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Stress-Induced Adaptive Islet Cell Identity Changes

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Abstract

The different forms of diabetes mellitus differ in their pathogenesis but, ultimately, they are all characterized by progressive islet β -cell loss. Restoring the β -cell mass is therefore a major goal for future therapeutic approaches. The number of β -cells found at birth is determined by proliferation and differentiation of pancreatic progenitor cells, and it has been considered to remain mostly unchanged throughout adult life. Recent studies in mice have revealed an unexpected plasticity in islet endocrine cells in response to stress; under certain conditions, islet non- β -cells have the potential to reprogram into insulin producers, thus contributing to restore the β -cell mass. Here, we discuss the latest findings on pancreas and islet cell plasticity upon physiological, pathological and experimental conditions of stress. Understanding the mechanisms involved in cell reprogramming in these models will allow the development of new strategies for the treatment of diabetes, by exploiting the intrinsic regeneration capacity of the pancreas.

Keywords

adaptive cell plasticity; cell conversion; cell dedifferentiation; cell fate change; cell identity; cell transdifferentiation; cell reprogramming; diabetes; diabetes treatment; islet; pancreas; transgenic

Introduction

Out of the four pancreatic islet endocrine cell types, β -cells are arguably the most important. Indeed, their physical or functional loss is one of the hallmarks of diabetes, a group of metabolic diseases characterized by the persistence of high unregulated blood glucose levels, and estimated to affect 592 million adults by 2035 [1]. The extent of β -cell loss differs in the different forms of the disease, even in diabetes resulting from autoimmunity [2].

β -Cell numbers have always been thought to remain stable after birth, and undergo only minor variations throughout life [3]. However, multiple studies have shown that the β -cell mass dynamically adjusts when metabolic demand increases or upon injury, at least in rodents but likely in humans as well. Under most circumstances, *proliferation* of pre-existing

β -cells is the major driver of postnatal islet cell expansion [4]. *Neogenesis*, i.e. differentiation of undefined adult progenitors or stem cells, has also been proposed, but the identity and nature of such precursor cells remain controversial [5]. Interestingly, recent studies report that extra-islet (acinar and ductal) and intra-islet (α - or δ -) cells contribute to β -cell mass restoration under stress conditions by *reprogramming* into insulin production [6-9]. These observations have been possible thanks to the development of different *in vivo* cell lineage tracing tools [10], which allow conditional or inducible (through doxycycline or tamoxifen administration) “tagging” of specific cell types in order to track their fate changes.

Callout n° 1: “Cell lineage tracing is a powerful method to irreversibly and selectively mark a given cell type *in vivo*, allowing us to follow its fate.”

The adaptive cell fate “switch” occurs either through direct conversion, with the acquisition of a transitional hybrid intermediate phenotype, or indirectly, through de-differentiation, proliferation and re-differentiation [11]. Despite some of these processes occur spontaneously after injury, their extent is very limited and often insufficient to entirely restore the β -cell mass.

Callout n°2: “In conditions of physiologic or induced β -cell death or dysfunction, the pancreas displays an unsuspected degree of cellular plasticity.”

The ability of pancreatic insular and exocrine cells to convert into insulin producers is a promising finding, as it potentially represents a way to replenish the lost β -cells in Type 1 and Type 2 diabetes (T1D and T2D). Currently, diabetic patients rely on insulin therapy. In most extreme situations of T1D, the only treatment is the transplantation of islets or whole pancreas [12]. One drawback of transplantation is the paucity of donors. Because of this shortage, many laboratories focus on the generation of insulin-producing cells *in vitro* from embryonic stem (ES) cells or, even better, from patient-derived induced pluripotent stem (iPS) cells [12]. These approaches imply the *ex vivo* manipulation of cells, and are hampered by problems linked to inefficient yields, variability in functionality and among donors, as well as viability, immunity and tumorigenesis risks after transplantation *in vivo*.

