming protocol need to be investigated	3–4	Notch signaling pathway	(122, 123, 124, 125)
Gastrointestinal cells						
Intestinal cells	Ectopic expression of <i>Pdx1</i> , <i>MafA</i> , and <i>Ngn3</i> ; Excision of transcription factor <i>FoxO1</i> ; Peptide (GLP-1); ‘Small intestinal organ’	Simple, non-invasive, easy to access; Sufficient source and cultivate easily; Great regeneration and	Low conversion rate	1–3	PI3K/Akt/FoxO1 pathways	(126, 128, 129)
Glandular cells	Ectopic expression of <i>Pdx1</i> , <i>MafA</i> , and <i>Ngn3</i> ; ‘Stomach mini-organs’	conversion ability; Similar glucose sensitive system and secretion mechanism with islet cells		3–6		(134, 136, 138)



and acinar cells (106) to β-cells can be induced by *MafA*, *Pdx1*, and *Ngn3/NeuroD*, while induction of *Pax6* was also needed to reprogram human ductal cells into β-cells (107). GLP-1 (108) may transform α-, duct, and acinar cells into cells with a β-cell-like phenotype. GABA (109) and artemisinins (110) promote the transdifferentiation of α-cells into β-cells. EGF in combination with ciliary neurotrophic factor (CNTF), nicotinamide, and leukemic inhibitory factor (LIF) (111, 112, 113) can also induce transdifferentiation of pancreatic acinar cells to insulin-producing cells in culture. Other growth factors such as gastrin and transforming growth factor-α (TGF-α) (114) as well as the DNA methyltransferase inhibitor 5-aza-2'-deoxycytidine (115) induced *Pdx1* expression in ductal cells to promote endocrine differentiation. These processes may be related to the PI3K/AKT/FOXO1 (116) and MAPK/STAT3 signaling pathways (117). The aforementioned results indicate that overexpression of endocrine genes, inhibition of exocrine genes, and treatment with cytokines or small molecules are promising strategies for β-cell regeneration. Results are variable due to the different models and methods; conversion rates are often low. More data are needed to identify safe and effective transcription factors and drugs to promote transdifferentiation for clinical use.

### Transdifferentiation of hepatocytes and biliary cells to β-cells

Due to the proliferative ability and tissue specificity of liver tissue, as well as the common endodermal origin shared between hepatocytes and pancreatic cells, the clinical manipulation of genetic factors in combination with small molecules targeting specific pathways could render the human liver an ideal source of functional insulin-producing cells (118). Some hepatocytes display ectopic expression of *Pdx1*. These hepatocytes are typically located near central veins and seem predisposed to transdifferentiation into β-cells (119). Ectopic overexpression of *Pdx1* and *NeuroD1*, downregulation of the expressions of hepatic transcription factors *HNF1α* and *HNF4α*, the reprogramming of hepatocytes into insulin-producing cells, and activation of the Wnt signaling pathway are necessary conditions for maintaining this plasticity (119, 120). Other factors such as GLP-1R agonists, Notch inhibitors, and transforming growth factor-β (TGF-β) inhibitors could enhance hepatocyte transdifferentiation (121). A notable finding is that the plasticity of the extrahepatic biliary tree enables intrahepatic biliary epithelial cells to express *Pdx1*, *NeuroD*, *Pdx1/VP16*, the insulin gene *Ins*, and *Glut2* (122). In *Hes1* knockout models, biliary epithelium differentiated into pancreatic exocrine and endocrine cells that formed acinar

and islet-like structures in bile ducts with upregulated expression of *Ngn3* (123, 124). Murine gallbladder epithelial cells were reprogrammed into β-like cells through the overexpression *Pdx1*, *Ngn3*, and *MafA*, that led to increased expressions of pancreatic endocrine genes (insulin, *NeuroD1*, *Nkx2.2*, and *Isl1*) (125). The overexpression of critical transcription factors combined with suppression of inhibitory factors may potentially enhance the efficiency of cell reprogramming.

### Transdifferentiation of gastrointestinal cells to β-cells

The gastrointestinal tract is a highly regenerative organ system rich in endocrine cells that are highly similar to pancreatic β-cells and is also an immune-privileged site. Based on these advantages, the gastrointestinal tract can be used as a site for either transdifferentiation to produce β-like cells or for the engraftment of regenerated cells that mimic β-cell function (126, 127).

