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REVIEW

The Quest for Tissue Stem Cells in the Pancreas and Other Organs, and their Application in Beta-Cell Replacement

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■ Abstract

Adult stem cell research has drawn a lot of attention by many researchers, due to its medical hope of cell replacement or regenerative therapy for diabetes patients. Despite the many research efforts to date, there is no consensus on the existence of stem cells in adult pancreas. Genetic lineage tracing experiments have put into serious doubt whether β-cell neogenesis from stem/progenitor cells takes place postnatally. Different *in vitro* experiments have suggested centroacinar, ductal, acinar, stellate, or yet unidentified clonigenic cells as candidate β-cell progenitors. As in the rest of the adult stem cell field, sound and promising observations have been made. However, these observations still need to be replicated. As an alternative to committed stem/progenitor cells in the pancreas, transdifferentiation or lineage reprogramming of exocrine acinar and endocrine α-cells may be used to generate new β-cells. At present, it is unclear which approach is most medically promising. This article highlights the progress being made in knowledge about tissue stem cells, their existence and availability for therapy in diabetes. Particular attention is given to the assessment of methods to verify the existence of tissue stem cells.

Keywords: stem cell · duct · progenitor · beta-cell · acinar · transdifferentiation · lineage tracing · reprogramming

Introduction

Islet cell transplantation with the Edmonton and related protocols has provided proof-of-principle for cell replacement therapy as a valid approach for the treatment of type 1 diabetes [1-5]. One limitation in its clinical application is the shortage of donor organs, from which islet cells can be isolated. Therefore, alternative sources are needed for transplantable β-cells, or β-like cells. Pluripotent stem cells like embryonic stem (ES) cells, or induced pluripotent stem (iPS) cells, would be attractive sources, given their unlimited capacity for expansion. However, it is assumed that these undifferentiated cells need to go through numerous successive stages of differentiation, as during embryonic development, to become appropriately differentiated cells like β-cells. This makes the differentiation protocol very complex. But one could hope to shortcut this by using cells already committed to the pancreatic fate. Such cells could be adult pancreatic stem cells. These cells could provide targets for pharmacological intervention aiming at β-cell regeneration in diabetes patients. However, despite a wealth of studies in the last decade, it is still uncertain whether stem cells, or endocrine progenitor cells, actually reside in the adult pancreas (Figure 1). We distinguish between histological studies aimed at identifying progenitor cells inside pancreatic tissue, and *in vitro* studies performed on dissociated pancreatic tissue.

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Are there progenitor cells in adult pancreatic tissue?

Interest in adult islet cell progenitors, or precursors, predates the stem cell concept. Even in the earliest histological studies on pancreatic islets, a relationship was proposed between exocrine and endocrine tissue. Some proponents of this relationship suggested acinar cells, and others ductal cells, as possible islet precursors [6-12]. In the wake of the stem cell "hype" that occurred around the turn of the last century, "dormant" or "facultative" stem cells were thought to reside in the adult gland, possibly in the exocrine ducts [13]. Alternatively, it was proposed that fully differentiated exocrine acinar, and/or duct cells, were able to transdifferentiate into endocrine islet cells [14]. Histological evidence, mostly indirect or correlative, was used to support these hypotheses for β-cell neogenesis. The problem remains that histological studies only give snapshot images, from which it is very difficult to reconstruct the entire picture.

In 2004, Dor *et al.* used the innovative approach of genetic pulse-chase labeling to test the neogenesis hypothesis in adult mice [15]. Their approach was based on an insulin promotor-driven Cre-lox labeling system that is dependent on a tamoxifen-pulse for specific labeling of β-cells at a chosen time point (insulin-CreERT). After that time point, β-cells and their progeny remained stably labeled and could be chased. The authors observed, that within a time frame of one year post-labeling, the proportion of labeled β-cells remained constant. Whereas, it should have decreased if new β-cells had originated from stem cells, or other cell types. In fact, putative progenitor cells are not expected to transcribe insulin at the time of labeling. Therefore, they should provide unlabeled progeny that could "dilute" the labeled islet cells. In the same study, partial pancreatectomy was used as a stimulus for partial regeneration. However, there was no dilution of labeled β-cells in the regenerating tissue. This observation made neogenesis from progenitor cells unlikely (Table 1).

