

## Transdifferentiation of pancreatic $\alpha$ -cells into insulin-secreting cells: From experimental models to underlying mechanisms

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rently being investigated to create insulin-producing  $\beta$  cells to replace deficient  $\beta$  cells, including the differentiation of either stem or progenitor cells, and the newly uncovered transdifferentiation of mature non- $\beta$  islet cell types. However, in order to correctly drive any cell to adopt a new  $\beta$ -cell fate, a better understanding of the *in vivo* mechanisms involved in the plasticity and biology of islet cells is urgently required. Here, we review the recent studies reporting the phenomenon of transdifferentiation of  $\alpha$  cells into  $\beta$  cells by focusing on the major candidates and contexts revealed to be involved in adult  $\beta$ -cell regeneration through this process. The possible underlying mechanisms of transdifferentiation and the interactions between several key factors involved in the process are also addressed. We propose that it is of importance to further study the molecular and cellular mechanisms underlying  $\alpha$ - to  $\beta$ -cell transdifferentiation, in order to make  $\beta$ -cell regeneration from  $\alpha$  cells a relevant and realizable strategy for developing cell-replacement therapy.

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**Key words:**  $\alpha$ -cell;  $\beta$ -cell; Transdifferentiation; Diabetes mellitus; Cell-replacement therapy

**Core tip:** Recent works highlighted the phenomenon of transdifferentiation of pancreatic  $\alpha$  cells into  $\beta$  cells, which has drawn much attention in the field. Considering that  $\alpha$ -cell transdifferentiation could be used as a new strategy of cell replacement therapy for the treatment of diabetes, because of the presence of  $\alpha$  cells in the pancreas of both type 1 and 2 diabetics, we believe that it is relevant to elucidate the cellular and molecular events in  $\alpha$ - to  $\beta$ -cell conversion. Our review focuses on the recent experimental  $\alpha$ -cell transdifferentiation models, highlighting the insight provided by these works into the candidates and contexts revealed to be involved in this process.

### Abstract

Pancreatic insulin-secreting  $\beta$ -cells are essential regulators of glucose metabolism. New strategies are cur-

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## INTRODUCTION

Pancreatic  $\beta$  cells are vital for glucose homeostasis. They are capable of producing and secreting insulin, a peptide hormone, in response to high blood glucose levels. Insulin acts on diverse tissues to stimulate the metabolism of glucose<sup>[1,2]</sup>. Diabetes mellitus, becoming an epidemic in different parts of the world and a major public health challenge, is a carbohydrate metabolic disorder arising from failure of glucose homeostasis, with consequent hyperglycemia, resulting in severe complications affecting numerous tissues. The International Diabetes Federation estimated that 336 million individuals worldwide had diabetes in 2010. By 2030, this will have risen to 552 million<sup>[3]</sup>. The disease is characterized by either defective  $\beta$ -cell function as seen in Type 1 diabetes patients who have insufficient or even no  $\beta$  cells, or increased insulin resistance as observed in Type 2 diabetics who fail to maintain glycemic control because of, at least partially, insufficiency in  $\beta$ -cell mass or function. Consequently, there is an urgent need to search for efficient strategies to generate functional  $\beta$ -cells for cell replacement therapy.

The current strategies of generating new  $\beta$  cells can be outlined mainly in the following three ways<sup>[2,4]</sup>: (1) pluripotent stem cell differentiation: with the combined use of different factors, a pluripotent stem cell can be directed to differentiate into the cells with insulin-producing capability. Although such a directed differentiation seems to mimic normal pancreatic development, functional  $\beta$  cells can currently only be differentiated through a lengthy transplantation step; (2) inducing cell replication in existing  $\beta$  cells: this may be conducted either *in vitro* or *in vivo* using different agents or factors, but caution should be taken to avoid neoplastic transformation; and (3) reprogramming a differentiated cell by using genetic factors to induce a pluripotent state and factors driving a specific differentiation program. Reprogramming of acinar cells to generate  $\beta$  cells has proved to be successful *in vivo*<sup>[5]</sup>. More recently, a new strategy, the transdifferentiation of fully differentiated  $\alpha$  cells into  $\beta$  cells, has emerged.

