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Regenerating pancreatic β -cells: Plasticity of adult pancreatic cells and the feasibility of *in vivo* neogenesis

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Abstract

Purpose of review—Diabetes results from inadequate functional mass of pancreatic β -cells, and so replenishing with new glucose-responsive β -cells is an important therapeutic option. In addition to replication of pre-existing β -cells, new β -cells can be produced from differentiated adult cells using *in vitro* or *in vivo* approaches. This review will summarize recent advances in *in vivo* generation of β -cells from cells that are not β -cells (**neogenesis**) and discuss ways to overcome current limitations of this process.

Recent findings—Multiple groups have shown that adult pancreatic ducts, acinar and even endocrine cells exhibit cellular plasticity and can differentiate into β -cells *in vivo*. Several different approaches, including misexpression of transcription factors and tissue injury, induced neogenesis of insulin-expressing cells *in vivo* and ameliorated diabetes.

Summary—Recent breakthroughs demonstrating cellular plasticity of adult pancreatic cells to form new β -cells are a positive first step towards developing *in vivo* regeneration based therapy for diabetes. Currently, neogenesis processes are inefficient and do not generate sufficient amounts of β -cells required to normalize hyperglycemia. However, improved understanding of mechanisms regulating neogenesis of β -cells from adult pancreatic cells and of their maturation into functional glucose-responsive β -cells can make therapies based on *in vivo* regeneration a reality.

Keywords

In vivo regeneration; neogenesis; transdifferentiation; β -cells; diabetes

Introduction

Replenishing β -cell mass by transplantation, or better yet by *in vivo* regeneration, is an important strategy to ameliorate diabetes. Recent demonstrations of pancreatic ducts, acinar and even endocrine cells to acquire new cell fates *in vivo* suggests *in vivo* regeneration could replenish inadequate β -cell mass (1-5**). Some of these cell types **transdifferentiate** [conversion of a differentiated cell of one developmental commitment into a differentiated cells of another lineage without first reverting to a more primitive stem cell or progenitor (6-8)] into β -cells, while others achieve this goal by dedifferentiation and redifferentiation (1-5,9,10). We will briefly review *in vitro* differentiation strategies and then focus on the limitations and strengths of recent studies demonstrating *in vivo* neogenesis of β -cells.

Cell sources for *in vitro* generation of β -cells

In vitro differentiation of stem/progenitor cells into β -cells is an important approach to generate a reliable and replenishable cell source of β -cells. A significant effort is underway to differentiate embryonic stem cells into glucose-responsive β -cells (11*-19*). Several reports showed successful isolation and differentiation of putative stem/progenitor cells from adult and fetal pancreatic tissues into insulin-expressing cells. These include clonal cells isolated from adult islet and ducts (20) and prospective isolation from adult and fetal pancreas using FACS (21,22). Progenitors isolated from fetal tissue could be true progenitors/stem cells, but since the *in vivo* identity of progenitors isolated from adult pancreas is not known, they may represent dedifferentiated mature cells.

Interestingly, some fetal and differentiated adult cells from non-pancreatic sources, including hepatic cells, can differentiate into insulin-expressing cells. Fetal liver cells, adult hepatocytes, hepatic cell lines, and biliary epithelial cells were shown to induce insulin expression upon expression of key pancreatic transcription factors (23-30). Similarly, primary intestinal epithelial cells and cell lines expressing pancreatic transcription factors expressed insulin mRNA when treated with GLP-1 and betacellulin (31,32). These observations suggest that expression of pancreatic transcription factors in some non-pancreatic cells in presence of a few signaling molecules can induce insulin expression. However, the amounts of insulin produced by these cells were often several orders of magnitude lower than mature β -cells.

Pancreatic ductal cell lines and primary ductal cells have been successfully differentiated into insulin-expressing cells by *in vitro* approaches, including treatment with growth factors (e.g., EGF, Gastrin, exendin), expression of pancreatic transcription factors, and aggregation (9,10, 33-37). Neogenesis of insulin-producing cells from differentiated pancreatic ductal cells results from their dedifferentiation into progenitors, expressing markers like PDX1, which redifferentiate into insulin-producing and other pancreatic cells. Hence, “terminally” differentiated ductal cells can be considered facultative stem cells (34). Like ductal cells, lineage-marked acinar cells in response to EGF underwent *in vitro* differentiation into insulin-expressing cells (38). A role for acinar-to-ductal transdifferentiation has also been suggested in conversion of acinar cells into endocrine cells (39). These observations demonstrate that multiple cell sources can differentiate into insulin-producing cells under *in vitro* culture conditions.