Similar findings were obtained in mice, in which 70-80% of the β-cells had been genetically ablated [16] (Table 1). Another innovative non-genetic lineage tracing technique, based on serial thymidine analog labeling, confirmed these findings in normal adult, pregnant, 50% partial pancreatectomized, and exendin-4 (Ex4) treated mice [17] (Table 1). In a recent study, Blaine *et al.* showed that insulin-positive cells adjacent to hyperplastic ductal epithelium arose from pre-existing insulin-positive cells, after transforming growth factor α (TGF-α) stimulation *in vivo* [18] (Table 1). Previously, it had been suggested that these endocrine cells arose from the 'multipotent' metaplastic ductal epithelium [19, 20]. These experiments demonstrated that in postnatal life, β-cells do not derive from stem cells, but rather renew and expand by the proliferation of β-cells already present shortly after birth.

Abbreviations:
ATP - adenosine triphosphate
bHLH - basic helix-loop-helix
CA-II - carbonic anhydrase II
CD133 - cluster of differentiation 133 (surface glycoprotein, expressed by different stem and progenitor cells)
c-Met - N-Methyl-N'-nitro-N-nitroso-guanidine HOS tranforming gene (encodes Met, also called hepatocyte growth factor receptor)
c-Myc - cellular version of the myelocytomatosis oncogene (transcription factor regulating expression of many genes)
Cre recombinase - type I topoisomerase (catalyzes site-specific recombination of DNA between loxP sites)
CreERT - tamoxifen-induced Cre recombinase (fusion protein with the human estrogen receptor (ER) to induce recombinase activity)
Cre/lox - recombination system to delete DNA sequences in living organisms (also termed Cre/loxP)
DT - diphteria toxin
EGF - epidermal growth factor
ES cells - embryonic stem cells
Ex4- exendin-4
Glucagon-TetO system - glucagon tetracycline operator system
Hnf1b - hepatocyte nuclear factor 1 beta
Hnf1b-CreERT - mouse hepatocyte nuclear factor 1 beta (Hnf1b) promoter Cre-lox pulse-chase system
huCAII-CreERT - human carbonic anhydrase-II (CA-II) promoter Cre-lox pulse-chase system
iPS cells - induced pluripotent stem cells
Kras - Kirsten rat sarcoma (tissue signaling protein propagating growth factor)
LIF - leukemia inhibitory factor
LoxP - locus of crossover in P1 (can be catalyzed by Cre)
MafA - v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (transcription factor necessary for beta-cell maturation)
MAPK - mitogen-activated protein kinase
Ngn3 - neurogenin 3 (member of the bHLH family of transcription factors expressed in the nervous system)
Pax4 - paired box gene 4 (transcription factor involved in fetal and pancreas development)
PDL - partial duct ligation
Pdx1 - pancreatic and duodenal homeobox 1 (transcription factor necessary for pancreas development)
siRNA - short interfering ribonucleic acid
STAT3 - signal transducer and activator of transcription 3
TGF-α - transforming growth factor α

The experiments discussed above do not rule out the possibility that under specific conditions "dormant" progenitor cells might become activated to generate new β-cells, i.e. performing β-cell "neogenesis". It was considered that generation of new β-cells occurred in the partial duct ligation (PDL) model. PDL is an experimental model, in which tissue injury is caused to part of the pancreas. In this model, β-cell number reduplicated within a week post-ligation in the ligated portion of the pancreas [21].