Transdifferentiation was originally defined as the change in a given adult cell from its initial differentiated state into another<sup>[2]</sup>. The most well known cell transdifferentiation phenomenon comes from the regenerative ability seen in urodele amphibians, which can regenerate their limbs, jaws, lens and large sections of their hearts. It is generally thought that transdifferentiating cells may go firstly through dedifferentiation, then proliferation and finally redifferentiation stages. Transdifferentiation can be distinguished from the above-mentioned directed stem

cell differentiation by the fact that the initial cells are not “undifferentiated”. Consequently, transdifferentiated cells are not systematically clonogenic. Although different examples of transdifferentiation were cited<sup>[2]</sup>, it remains uncertain whether “natural” transdifferentiation can actually occur in mammals. More interestingly, recent studies have reported several experimental transdifferentiation models triggered either by drastically changing cellular and/or tissue contexts, or by directly altering molecular programs governing the cellular differentiation state (often referred to as cell conversion). Most notably, it is known that acino-ductal transdifferentiation can be seen in the case of severe tissue injury in the pancreas<sup>[6,7]</sup>. The treatment of rats with a copper-deficient diet resulted in the appearance of hepatocytes in the pancreas, whereas a reversed transdifferentiation was observed in the treatment of rats with polychlorinated biphenyls<sup>[8]</sup>. Experimental works have shown that either the pancreatic acinar tumor cell line AR42-J<sup>[9]</sup>, or freshly isolated adult acinar cells<sup>[10]</sup> can transdifferentiate into hepatocytes *in vitro*. It was also reported that, under certain cell culture conditions, AR42-J cells were seen to display endocrine cell features<sup>[11,12]</sup>. Similarly, with the use of epidermal growth factor- and leukemia inhibitory factor-supplemented cell culture medium, it was reported that pancreatic exocrine cells were transdifferentiated into insulin-producing cells<sup>[13]</sup>. The phenomenon may also occur *in vivo*, the cells coexpressing transiently exocrine and endocrine markers being observed in rats that were subject to duct ligation<sup>[14-16]</sup>, and in mice treated with alloxan<sup>[17]</sup>. Considering the particular role of Ngn3, its ectopic expression has been explored to trigger transdifferentiation of adult human duct cells into endocrine cells<sup>[18]</sup>. Finally, it is also speculated that  $\beta$ -cell mass increase seen in rats chronically infused with glucose may imply transdifferentiation as mechanisms of adaptation<sup>[19,20]</sup>.

More interestingly, several laboratories have reported the phenomenon of transdifferentiation of pancreatic  $\alpha$  cells into insulin-secreting cells (Table 1), which has been observed in different experimental settings<sup>[21-36]</sup>. Because of the close developmental and physiological relationship between these two cell lineages, and the presence of  $\alpha$  cells in the pancreas of Type 1 and 2 diabetes patients,  $\alpha$ -cell transdifferentiation draws much attention in the field of  $\beta$ -cell regeneration. Here, we review in detail these different models.

## EXPERIMENTAL MODELS DISPLAYING $\beta$ -CELL TRANSDIFFERENTIATION

### *Altered cross-regulatory circuit between Arx and Pax4*

A number of studies have demonstrated that, during development, the influence of several transcription factors successively directs progenitor cells toward pancreatic, and ultimately islet endocrine cell fates. A complex network of transcription factors, including Arx and Pax4, progressively and differentially promotes particular endocrine fates<sup>[21,22]</sup>. In mice lacking Arx,  $\beta$ - and  $\delta$ -cell