Thus, we suggest that like ES cells, adult pancreatic and non-pancreatic cells should be considered as potential *in vitro* cell sources for generating insulin-producing cells.

Cell sources for *in vivo* generation of β -cells

Over the last decade significant advances have been made in developmental biology of the pancreas and other endoderm-derived organs (25,40-43). We gained a greater understanding of transcription factors and signals involved in the formation of β -cells and determined the molecular events that regulate differentiation of endoderm into pancreas, liver, and intestine. Analyses of lineage-commitment in transgenic and knockout mice demonstrated that altering expression of key factors during pancreatic development can alter cell fate decisions (44-46), and in some cases, cells destined to be part of the pancreas become part of a different organ (47). These findings show plasticity of pancreatic progenitors in acquiring different cell fates during embryonic development. Recently *in vivo* neogenesis of β -cells from pancreatic and non-pancreatic cells has been reported in adults. We will discuss these results and factors that can improve the efficiency of *in vivo* regeneration of glucose-responsive β -cells.

Neogenesis of β -cells from non-pancreatic cells

Liver represents an attractive *in vivo* source for generating β -cells due to its related developmental origin to the pancreas and its ease of genetic and surgical manipulation. *In vivo* conversion of pancreatic cells into hepatocyte-like cells was shown in copper-deprived rats (48,49), while that of hepatic cells into acinar cells was reported in rats treated with polychlorinated biphenyls (50). Expression of transcription factor Pdx1 in adult mouse liver triggered the formation of insulin-expressing cells (51), while in liver of *Xenopus* tadpoles, expression of Pdx1-VP16, but not Pdx1 alone, formed ectopic pancreas containing both exocrine and endocrine compartments (52). The VP16 activation domain was essential for transdifferentiation of liver cells by another key pancreatic transcription Ptf1a, but it only formed acinar cells. However, expression of Ptf1a alone in stomach and duodenum generated both pancreatic acinar and endocrine cells (53). Combination of Pdx1, NeuroD1 and MafA expression, or NeuroD1 expression with betacellulin transdifferentiated liver cells into insulin-expressing cells, which could ameliorate streptozotocin-induced diabetes (51,54,55). Insulin-producing cells generated upon Adenoviral-mediated-expression of Pdx1 in the liver improved the blood sugar levels in 43% of cyclophosphamide-accelerated diabetic NOD mice (56). These results support the feasibility of using non-pancreatic cells for *in vivo* generation of insulin-producing cells.

Plasticity of pancreatic cells and *in vivo* neogenesis of β -cells

Strong evidence to support the presence in adult pancreas of a population of undifferentiated stem/progenitor cells that can give rise to all pancreatic cell types is still lacking. It has been shown that replication of differentiated β -cells contribute new β -cells during normal adult life (57). However, neogenesis of islets also occurs during normal development (at least during the first month) and in response to physical and physiological stress (34,58). In this review, we will limit our discussion to neogenesis of β -cells in adults.

Neogenesis of β -cells from pancreatic ductal cells

Morphological analysis of pancreas following various manipulations, as well as *in vitro* differentiation of pancreatic ductal cells, suggest that pancreatic “progenitors” reside in ducts. In an elegant study using lineage-tracing and pancreatic ductal ligation in adult mice, Xu et al. demonstrated that the neogenesis of β -cells accompanied the induction of Ngn3-expressing endocrine progenitors in the ductal lining in the regenerating portion but not in the non-injured pancreas (4**). However, a recent publication reports a low level expression of Ngn3 in adult endocrine cells (59*), raising concerns about using Ngn3 expression as a marker of endocrine progenitors and neogenesis in adult pancreas. Interestingly, the Ngn3-expressing cells seen in the Xu et al. study did not express hormones, yet the increase in β -cell mass after ductal ligation depended on Ngn3 expression (4**). These observations confirm that the neogenesis, i.e., formation of β -cells from non-islet cells, occurs in adult pancreas.

By a direct lineage-tracing approach using a duct-specific human CAII promoter driving Cre or Cre^{ERT}, one adult pancreatic cell type that gives rise to multipotent progenitors was identified (5**). CAII expression begins late in gestation (embryonic day 18.5) and is seen throughout the ductal tree but not in β -cells. Bigenic (CAII^{Cre};R26R) mice at birth had lineage marker expression restricted to ductal cells, but by four weeks marker was also seen in endocrine cells (both α - and β -cells) and in acinar cells; 38% of the islets examined showed expression of the reporter gene. In inducible CAII^{CreERT};R26R mice ductal ligation after marking the adult ductal cells resulted in lineage-marked islets and acinar cells. Impressively, two weeks after duct ligation, 42% of islets and 24% of β -cells had lineage marker. These results clearly demonstrate that a significant proportion of adult pancreatic ductal cells retain the potential to differentiate into other pancreatic cell types including β -cells.