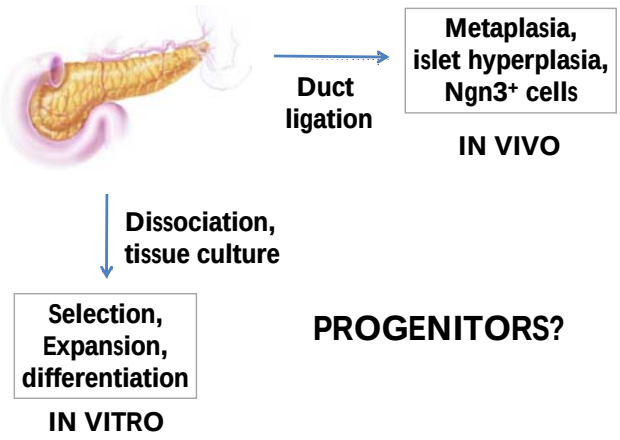

Figure 1. Approaches to identify progenitor cells in the adult pancreas. Two major approaches have been applied to find evidence for adult β-cell neogenesis in the adult pancreas (i.e. formation of new β-cells from progenitor cells). One approach consisted of isolating progenitor cells, and stimulating their neogenic activity *in vitro*. This gave rise to c-Met- [32] or aldehyde dehydrogenase [33] expressing cells, for example. The other approach was based on causing tissue injury to stimulate progenitor cells *in vivo*, for example, cells that express Ngn3 following duct ligation [25]. In both cases, genetic lineage tracing still needs to be done to identify the stem/progenitor cell source.

Inada *et al*. used another genetic lineage tracing model, with human carbonic anhydrase-II (CA-II) promoter acting as a driver of the Cre-lox pulse-chase system (huCAII-CreERT) [22]. This was intended to allow tracing of duct cells which express CA-II, and was applied to duct-ligated mice. The authors found that a significant proportion of β-cells expressed the label in the duct-ligated pancreas. Also, labeled β-cells and acinar cells were found in normal neonates a few weeks after tamoxifen pulse (Table 1). However, more recently, Solar *et al*. found completely opposite results, using a genetic lineage tracing system for duct cells. Their system was based on the mouse hepatocyte nuclear factor 1 beta (Hnf1b) promoter as driver (Hnf1b-CreERT) [23] (Table 1). Hnf1b is a transcription factor that is expressed specifically by all duct cells in embryonic and postnatal pancreas. The authors found that early embryonic duct cells were multipotent progenitors for all pancreatic lineages, but their differentiation potency became gradually restricted. At the end of gestation and after birth, duct cells were only capable of generating other duct cells, and they did not contribute further to β-cell mass, or to other lineages. According to this study, duct cells could not generate β-cells in normal neonatal and adult life, after pancreatic duct ligation, and in a model of β-cell regeneration following alloxan-destruction.

At present, the discrepancy between the Inada and the Solar finding is difficult to explain. A drawback of the Inada study is that it used a human fragment of the CAII promoter to direct Cre-recombinase expression in mice. This could have caused misexpression in cell types other than duct cells. Solar *et al*. ascertained that expression of Cre-recombinase enzyme and reporter enzyme beta-galactosidase was restricted to duct cells. Furthermore, they demonstrated by immunohistochemistry that all duct cells expressed Hnf1b and Cre-enzyme. This ruled out the existence of a postnatal duct cell subset that might have been overlooked by the tracing system that did not ascertain 100% beta-galactosidase labeling of duct cells due to inefficient recombination. More recently, another study appeared, using Mucin-1 genetic labeling to trace exocrine acinar and duct cells that express this gene [24]. This study failed to provide evidence for a contribution by these cells to the islet cell compartment, in normal neonatal and adult life; thereby confirming the conclusion of the Solar study (Table 1).

In another study of duct-ligated pancreas, Xu *et al*. reported the appearance of neurogenin 3 (Ngn3)-positive cells in the duct-ligated pancreas (Figure 1) [25]. These cells were shown to be endocrine progenitors. This conclusion was based on the observation that the cells were able to differentiate into different endocrine cell types *ex vivo*, when isolated and transferred into embryonic pancreatic explants as a suitable microenvironment. Ngn3 expression was required for the observed β-cell mass expansion after duct ligation, since Ngn3-silencing with short interfering (si)RNA partially inhibited the β-cell numerical increase. How-

Table 1. Overview of lineage tracing evidence for cellular conversion in the pancreas (beta-cell and duct cell proliferation and conversion)