**Table 1** List of some experimental models of  $\beta$ -cell transdifferentiation

Experimental model	Phenotype	Intermediate cells	$\alpha$ -cell proliferation	Ref.
Pax4 overexpression	Converts progenitor cells into $\alpha$ and subsequently $\beta$ cells	Very few	-	[24]
Arx inactivation	$\alpha$ - to $\beta$ -like conversion	+	-	[25]
Arx inactivation; Pdx1;Arx double mutant	$\alpha$ - to $\beta$ -like conversion	+	-	[26]
Pdx1 overexpression	$\alpha$ - to normal $\beta$ cell conversion	Numerous mantle-located Gcg + Ins + cells were detected in P1-P12	-	[28]
PDL + alloxan	A large number of new $\beta$ cells arising from adult $\alpha$ cells within 14 d	58% of Ins+ cells coexpressed glucagon	-	[30]
Extreme $\beta$ -cell loss	$\alpha$ - to $\beta$ -cell transdifferentiation	+	-	[29]
Treatment with histone methyltransferase inhibitor	$\alpha$ - to $\beta$ -cell conversion	Colocalization of both glucagon and insulin in human and mouse islets	-	[36]
Ablation of glucagon gene	Normoglycemia and hyperplasia of pancreatic $\alpha$ cells	+	+	[31]
Ablation of glucagon receptor (Gcgr <sup>-/-</sup> )	Lower blood glucose, hyperglucagonemia, and pancreatic $\alpha$ -cell hyperplasia	Few scattered Gcg + Ins + cells or not mentioned	+	[27,32-34]
Impaired glucagon synthesis (SPC2 <sup>-/-</sup> )	Normoglycemia, hyperplasia of pancreatic $\alpha$ and $\delta$ cells	Not mentioned	+	[37]
Disturbed glucagon pathway [Liver-specific G(s)alpha deficiency]	Hypoglycaemia, hypoinsulinemia, pancreatic $\alpha$ -cell hyperplasia		+	[38]
Men1 inactivation	$\alpha$ -cell transdifferentiation, $\alpha$ -cell hyperplasia and development of glucagonoma and insulinoma	+	+	[39]

fates were found to be favored at the expense of  $\alpha$ -cell genesis, while the total endocrine cell content remained normal<sup>[21]</sup>. Conversely, in the absence of Pax4,  $\beta$ -cell loss was observed accompanied by an increase in  $\alpha$ -cell number<sup>[22]</sup>, indicating an inhibitory, cross-regulatory circuit between Arx and Pax4<sup>[23]</sup>.

Interestingly, Collombat *et al.*<sup>[24]</sup> demonstrated that ectopically expressed Pax4 in endocrine precursor cells and  $\alpha$  cells in the mouse resulted in the conversion of these cells into insulin-producing cells. As early as 1 wk postpartum, a 50% enlargement in islet size was outlined, with the islets containing increased numbers of insulin- and Pax4-positive cells compared with controls, and the number of glucagon-producing cells reduced by 77%. An age-dependent increase in islet size and the number of insulin-producing cells was observed. The latter exhibited most  $\beta$ -cell features, suggesting that, upon Pax4 ectopic expression, adult glucagon-expressing cells were continuously converted into cells exhibiting a  $\beta$ -cell phenotype. The lack of glucagon-producing cells resulted in an apparent adaptive neogenesis of  $\alpha$  cells. The authors provided evidence suggesting that such a conversion triggered by Pax4 ectopic expression in  $\alpha$  cells was sufficient to alleviate the diabetic condition resulting from massive  $\beta$ -cell destruction in the mouse.