By screening adult cell types capable of becoming insulin-producing cells *in vivo*, we found that due to the ectopic expression of *Pdx1*, *MafA*, and *Ngn3* in the intestinal crypts, intestinal cells can form β-like cells and may represent an accessible and abundant source of functional insulin-producing cells (128). Under the influence of *Pdx1*, *MafA*, and *Ngn3* (PMN), enterocytes are capable of acquiring β-like characteristics that include the abilities to process preproinsulin into its mature form (with the release of C-peptide); to upregulate genes encoding the β-cell KATP channel subunits Kir6.2 and Sur1, and to display distinctive β-granules (128). Ablation of the *FoxO1* transcription factor in enteroendocrine cells produces functional β-like cells (126). GLP-1 treatment induces insulin production in developing enterocytes, and to a lesser extent, in adult enterocytes both *in vitro* and *in vivo*; this process is mediated by the activation of *Ngn3* and its downstream genes (128, 129).

However, the success rate of intestinal epithelial transdifferentiation is low, the transformation of enterocytes is relatively incomplete and the lifespan of intestinal insulin<sup>+</sup> cells is shorter than that of sinus insulin<sup>+</sup> cells due to *CDX2*, the intestine-specific cell surface marker, which prevents enterocytes from reprogramming into effective β-cells (130). Although these studies have revealed the feasibility of producing β-like cells from the intestinal epithelium, intestinal insulin<sup>+</sup> cells cannot be regarded as completely regenerated β-cells because of their deficient *NKX6.1* expression and unstable phenotype (131, 132, 133). A new framework that promotes a complete

reprogramming of intestinal epithelial cells to become fully functioning  $\beta$ -cells is urgently needed.

However, the stomach represents a potential source of reprogrammable cells that may be transdifferentiated to produce insulin-secreting cells (134). The transformation mechanisms of different gastric cell types are varied. Based on the regenerative capacity of the antrum, native stem cells can supplement the increased demand for antral insulin<sup>+</sup> cells and provide an excellent source of functional  $\beta$ -like cells. Antral endocrine cells mediated by *PMN* can be effectively reprogrammed into insulin<sup>+</sup> cells, with robust expressions of key  $\beta$ -cell genes such as *Sur1*, and *Glp1R*, resulting in substantially improved glucose responsiveness. The gastric corpus contains a small amount of *Ngn3*-derived endocrine cells which differ from those of the antrum or intestinal tract and are not derived through *PMN*-mediated  $\beta$ -cell transformation (134, 135, 136). To summarize, cell plasticity and mechanisms of transdifferentiation differ in various segments of the gastrointestinal tract. The advantages of easy accessibility and transformability of gastrointestinal cells have attracted increasing attention. Further developments in biotechnology are expected to yield functional  $\beta$ -cells.

### Bioengineered approaches

The *in situ* induction of  $\beta$ -cells from the native gastrointestinal tract may be limited due to a physiological environment that may disrupt normal endocrine homeostasis. In addition, the functionality of reprogrammed gastrointestinal cells remains to be determined. Unresolved issues include whether they express all key beta cytokines, whether their physiological function is complete, and whether they can reliably control insulin production in response to various physiologic stimuli. The development of regenerative engineering offers a potential replacement option described as organoids or functional 3D structures assembled with cell types from different sources. This strategy is widely used in regenerative medicine for tissue replacement or repair (137). However, the tissue-engineered stomach represents a versatile *in vivo* tool. ‘Stomach mini-organs’ that contain genetically engineered antral tissue enable both the formation and protection of transformed cells to constitute a new  $\beta$ -cell reservoir (134). Bioengineered gastric spheres isolate newly derived  $\beta$ -mimetic cells from the native organ, maintaining the physiological stability of the endocrine cell population in the intestinal tract, while on the other hand protecting the deposited  $\beta$ -mimetic cells from inappropriate glucose responses under stress (138).

Recent advances in genetic engineering have expanded the accessibility of gastric organoids. The potential research (139) and therapeutic applications of genetically engineered organoids are substantial.