Acinar cell transdifferentiation to β -cells

Acinar and endocrine cells probably have a similar epigenetic profile as they share a common multipotent progenitor (60), which should make transdifferentiation of acinar cells into β -cells easier than from non-pancreatic cells. Yet, adult lineage-marked acinar cells were not seen to transdifferentiate into other pancreatic cell type in several experimental paradigms (61). Acinar cells can regenerate, as shown in chemical induced pancreatitis (caerulein) model, predominantly through their dedifferentiation and redifferentiation (62). However, misexpression of pancreatic developmental regulators in adult acinar cells using adenoviruses encoding Ngn3, Pdx1, and MafA transduced nearly 20% of acinar cells expressing these factors into insulin-producing β -cells that were indistinguishable from endogenous islet β -cells in size, shape and structure (2**). These new β -cells did not organize into islets and remained isolated. They improved, but not normalized, glycemia in diabetic mice. Additional studies will be needed to determine why only a fraction of acinar cells transdifferentiate into β -cells.

Transdifferentiation from other endocrine cells

Can pancreatic endocrine cells, like pancreatic ductal and acinar cells, transdifferentiate or dedifferentiate/redifferentiate into other cell types? Analyses of pancreas from some pancreatic transcription factor (e.g., *Nkx2.2*, *Pax4*, and *Arx*) knockout mice demonstrate that lineage commitment of endocrine progenitors can be reprogrammed (44-46). *Nkx2.2* knockout mice lack β -cells and have reduced α - and PP-cells but increased ghrelin-expressing cells (46). Similarly, the relative expression of transcription factors, *Pax4* and *Arx*, in endocrine progenitors determined the lineage of a differentiated cell. In *Pax4* knockout mice, loss of β - and δ -cells was compensated by a proportional increase of α -cells (44), while in *Arx*-deficient mice the loss of α -cells was compensated by a proportional increase in β - and δ -cells (45). Interestingly, committed endocrine progenitors can also differentiate into other pancreatic cell types. In *Pax4*:constitutively activated Notch mice, *Pax4*-expressing endocrine progenitors differentiated into ducts (63). Thus, during embryonic development, lineage commitment of endocrine progenitors can be regulated.

Altering expression of transcription factors in differentiated endocrine cells can also change their endocrine fate. *Arx* misexpression in β -cells, using either an *Ins*^{Cre} or in adult β -cells using an inducible *Pdx1*^{CreERT} (64), reduced insulin-expressing cells and increased α - and PP-cells. In both cases, the vast majority of α - and PP-cells contained lineage marker confirming their derivation from insulin/*Pdx1*-expressing β -cells. Interestingly, expression of *Pax4* in mature α -cells using *Gcg*^{Cre}, increased β -cell mass and dramatically reduced that of α -cells (3**). The increase could not result from proliferation of new β -cells nor from the normal number of α -cells in controls. Most β -cells contained the lineage marker indicating their derivation from α -cells. In spite of increased β -cell mass and initial hypoglycemia, these mice gradually became hyperglycemic. This system was also able to reconstitute β -cells in streptozotocin-treated mice of different ages. Younger mice showed preferential survival, possibly by inducing neogenesis of α -cells in response to reduced glucagon signaling, and reprogramming them in the presence of *Pax4* to β -cells. Thus, these results demonstrate that adult α -cells, like pancreatic ductal and acinar cells, can serve as *in vivo* source for generating β -cells.

Steps to enhance *in vivo* regeneration of β -cells

As reviewed above, adult cells in response to appropriate *in vitro* and *in vivo* factors/signals can differentiate into new β -cells (Figure 1). Although one should be careful to equate results from animal studies with human situation, the reviewed literature provides rationale for developing *in vivo* regeneration-based treatments for diabetes. Acinar and ductal cells constitute over 95% of the pancreas, and their ability to proliferate in response to various stimuli

makes them attractive targets for *in vivo* regeneration of β -cells. Developing efficient strategies to deliver regulatory factors to these cells, either on their own or when combined with short-term growth stimulus, should result in increasing β -cell mass. Transdifferentiation of α -cells into β -cells is an equally attractive approach for *in vivo* regeneration due to their location and the neogenic response of α -cells to impaired glucagon signaling. To realize *in vivo* regeneration based on this approach will require developing strategies to induce Pax4 expression in α -cells and combining it with approaches to inhibit glucagon signaling. However, before we make a significant investment in developing therapies based on acinar, ductal or α -cells, it will be important to determine the maximum extent of neogenesis that can be achieved using these cell types.