Model	β-cell proliferation	Duct cell to β-cell conversion	No duct cell to β-cell conversion	Duct cell proliferation	Duct cell to acinar cell conversion	No duct cell to acinar cell conversion
Neonatal		Inada [22] huCAII-Cre ↔	Solar [23] Hnf1bCreERT2 Kopinke [24] Muc1-CreERT2		Inada [22] huCAII-Cre ↔	Solar [23] Hnf1b-CreERT2
Adult	Dor [15] RIP-CreERT; Teta [17] CldU/IdU		Solar [23] Hnf1bCreERT2 Kopinke [24] Muc1-CreERT2			Solar [23] Hnf1b-CreERT2
Pregnant	Teta [17] CldU/IdU					
Partial Ppx	Dor [15] RIP-CreERT; Teta [17] CldU/IdU					
Pancreatitis						
PDL		Inada [22] huCAII-CreERT ↔	Solar [23] Hnf1bCreERT2	Solar* [23] Hnf1b-CreERT2	Inada [22] huCAII-CreERT ↔	Solar [23] Hnf1b-CreERT2
Allox+E/G			Solar [23] Hnf1bCreERT2			
Exendin-4	Teta [17] CldU/IdU					
TGFα	Blaine [18] Ela500-Cre Villin-Cre					
Genetic β-cell ablation	Nir$ [16] RIP-CreERT					

Legend: Several studies with lineage tracing evidence for cellular conversions in the pancreas have been performed. Different models have been used: *in vivo*, and *in vitro*, from normal development to genetic alterations. Most studies are mutually confirmatory, but some are controversial. Whilst the study of Inada *et al.* proposed adult duct cells as multipotent progenitors [22], the studies by Solar *et al.* and Kopinke *et al.* found no evidence for a ductal derivation of other adult pancreatic cell types [23, 24]. There are no published studies based on lineage tracing of adult stem or progenitor cell markers. Indicated are: the first author, the reference number, and the (promoter) construct used for lineage tracing. Studies shown in green on the same row confirm each other. Studies shown in red, are conflicting (double arrows indicate studies with opposing results). * In PDL, the studies of Solar *et al.* [23] and Desai *et al.* [30] seem to conflict on the contribution of acinar cells to the ductal complexes. However, in Desai *et al.* [30], no quantification was performed. $ Nir *et al.* [16] and Thorel *et al.* [67] appear to be contradictory. However, they used different genetic β-cell ablation models (70-80% ablation in insulin-rtTA TET-DTA, and more than 99% in RIP-DTR mice, respectively). Neonatal: analysis during neonatal period with labeling around birth and analysis at least 2 weeks later. Adult: analysis during adult life. Pregnant: analysis during pregnancy. Partial Ppx: partial pancreatectomy (50-80%). Pancreatitis: acute and chronic pancreatitis induced by caerulein injections. PDL: partial duct ligation. Allox+E/G: β-cell-specific ablation by alloxan followed by EGF/gastrin treatment. Exendin-4: exendin-4 injections. TGF-α: TGF-α overexpression. Genetic β-cell ablation: β-cell-specific ablation in insulin-rtTA TET-DTA or RIP-DTR mice. Pax4: Pax4 overexpression. Ngn3, Pdx1, MafA: overexpression of Ngn3, Pdx1, and MafA by adenoviruses. Kras mutation: mutated Kras (oncogene) overexpression. In vitro: culture of rat or mouse exocrine cells.

ever, it is not clear where these Ngn3$^+$ cells originated. They were frequently observed in the vicinity of ducts, but this could be misleading, as ducts and islets are the only epithelial structures remaining after duct ligation due to acinoductal metaplasia.

The recent demonstration of Ngn3 expression in islet β-cells [26] might challenge the earlier conclusion that Ngn3$^+$ cells are true progenitors. It remains to be ruled out that pre-existing β-cells might upregulate Ngn3 (and proliferate) in the partial duct ligation model. In insulin-Cre tracer mice, labeled Ngn3-positive cells were not detected by immunohistochemical staining. This result suggested that the cells were not derived from β-cells. However, Ngn3 detection by immunohistochemistry is difficult [27]. A better way to examine the possibility that β-cells can give rise to Ngn3-positive cells would be to cross insulin-Cre tracer mice with Ngn3-reporter mice. Also, adult acinar

cells can be stimulated to express Ngn3 [28, 29] (Figure 2). This finding suggests that acinar cells can be a source of Ngn3^{+} precursors in the PDL model. On the other hand, Desai *et al.* found no evidence for a contribution of acinar cells to β-cells after PDL in elastase-tracing mice [30] (Table 2).

In summary, at present there is no clear evidence for the hypothesis that new islet cells originate postnatally from ducts. Insulin promoter-driven cell tracing has not yet been used to demonstrate the existence of β-cell neogenesis postnatally after duct ligation or in other conditions.