Emerging advances in synthetic biology have enabled the construction of specialized cells capable of performing vital functions. Conditioned media and the addition of a glucose sensing medium have been employed to synthesize  $\beta$ -cell-mimetic designer cells (140). We engineered a glucose-inducible transcriptional system by coupling a  $\beta$ -cell-mimetic cascade of glycolysis-mediated calcium entry to a synthetic excitation transcription coupling system in human embryonic kidney 293 (HEK-293) cells. These engineered cells are capable of glucose sensing and concentration-dependent expression of insulin and GLP-1. Injection of microencapsulated cells increased insulin secretion and improved hyperglycemia in diabetic mice (141). A semi-autonomous light control system stimulated the secretion of glucose-lowering hormones by photoactivated HEK293 cells implanted in diabetic mice, thus restoring glucose homeostasis (142, 143). Glucose-sensing devices loaded with biomimetic cells displayed glucose-induced insulin release comparable to endogenous  $\beta$ -cells; these constructs may be modified to secrete therapeutic proteins such as GLP-1 required for T2D therapy. However, the elucidation of the long-term *in vivo* effects of biomimetic cells will require additional studies.

Future research could explore the potential advantages of combining bioengineering (utilizing polymers such as PTFE or polycaprolactone, and/or microencapsulation of  $\beta$ -cells in materials such as alginate, polyacrylate, collagen or agarose) (144); gene-editing tools (CRISPR-Cas9) (145, 146); immune tolerance induction (147, 148); the adaptation of cell lines for enhanced mass production in bioreactors; optimization of biological process parameters and bioreactor environments; the promotion of cell growth and differentiation to augment biological function. The efficient mass production of cells that are stable, functionally mature, and that can mitigate functional deficiencies will accelerate the development of long-term cell replacement therapy for DM.

Acellular bioengineered constructs may offer alternatives to avoid design complexities and bioincompatibilities of reprogrammed cells. These non-living biomimetic assemblies include vesicles that carry drug payloads in cell membrane-cloaked nanoparticles that deliver insulin in a dynamic response to hyperglycemia and could theoretically act as  $\beta$ -cell surrogates for DM therapy (149). A disadvantage of acellular constructs is that they only provide basic  $\beta$ -cell functions such as insulin

secretion. Capacities for insulin synthesis, its modulation through amplifying signal pathways, and the fine control of relative insulin content in response to external stimuli are absent (13). However, these constructs represent an exciting advance in the search for β-cell alternatives.

## Conclusion

Dedifferentiation is a key mechanism of pancreatic β-cell failure. β-cell dysfunction induced by stress is driven by a complex set of reversible environmental factors. Improved understanding of the mechanisms of β-cell dedifferentiation will inform its reversal. Augmentation of β-cell volume and mass is essential to maintain normal glucose homeostasis and treat DM.

Recent studies have shown that supplementation of endogenous β-cells by transdifferentiation of other cell types may be a better approach than the differentiation of pluripotent stem cells and induced pluripotent stem cells with reduced proliferative capacity. The transdifferentiation of endogenous cells to produce β-cell mimetics is considered a safer approach. Recent studies have reported different conversion efficiencies of various cell types. Conversion rates range from 10 to 20% in pancreatic ductal cells; 0.2 to 70% (typically 20 to 30%) in acinar cells; 30% in gastrointestinal cells; 5 to 20% in biliary cells; 10 to 30% in hepatocytes. In addition, the different combinations of islet transcription factors such as *Pdx1*, *Ngn3*, *MafA*, and *NeuroD1* affect the efficiency of transdifferentiation, and the co-expression of islet transcription factors and EGF/TGFβ growth factors promotes insulin gene expression. Studies of the effects of GABA, artemisinin, and GLP-1 on β-cell transdifferentiation have yielded contradictory results. Drug effects need further validation because of the different cell types, animal models, study intervals, and methods used in previous studies. Numerous strategies have been developed to improve the efficiency of cellular transformation, but there are also significant challenges to be addressed. Although there are obstacles to the reconstitution of β-cell populations through dedifferentiation and transdifferentiation, the development and application of regenerative medicine may provide a wide range of options for the generation of targeted and effective DM treatments.

### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

### Funding

This work was supported by 2015 National Natural Science Foundation of China (81471028).

### Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

### Author contribution statement

Literature collection and writing: W R; revision: C Z. All authors have read and agreed to the published version of the manuscript.

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Received in final form 21 June 2021

Accepted 21 July 2021

Accepted Manuscript published online 21 July 2021