At present these approaches cannot normalize hyperglycemia, and at least in some cases (3**), animals gradually redeveloped hyperglycemia, demonstrating the inability of these approaches to generate/maintain sufficient amounts of glucose-responsive β -cells. The limited success of *current approaches* could result from: 1) incomplete dedifferentiation of terminally differentiated cells to a progenitor state, 2) inefficient redifferentiation of dedifferentiated progenitors into β -cells, and/or 3) inability of insulin-producing cells to survive/proliferate and mature into glucose-responsive β -cells.

The yield of β -cell neogenesis from ducts is possibly limited by their first acquiring a dedifferentiated fate and then redifferentiating into multiple cell types (endocrine, acinar and ducts) (4*,34,58). In case of acinar reprogramming, 20% of infected acinar cells became β -cells, without significant proliferation or expression of markers of a common embryonic acinar-endocrine progenitor (2**). However, it is still likely that these cells partially dedifferentiated and that affected the efficiency of neogenesis. In contrast, the majority of α -cells that expressed Pax4 transdifferentiated into β -cells possibly without dedifferentiating into a progenitor stage (3**). Thus, in order to enhance the efficiency of *in vivo* neogenesis of β -cells, it will be essential to understand the process of dedifferentiation and redifferentiation and to determine which of these steps is rate-limiting. Identification of factors that can efficiently dedifferentiate adult pancreatic cells into progenitors having an epigenetic profile similar to that of pancreatic/endocrine progenitors will be important. Our ability to monitor at the single cell level the loss of differentiated phenotype, induction of progenitor and β -cell markers, and changes to the epigenetic profile will be key to enhancing the efficiency of neogenesis. Such analyses will be crucial for determining whether all, or only a few, cells can acquire “true” epigenetic makeup of pancreatic/endocrine progenitor and thus can differentiate into β -cells.

It is also possible that the newly generated β -cells are immature and functionally impaired, which would limit their ability to normalize hyperglycemia, leading to suppression of several key genes in β -cells and further exacerbating the impairment. Newly-formed insulin-producing cells undergo maturation steps that include reduction in the expression of transcription factors MafB and Pax4 and induction of MafA expression (65,66). MafA knockout mice had impaired glucose-stimulated insulin secretion (GSIS) and gradually developed diabetes (67). Furthermore, MafB-expressing insulin-positive cells generated from differentiation of human ES cells were not glucose-responsive (18). These results suggest a need to examine the newly generated β -cells whether they underwent proper maturation. An increasing body of evidence suggests that β -cells in the pancreas are heterogeneous in nature and can be specified by more than one pathway (65,66,68*,69*). It was recently shown that zebrafish β -cells derived from the dorsal pancreatic bud are different from those derived from the ventral bud (69*). Dorsal bud-derived β -cells were quiescent and had reduced expression of insulin, while those from ventral bud proliferated actively and expressed more insulin, Pdx1, NeuroD1, Pax6 and MafA mRNA at 12 days-post-fertilization than their dorsal counterpart (69*). Intriguingly, dorsal bud-derived β -cells expressed more MafB than the β -cells from ventral bud. These results suggest that the origin of β -cells may govern their ability to proliferate and function. However,

a study examining the replication potential of adult β -cells showed that all β -cells contribute equally to islet growth and maintenance (70). This result needs to be reconciled with the zebrafish data unless the proliferation of newly-formed β -cells differs from that of adult β -cells. We suggest that understanding of pathways regulating specification and maturation of β -cells will help determine whether insulin-producing cells generated from differentiation of adult cells have the same capacity to proliferate and mature into glucose-responsive β -cells. This knowledge, combined with improved efficiency of dedifferentiation and redifferentiation of adult pancreatic cells into β -cells, should result in new ways to treat diabetes.