Table 2. Overview of lineage tracing evidence for cellular conversion in the pancreas (acinar cell proliferation and conversion)

Model	Acinar-cell proliferation	Acinar cell to β-cell conversion	No acinar cell to β-cell conversion	Acinar cell to duct cell conversion	No acinar cell to duct cell conversion	Acinar cell to hepatocyte conversion	Alpha-cell to beta-cell conversion
Neonatal							
Adult			Desai [30] Ela1-CreERT2				
Pregnant							
Partial Ppx	Desai [30] Ela1-CreERT2		Desai [30] Ela1-CreERT2		Desai [30] Ela1-CreERT2		
Pancreatitis	Strobel [60] Ela1-CreERT		Desai (30) Ela1-CreERT2	Strobel [60] Ela1-CreERT ↔	Desai [30] Ela1-CreERT2		
PDL			Desai [30] Ela1-CreERT2	Desai* [30] Ela1-CreERT2			
Allox+E/G							
Exendin-4							
TGFα			Blaine [18] Ela500-Cre Villin-Cre	Blaine [18] Ela500-Cre Villin-Cre			
Genetic β-cell ablation							Thorel$^{\$}$ [67] RIP-CreERT Gluc-rtTA
Pax4							Collombat [27] Gluc-Cre
Ngn3,Pdx1, MafA		Zhou [52] Cpa1-CreERT2					
Kras mutation				De La O [61] Ela1-CreERT; Guerra [62] Ela-tTA; Habbe [63] Mist1-CreERT2, Ela-CreERT; Morris [64] Ela1-CreERT2; Shi [65] Mist1-CreERT2			
In vitro		Baeyens [28] WGA lectin, Minami [54] Ad-Ela1-Cre Ad-Amy2-Cre, Okuno [55] Ad-Amy2-Cre		Means [57] Villin-Cre Ela-CreERT2		Wu [59] Ela-CreERT2	

Legend: Indicated are: the first author, the reference number, and the (promoter) construct used for lineage tracing. Studies shown in green on the same row confirm each other. Studies shown in red, are conflicting (double arrows indicate studies with opposing results). * In PDL, the studies of Solar *et al.* [23] and Desai *et al.* [30] seem to conflict on the contribution of acinar cells to the ductal complexes. However, in Desai *et al.* [30], no quantification was performed. $ Nir *et al.* [16] and Thorel *et al.* [67] appear to be contradictory. However, they used different genetic β-cell ablation models (70-80% ablation in insulin-rtTA TET-DTA, and more than 99% in RIP-DTR mice, respectively). See also legend of Table 1.

Also, there is no conclusive histological evidence for the existence of pancreatic or endocrine stem/progenitor cells in the adult organ.

Can the existence of pancreatic/endocrine progenitor cells be demonstrated *ex vivo*?

It is possible that adult pancreatic tissue harbors a not yet identified population of stem/progenitor cells. Such "dormant" progenitors could represent an additional source of cells that may be capable of activation in artificial culture conditions, e.g. in the presence of necessary growth factors, feeder cells, or extracellular matrix components. Therefore, researchers have tried several culture conditions on dissociated pancreatic tissue to find out if any of the pancreatic cells are able to differentiate into β-cells. This procedure is called the "gardening approach" (Figure 1). Sometimes, this approach was based on protocols that work with other tissue stem cell types, and at other times, different combinations of growth factors, hormones, or cytokines were tested randomly.

Clonally proliferating cells have been found that showed some differentiation plasticity *in vitro*, evidenced by exhibiting neural and endocrine cell markers [31]. In another study, the c-Met receptor for hepatocyte growth factor was used prospectively to sort cells that could form clonal colonies expressing multiple pancreatic and non-pancreatic markers *in vitro*. The latter included liver, stomach, and intestine markers [32]. Sorted cell preparations expressing aldehyde dehydrogenase enzymatic activity, and that were enriched in centroacinar and ductal cells, were able to form self-renewing "pancreatospheres" in suspension culture. In these organoids, both endocrine and exocrine differentiation occurred [33]. Also, in the study of Ramiya *et al*., digested pancreatic ductal tissue (from prediabetic non-obese diabetic mice) could be subcultured over a long period [34]. This tissue could be induced to produce functioning islets containing α, β, and δ cells. Unfortunately, no lineage tracing was performed to unequivocally identify the origin cells.