More recently, Wilcox *et al.*<sup>[25]</sup> showed that ablation of Arx in neonatal  $\alpha$ -cells resulted in an  $\alpha$ -to- $\beta$ -like conversion through an intermediate bihormonal state, while short-term ablation of Arx in adult mice did not. However, Courtney *et al.*<sup>[26]</sup> showed that selective Arx disruption in  $\alpha$  cells at any age could elicit the conversion. It is important to note that such a conversion induced duct-lining precursor cells to differentiate to endocrine cells. The  $\alpha$  cells thus generated were subsequently converted

into  $\beta$ -like cells because of Arx inactivation. Using conditional Arx and Pax4 double mutants, Courtney *et al.*<sup>[26]</sup> provided evidence showing that Pax4 was dispensable for this regeneration process, suggesting that Arx could be the main trigger of  $\alpha$ -cell conversion into  $\beta$ -like cells. Importantly, Arx disruption in  $\alpha$  cells was able to reverse mouse diabetes resulting from  $\beta$ -cell depletion.

#### $\alpha$ to $\beta$ cell reprogramming by forced PDX1 expression

Vuguin *et al.*<sup>[27]</sup> performed ectopic Pdx1 expression from Ngn3-positive endocrine progenitors (*Neurog3<sup>Cre</sup>-Pdx1<sup>OE</sup>* mice). They detected a slight increase in  $\beta$ -cell number accompanied by a reduced  $\alpha$ -cell number during the embryonic period<sup>[28]</sup>. At each stage, the combined number of  $\alpha$  and  $\beta$  cells in *Neurog3<sup>Cre</sup>-Pdx1<sup>OE</sup>* mice was similar to that in controls, despite a significant difference in the  $\alpha$ - to  $\beta$ -cell ratio, strongly suggesting a scenario of lineage diversion, where one cell population expands at the expense of the other under a constant total cell number. Two phases of lineage conversion were identified, contributing to a complete  $\alpha$ -cell loss by the early adult stage. First, a significant decrease in glucagon-positive cell number (47% in the control reduced to 35% in mutant mice) and accompanying increase in insulin-positive cells was detected in the E16.5 *Neurog3<sup>Cre</sup>-Pdx1<sup>OE</sup>* pancreas, shortly after the peak of Neurog3 expression at approximately E15. Second, a major progressive loss of glucagon-positive cells in parallel with increased insulin-positive cell numbers was detected at P1-P12. Coexpression of insulin and  $\alpha$ -cell-specific factors such as Arx, suggesting an early movement toward  $\beta$ -cell-directed transdifferentiation, was not detected at the first stage. Importantly, numerous mantle-located glucagon- and insulin-positive cells were detected in the second stage, representing intermediate

state  $\alpha$  cells undergoing conversion, suggesting that the suppression of glucagon and the induction of insulin occurred concurrently. Intriguingly, when activating Pdx1 in the differentiated or mature glucagon-expressing  $\alpha$  cell, the efficiency of the occurrence of  $\alpha$ -to- $\beta$  conversion was very much impaired, even absent. The work suggests that Pdx1 alone may play a strong role in regulating the cell differentiation program of islet-cells.

### **Near complete $\beta$ -cell ablation**

Thorel *et al.*<sup>[29]</sup> have generated an elegant mouse model which allows nearly total  $\beta$ -cell ablation using the diphtheria toxin receptor system. The massive  $\beta$ -cell destruction thus obtained resulted in heterologous  $\beta$ -cell formation. Surprisingly, the majority of newly formed  $\beta$  cells originated from former glucagon-producing cells. By using cell lineage tracing, they demonstrated that, upon near total loss of  $\beta$  cells, genetically marked  $\alpha$  cells rapidly began firstly to coexpress Nkx6.1, then coexpress insulin and the adult  $\beta$ -cell markers Pdx1, Nkx6.1 and Glut2, subsequently forming the majority of the regenerated  $\beta$  cells. Importantly, when  $\alpha$  cells were ablated together with  $\beta$  cells, bihormonal cells expressing both glucagon and insulin were no longer observed. The work may also suggest that, in this particular experimental setting, a complete lack of local insulin signaling would elicit the interconversion between  $\alpha$ - and  $\beta$ -cells. It would be interesting and challenging to use this model to further study the process and the mechanisms of  $\alpha$ -cell transdifferentiation.