Conclusion

Significant efforts are underway to generate β -cells *in vitro* for cell-based therapies. Over the past few years, significant progress has been made that supports the inclusion of *in vivo* regeneration of β -cells as a potential therapy for diabetes. These studies successfully demonstrate that *in vivo* neogenesis of β -cells could ameliorate STZ-induced and autoimmune diabetes. Combining an efficient delivery system for regulatory factors with ways to enhance efficiency of neogenesis of glucose-responsive β -cell from *in vivo* differentiation of adult cells will be critical for developing novel therapies for diabetes.

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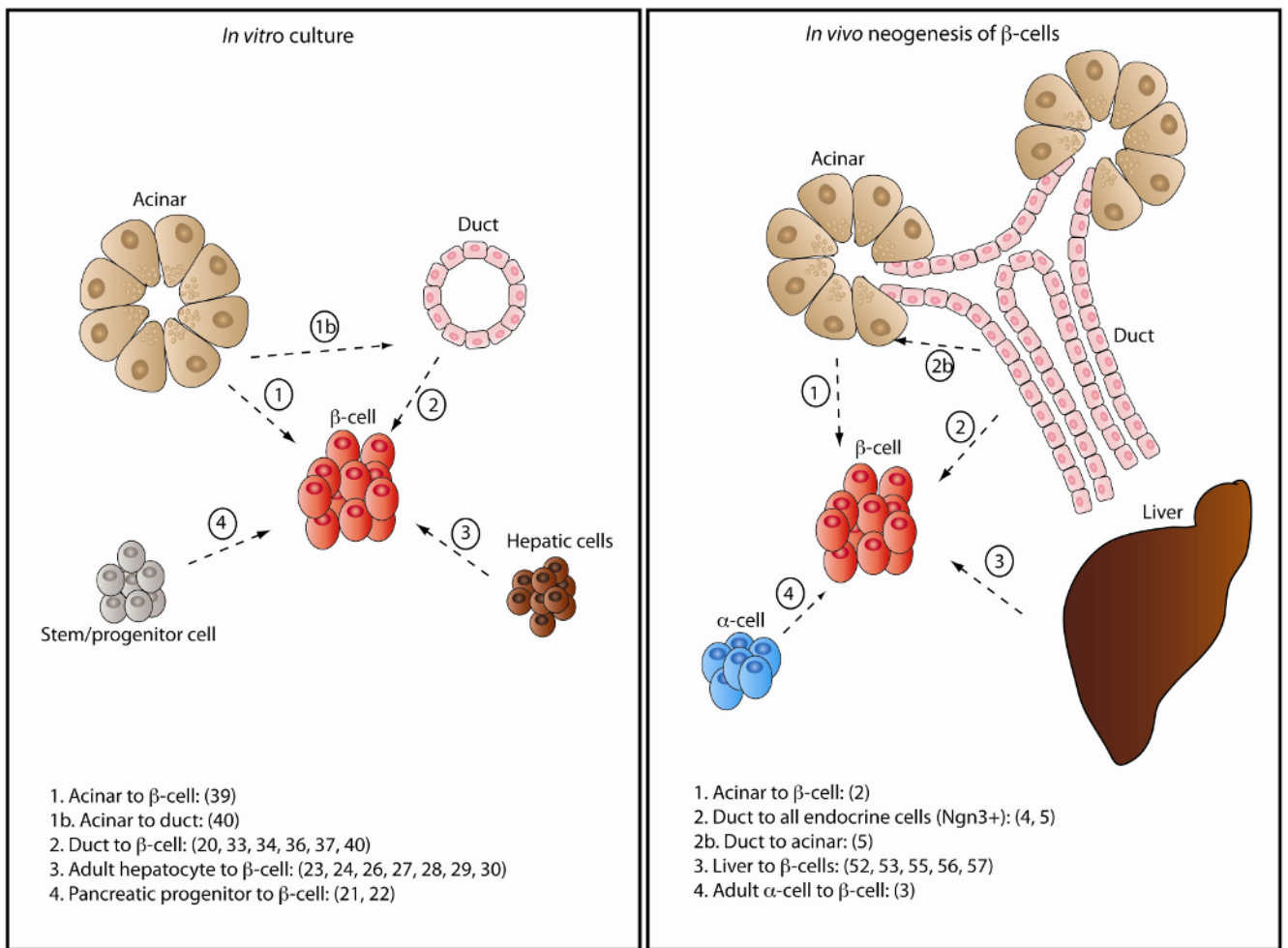


Figure 1. Schematic showing different cell sources that under *in vitro* or *in vivo* differentiation conditions transdifferentiate or dedifferentiate/redifferentiate into insulin-producing cells. Numbers in parenthesis refer to reference numbers.