CD133 expression is a marker frequently associated with stem cells. Based on this marker, cells were obtained from adult mouse pancreas. The cells showed a high proliferative capacity, but were committed to the ductal lineage [35]. Cells with similar characteristics, but isolated perinatally, showed a certain degree of differentiation plasticity. This observation is in accordance with the finding that duct cells gradually lose their differentiation plasticity during embryonic-fetal life, and become restricted to the ductal lineage postnatally [23]. Also, the CD133 marker has been shown to be expressed on all differentiated centroacinar and duct cells in adult pancreas [36]. This means that it cannot be used for prospective isolation of putative pancreatic stem cells.

Mato *et al*. purified a population of pancreatic stellate cells from lactating rats [37]. These cells expressed the ATP-binding cassette transporter frequently associated with stem cells, and could be grown for over 2 years as a fibroblast-like monolayer. When plated on extracellular matrix and given a cocktail of growth factors, the cells expressed phenotypic markers characteristic of β-cells. In another study, nestin-positive cells from pancreatic islets and ducts showed extended proliferative capacity, and appeared to be multipotent [38]. These cells showed both liver and exocrine pancreas markers, and displayed a ductal/endocrine phenotype after differentiation.

It is largely irrelevant whether the abovementioned observations are material to pathophysiological conditions, or represent *in vitro* artefacts, provided that this "bioengineering" approach can lead us to the derivation of transplantable β-like cells. However, such studies often lead to β-like cells, in which the expression of β-cell marker genes and proteins occurs at very low levels compared to genuine islet β-cells. More studies are needed demonstrating that the obtained insulin-expresssing β-like cells are capable of regulating blood glycemia *in vivo*.

It is also still questionable whether progenitor cells reside within the human pancreas. In a study with duct tissue from human pancreas, using defined culture medium and extracellular matrix, insulin-producing cells originated from the epithelium [39]. When the same conditions were applied to human preparations, from which the contaminating β-cells had first been removed, no new β-cells could be formed [40]. The reason for this discrepancy is unclear, but it emphasizes again the need to use lineage tracing in future experiments of this kind. This also applies to the study by Suarez-Pinzon *et al*. [41]. The authors reported an increase in the number of β-cells after treatment of a mixture of human islet, duct, and acinar cells with epidermal growth factor and gastrin. It is unclear whether the resulting β-cell are a consequence of neogenesis from duct cells.

Some studies have followed another approach to demonstrate the presence of islet progenitor

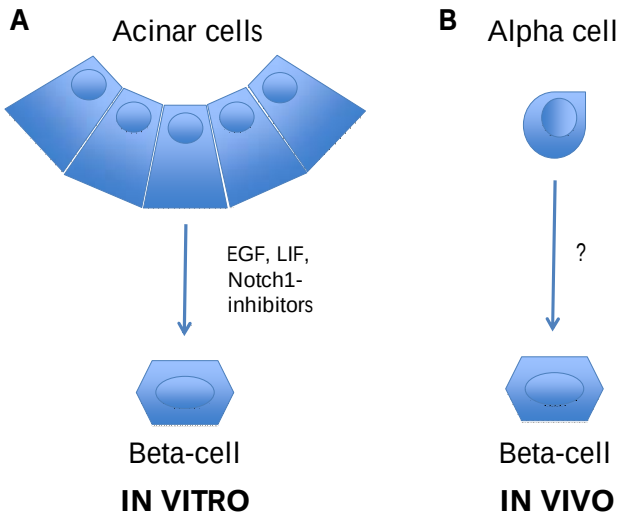

Figure 2. Transdifferentiation in the pancreas. Transdifferentiation, or reprogramming, is the conversion of one differentiated cell type into another. For example, in the presence of LIF and EGF, acinar cells have been shown to transdifferentiate into mature β-cells *in vitro*. This was significantly increased by inhibiting Notch1-Hes1 signaling [28, 53]. α-cells have been shown to transdifferentiate into β-cells *in vivo*, after genetic ablation of the majority of β-cells [67]. In the latter case, it is not known which factors stimulated this phenomenon. Non-genetic and genetic lineage tracing methods, respectively, have been used in these studies to identify the origin cells.