### **Pancreatic duct ligation + alloxan treatment**

Chung *et al.*<sup>[30]</sup> generated another pancreas and  $\beta$ -cell-deficient mouse model to study the origin and extent of adult  $\beta$ -cell regeneration. To this end, they used the  $\beta$ -cell specific toxin alloxan to ablate  $\beta$  cells, and, subsequently, carried out pancreatic duct ligation (PDL) to stimulate  $\beta$ -cell neogenesis. They reported that more than half (58%) of insulin-positive cells coexpressed glucagon one week after PDL and alloxan treatment. Moreover, they found that some glucagon-positive cells coexpressed  $\beta$ -cell-specific transcription factors, such as Pdx1 and Nkx6.1, suggesting a transitional stage during the conversion. Later, cells coexpressing insulin and glucagon were found. Interestingly, these insulin-positive cells expressed MafB, but afterward switched from MafB to MafA expression, suggesting that they were initially immature, and became mature over time. Unfortunately, cell lineage tracing was not performed in this model.

### **Glucagon pathway deficiency models**

Mice with glucagon signaling deficiency, due to the inactivation of either the *Glucagon* gene<sup>[31]</sup> or its receptor (*GCGR*)<sup>[27,32-34]</sup>, impaired glucagon synthesis<sup>[37]</sup>, or a disturbed glucagon pathway<sup>[38]</sup>, display common features. These include lower blood glucose levels, improved glucose tolerance with relatively normal insulin levels, and,

in particular,  $\alpha$ -cell hyperplasia and even tumorigenesis<sup>[33]</sup>, accompanied by hyperglucagonemia and, in some of these models, scattered intermediate cells coexpressing insulin and glucagon. However, full transdifferentiation of  $\alpha$  cells into  $\beta$  cells has never been demonstrated in the above models. Most probably, the fact that islets were often clustered near ductal tissue, and glucagon staining was seen along and budding from ductal epithelium or within exocrine tissue, suggests that the islet neogenesis could be the cause of increased  $\alpha$ -cell mass.

### **Transdifferentiation from $\alpha$ cells to insulin-expressing cells triggered by *Men1* disruption**

In our previous study, we demonstrated the phenomenon of transdifferentiation in a mouse model where the *Men1* gene, a tumor suppressor in many types of endocrine cells, is specifically disrupted in pancreatic  $\alpha$  cells<sup>[39]</sup>. Our analyses of pancreata from aging mutant mice showed that, in spite of the  $\alpha$ -cell specificity of the *GluCre* transgene, both glucagonomas and insulinomas, as well as mixed islet tumors, were observed in mutant mice older than 6 mo of age. More interestingly, starting from as early as 2 mo of age well before tumor onset, cells sharing characteristics of both  $\alpha$  and  $\beta$  cells, and coexpressing insulin and glucagon could be identified. Importantly, using a cell lineage tracing approach, we showed that these intermediate cells and insulinoma cells were both derived from *Men1*-deficient  $\alpha$  cells. Furthermore, our data suggest that Pdx1, MafA and Ngn3 expression did not seem to be involved in the initiation of this transdifferentiation<sup>[39]</sup>. Intriguingly, although many *Men1*-deficient  $\alpha$  cells transdifferentiated into insulin-secreting cells, some maintained their  $\alpha$ -cell identity. This may indicate that *Men1*-disruption *per se* does not systematically lead to  $\alpha$ -cell transdifferentiation, but rather affords the pathophysiological conditions to allow the transdifferentiation to occur. Other factors, independent of *Men1* disruption, may, therefore, play a crucial role in the initiation of the transdifferentiation. Using this model, where transdifferentiating cells are numerous before the development of tumors, to search for these factors would be of help in further deciphering the cellular and molecular basis of  $\alpha$ -cell transdifferentiation. The identification of such factors would be crucial to determine the conditions favorable for  $\alpha$ -cell transdifferentiation, while avoiding the known tumorigenic effect of *Men1* inactivation in islet cells.

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## **CLUES TO OTHER FACTORS AND UNDERLYING MECHANISMS IMPORTANT FOR TRANSDIFFERENTIATION**

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The data from the above mouse models displaying experimental transdifferentiation of  $\alpha$  cells into  $\beta$  cells suggest that  $\alpha$  cells could possess intrinsic abilities to allow their conversion under certain circumstances, giving rise to an adaptive response to  $\beta$ -cell loss or deficiency. While these

models highlighted the genetic factors directly involved in such a process, they also provided clues as to other factors that may or may not participate in  $\alpha$ -cell transdifferentiation.

### Cell dedifferentiation

It is generally considered that natural transdifferentiation occurs in two steps: the dedifferentiation of the cell, followed by the differentiation of the dedifferentiated cell into the new lineage<sup>[40]</sup>. Although it is still unclear whether experimental transdifferentiation follows a similar course, the fact that no completely dedifferentiated  $\alpha$  cells have been reported in the above experimental models seems to indicate that this may not be the case. Instead, it may be possible to directly convert one cell type into another. In this case, there could be a simultaneous switch from the inactivation of an old cell differentiation program into the activation of a new program. The existence of “intermediate cells” expressing both glucagon and insulin documented in several of these models even suggests that the initial activation of the new program may precede the complete inactivation of the old one. However, detailed cellular and molecular analyses are still required to allow a full understanding of the transdifferentiation procedure.

### Epigenetic factors

Epigenetic mechanisms are known to play an important role in establishing and maintaining cell differentiation programs. Interestingly, a recent study demonstrated that  $\alpha$  cells harbor bivalent chromatin signatures, containing both active and repressive histone markers, at genes that are active in  $\beta$  cells, such as Pdx1 and MafA<sup>[36]</sup>. The finding of  $\alpha$ -cell plasticity may be supported by the fact that  $\beta$ -cell specific genes are likely ready to be activated. Moreover, they found that the repressed Pdx1 and insulin expression in  $\alpha$  cells could be reactivated by treating islets with an inhibitor of histone methyltransferase. The work provides interesting clues into eventual cell reprogramming through epigenetic modifications<sup>[36]</sup>.

Along with the above data, two other studies have demonstrated that changing histone methylation marks by deleting the *Dnmt1/3a* gene resulted in the transdifferentiation of  $\beta$  cells into  $\alpha$  cells<sup>[41,42]</sup>. Indeed, detailed analyses showed that these two genes, together with other epigenetic factors, such as PRMT6, MeCP2 and HDAC1, play a crucial role in inhibiting the expression of transcriptional factors that may give rise to the activation of a cell differentiation program of other cell lineages, such as ARX in  $\beta$  cells. The loss of DNA methylation, therefore, results in the de-repression of these transcription factors, and the activation of the transcriptional program of other cell lineages. Thus, it would be interesting to investigate whether similar mechanisms could control  $\alpha$ -cell identity.