cells in adult pancreatic epithelium, namely by co-transplantation of adult pancreatic and fetal tissue. The rationale for this approach was that the combination of fetal cells, and the *in vivo* environment of the graft, might provide the necessary factors to promote differentiation of putative endocrine progenitors present in the adult pancreatic tissue. It is now twenty years since it was first reported that co-transplantation of rat non-endocrine pancreatic tissue with fetal tissue seemed to induce islet formation in the graft [42]. More recently, this was successfully demonstrated with human cells [43]. In the recent study, islet cells in the graft were from donor tissue, as evidenced by genetic labeling. Interestingly, in the previous studies, preparations may have still contained some contaminating β-cells at study start. Co-transplantation of affinity-purified human duct cells with stromal feeder cells was found to cause the appearance of β-cells in the graft [44]. These studies suggest that there may be cells endowed with a certain differentiation plasticity even in the adult human pancreas. Such cells might be harnessed to generate β-cells in defined culture conditions, although these conditions still remain a "black box" at present. The cellular progenitor characteristics are still unknown. The same applies for the question, whether they represent true self-renewing stem cells, or mature cells that are still endowed with a specific plasticity (see next section).

Transdifferentiation

Transdifferentiation is the conversion of one differentiated cell type into another (Figure 2). Although this approach has already been known for many years [45-47], it has become more popular recently under the term "cellular reprogramming". β-cell neogenesis may result from the differentiation of putative stem/progenitor cells, i.e. cells that have not yet reached a "terminally differentiated state". Alternatively, it could result from the transdifferentiation of mature pancreatic cell types.

Amongst other examples, it was found that introducing genes for three, or four, transcription factors, could convert somatic cells, like skin fibroblasts, into pluripotent stem cells [48-50], or into mature neurons, for example [51], depending on the nature of the transcription factors used. Similarly, *in vivo* delivery of two, or three, transcription factor-encoding genes in mouse pancreas, e.g. Ngn3, Pdx1, and MafA, led to the transdifferentiation of acinar cells into functional β-cells [52] (Table 2). Even more exciting is the possibility of inducing transdifferentiation with growth factors, or cytokines, that do not require viral vector or gene insertion. The *in vitro* conversion of normal rat exocrine acinar cells into functional β-cells was reported first by Baeyens *et al*. [53] (Figure 2). This group used a combination of two factors in the culture medium, namely epidermal growth factor (EGF) and leukemia inhibitory factor (LIF). After only 3 days of culture in this medium, approximately 10% of epithelial cells expressed insulin and other β-cell markers.

Other researchers have reported on *in vitro* transdifferentiation of mouse acinar cells into β-like cells, which was induced by EGF and nicotinamide. This was achieved with cells from normal mice, and from diabetic mice [54, 55] (Table 2). However, this approach was associated with lower efficiency. When EGF and LIF was supplemented, and Notch signaling was inhibited, up to two-thirds of rat exocrine cells acquired the β-cell phenotype [28]. In this study, non-genetic lineage

tracing was performed with a lectin that specifically labeled acinar cells, and which demonstrated the acinar origin of the newly formed β-cells (Table 2). The reprogrammed acinar cells were able to normalize blood glucose levels after transplantation into diabetic animals, thus demonstrating their functional maturity. Although the cells initially showed some phenotypic differences with normal islet β-cells, indicating relative immaturity; they became undistinguishable from normal β-cells one week after transplantation.