### Islet hormones and $\alpha$ -cell proliferation

**Glucagon, insulin and GLP1:** Glucagon was found to

inhibit the formation of  $\beta$  cells converted from  $\alpha$  cells upon Pax4 overexpression<sup>[24]</sup>. However, the phenomenon may be more directly related to the expansion of Ngn3 progenitors rather than the reprogramming itself, since virtually all  $\alpha$  cells were converted to insulin-expressing cells by ectopic Pax4 expression, and mutant mice displayed hypoglucagonemia. Furthermore, in the *Men1* disruption-mediated transdifferentiation model, the very high levels of glucagon did not prevent  $\alpha$ -cell transdifferentiation<sup>[39]</sup>. As for the potential inhibitory role of insulin deduced from the work by Thorel *et al.*<sup>[29]</sup>, the quasi absence of insulin does not seem to be a prerequisite for the occurrence of  $\alpha$ -cell transdifferentiation, since the majority of experimental transdifferentiation models mentioned above display substantial levels of insulin. The existence of intra-islet GLP1 in many of the experimental transdifferentiation models makes it a plausible candidate involved in  $\alpha$ -cell transdifferentiation. However, in aged GCGR knockout mice with extremely high levels of GLP1, only  $\alpha$ -cell expansion, likely due to neogenesis, but not transdifferentiation was observed<sup>[34]</sup>. Altogether, the above data from different experimental transdifferentiation models indicate that islet hormones themselves, including glucagon, insulin and GLP1, may not be sufficient to be critically involved in the process.

**$\alpha$ -cell proliferation:**  $\alpha$ -cell proliferation and hyperplasia, even neoplastic changes in some circumstances, were frequently found in various glucagon-deficient models. This raises the possibility that it may be required for, or even trigger, transdifferentiation. However, in the case of GCGR knockout mice, massive  $\alpha$ -cell proliferation and neoplastic alteration did not lead to  $\alpha$ -cell transdifferentiation. Importantly, a patient with a homozygote germline mutation of the GCGR gene displayed microglucagonoma and non-functional islet-tumor development, but no sign of  $\alpha$ -cell transdifferentiation<sup>[43]</sup>. The data suggest that  $\alpha$ -cell proliferation may be favorable for, but not systematically result in, the occurrence of transdifferentiation. At the same time, this highlights the potential deleterious effects of  $\alpha$ -cell proliferation due to drastic glucagon pathway deficiency and/or massive  $\alpha$ -cell loss.

### Timing

In a recent work reported by Wilcox *et al.*<sup>[25]</sup>, the authors observed that embryonic  $\alpha$  cells and adult  $\alpha$  cells may react differently towards *Arx* disruption. Whereas the former were driven to convert to  $\beta$  cells, the latter seemed completely nonresponsive to the lack of ARX. However, similar work by Courtney *et al.*<sup>[26]</sup> did not confirm this observation. The reason for the discrepancy remains unclear. Interestingly, another study, using ectopic Pdx1 expression in either pancreatic progenitors or in embryonic and mature  $\alpha$  cells to reprogram the cells into  $\beta$  cells, also demonstrated that the efficiency of the reprogramming decreased when forced Pdx1 expression occurred later in embryonic development or in adult mice<sup>[28]</sup>. Col-

lectively, these studies highlighted the importance of timing in  $\alpha$ -cell plasticity that should be taken into account for possible future clinical applications based on  $\alpha$ -cell transdifferentiation.

## CONCLUSION

Taken together, the above-mentioned recent studies highlighted the importance of both transcriptional factors and/or cofactors in maintaining cell differentiation status and in the physiological mechanisms involved in  $\alpha$ -cell transdifferentiation. It would be vital and challenging for future studies to pinpoint the decisive factors from these two axes, and to provide insight into detailed mechanisms responsible for  $\alpha$ -cell transdifferentiation. At the same time, past experience seems to indicate that some of the above-mentioned experimental conditions, such as PLD and glucagon pathway deficiency, may be more favorable for eliciting neogenesis, rather than  $\alpha$ -cell transdifferentiation.

Because of their close ontogenic relation with  $\beta$  cells and unusual plasticity in responding to internal and external alterations, pancreatic  $\alpha$  cells elicit much curiosity and clinical promise. In particular, the capacity for their transdifferentiation into insulin-secreting cells documented by several distinct models renders them a potentially relevant cellular basis for new strategies of  $\beta$ -cell regeneration. Deciphering detailed cellular and molecular mechanisms of the  $\alpha$ -cell transdifferentiation process will be challenging for the field and crucial for future clinical applications.

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