The reprogramming of acinar cells to β-cells requires the two factors, EGF and LIF. It also depends on activation of STAT3 and mitogen-activated protein kinase (MAPK) pathways, and on the expression of Ngn3 during an intermediate phase of the transdifferentiation process [29]. As reported earlier with acinoductal transdifferentiation (i.e. reprogramming of acinar cells into duct cells [56, 57] (Table 2)), the acinar cells seem to go through an intermediate phase of relative dedifferentiation. Thereby they acquire characteristics of progenitor cells. They can also transdifferentiate into hepatocyte-like cells [58, 59] (Table 2), which indicates the multipotency of acinar pancreatic cells, and their ability to become reprogrammed in different microenvironments. However, it remains unclear whether acinar cells can also exhibit this potential *in vivo* under pathophysiological conditions (without gene transduction). Genetic lineage tracing, allowing specific acinar cell labeling (elastase-CreERT), revealed that conversion of acinar cells into endocrine cells did not occur. Although acinoductal transdifferentiation was demonstrated by this acinar-specific tracing technique. This was evident in different experimental conditions such as acute and chronic pancreatitis, partial duct ligation, and TGF-α stimulation [18, 30, 60] (Table 2). Also, acinoductal conversion was demonstrated when mutated Kras was expressed in acinar cells [61-65] (Table 2). Bonal *et al.* suggested that inactivation of c-Myc in pancreatic cells, leads to the transdifferentiation of acinar cells into adipocytes [66]. However, their tracing was based on the pancreas progenitor-specific pancreatic and duodenal homeobox 1 (Pdx1)-Cre, and not on an acinar-specific tracing technique. All these studies indicate a high level of plasticity for acinar cells *in vivo,* and *in vitro.* Therefore, these cells could serve as a progenitor pool for several cell types. Analogous to the *in vitro* transdifferentiation of acinar cells to β-cells [53], it would be interesting to study the effect of factors like EGF, and LIF, on acinar cells *in vivo.* Also, the transdifferentiation capacity of human exocrine cells needs to be demonstrated, before this knowledge can be translated to cell replacement therapy.

Another interesting example of transdifferentiation was recently reported by Herrera's group, namely α-cells converting *in vivo* into β-cells [67] (Figure 2). This study made use of efficient genetic ablation of β-cells, using the diphteria toxin (DT) receptor under the insulin promoter. After DT administration, more than 99% of the β-cells were ablated. In mice that received exogenous insulin for survival, there was a slow and partial regeneration of β-cells. Genetic lineage tracing (glucagon-TetO system) revealed that α-cells contributed to this β-cell regeneration (Table 2). Also, α-cell to β-cell transdifferentiation was shown to occur in mice which overexpress Pax4 in mature α-cells [27]; and in a PDL plus alloxan model, where all β-cells that appeared after treatment were neogenic [68, 69] (Table 2).

In some studies, transdifferentiation of mature cells (like acinar or α-cells) with, or without, a prior dedifferentiation event, could explain observations attributed to stem cell differentiation. Now, it is clear that future studies examining β-cell neogenesis, and putative tissue stem cells, will require rigorous testing by genetic or other lineage tracing.

Non-pancreatic stem/progenitor cells

Some adult tissues contain stem/progenitor cells. These cells are responsible for normal tissue renewal. Usually, their differentiation potential is considered to be lineage-restricted i.e. confined to cells of the host tissue/organ. However, recent work has demonstrated that they can be transdifferentiated or transdetermined to other types of tissue. Transdetermination means switching of lineage commitment in lineage-determined, but not terminally differentiated, cells. This approach offers the opportunity for non-pancreatic tissues to generate insulin-expressing cells.

Multiple tissues have been shown to differentiate into insulin-expressing cells, including liver [70-75], bone marrow [76-82], intestine [83], neural tissue [84], epidermis [85], salivary gland [86], and fibroblasts [87]. These studies have been reviewed in this issue and elsewhere [88-90]. However, data on the plasticity of adult stem cells remain controversial. Some of the transdifferentiation and transdetermination events have been attributed to cell fusion. Others are claimed to have only a supporting role in the endogenous regeneration capacity of the pancreas. Also, lineage

tracing has not always been performed in these studies. Finally, many of these studies needed genetic manipulations, which are difficult to apply safely to the clinic.

Conclusions

Similar debates, concerning stem cells in other organs, highlight a major problem in the adult stem cell field. Namely, many research teams are not able to reproduce the seemingly promising results of others [91]. This may be the reason why we cannot yet make a clear conclusion on the existence, and identity, of adult stem cells in the pancreas. *In vivo* genetic lineage tracing experiments have indicated that stem cells do not participate to the β-cell mass postnatally.

However, *in vitro* experiments have provided promising observations on what might represent stem cells. There is a real need to explore further the relationships between the expression of stem cell markers like c-Met, CD133 and aldehyde dehydrogenase, the expression of Ngn3, clonigenicity, and/or self-renewal. Regarding the pancreas, we still await the formation of organoids containing the different mature cell types, from a single adult stem cell in culture, similar to the work already done with gut stem cells [92].

Transdifferentiation of other pancreatic cell types, like acinar cells and α-cell to β-cell, represents an alternative to stem cells. It may find its way to clinical application, if the results obtained in rodents can be translated to human cells.

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