Taking into account the recent experimental evidence proving the ability of adult exocrine and endocrine cells to reprogram and change their phenotype [13], the interest in *in situ* reprogramming as a strategy to treat diabetes is gaining momentum. For instance, the characterization of signals and factors influencing the intrinsic cell plasticity within the islet cell niche could lead to the development of therapeutic strategies to reconstitute autologous β -like cells, already located in their natural environment, thus overcoming the need of transplantation and the risk of rejection. In any case, nevertheless, a cure for T1D will require our efficient modulation of autoimmunity.

In this review we describe recent studies reporting various cell plasticity events in different stress conditions; we will mainly focus on islet cell-type interconversion phenomena, and have included unpublished data from our laboratory.

Physiological stress

Common physiological and pathological states, such as pregnancy and obesity, are frequently associated with insulin resistance and increased insulin demand [14]. To compensate for this situation and maintain normoglycemia, islets undergo several functional and morphological adaptations, which result in increased insulin secretion and expansion of the β -cell mass [14].

Pregnancy

The mechanisms contributing to β -cell mass expansion during pregnancy in rodents have been elucidated in part. Studies report a 3.8-fold increase in the β -cell mass in pregnant females, ascribed to β -cell hypertrophy and increased β -cell proliferation, with a peak at day 14.5 of gestation [15]. This process is highly dynamic and within ten days after delivery the β -cell mass returns to normal levels, through decreased proliferation, apoptosis and β -cell size reduction [16]. β -Cell expansion through proliferation has been shown to be regulated by hormones, such as serotonin, placental lactogens and prolactin [17]. Whether islet non- β -cells contribute to β -cell expansion is still not clear. In two recent studies, the authors used β -cell lineage tracing tools in pregnant mice to genetically label β -cells and their progeny with human placental alkaline phosphatase or RFP upon tamoxifen (TAM) or doxycycline (DOX) administration, respectively. Interestingly, both studies reported a reduction in the fraction of β -cells that were labeled in pregnant females, as compared with non-pregnant controls; this suggests that an unlabeled cell type, i.e. a non- β -cell population, might contribute to the observed increase in β -cell numbers during gestation [18,19]. Conversely, in another study, Xiao et al. searched for evidence of β -cell neogenesis from non- β -cells using mice in which non- β -cells express the red reporter protein mTomato, while β -cells express the green reporter GFP. In this system, any β -cell of non- β -cell origin expresses both fluorescent reporter proteins, mTomato and GFP, for a period of 40-48 hours. The study revealed the absence of cells expressing both Tomato and GFP on days 14.5–17.5 of gestation, thus suggesting that β -cell neogenesis does not occur in pregnancy [20]. The discrepancy between these studies might be explained by timing differences, implying that non- β -cell recruitment into insulin production could still occur early in gestation.

Studies with constitutive or inducible lineage tracing of non- β -cells have not been reported so far. In this respect, unpublished results from our laboratory, using *Glucagon-rtTA;TetO-Cre;R26-YFP* mice to irreversibly label α -cells upon DOX administration, suggest that lineage-traced α -cells do not reprogram into insulin expression in pregnant mice (not shown).

Interestingly, the analysis of pancreatic samples from deceased pregnant women revealed a 1.4-fold increase in the fractional β -cell area, with increased islet density and β -cells scattered in the exocrine tissue and in ducts. Although these were just snapshots of a dynamic process, these observations suggest that i) there is adaptive β -cell mass increase during human pregnancy, and ii) this could be achieved by differentiation of unidentified progenitors, rather than by duplication of pre-existing β -cells [21].

Obesity

In rodents, the main mechanism implicated in β -cell mass expansion during obesity and insulin resistance is the proliferation of fully differentiated β -cells [22]. All β -cells have been reported to have equal potency to proliferate [23]. However, Sharma and collaborators described that under insulin resistance conditions the β -cell proliferation capacity depends upon activation of the unfolded protein response, which senses an unmet insulin demand [24]. Compensatory β -cell proliferation was also recently described in mice in which insulin resistance is triggered with S961, an insulin receptor antagonist [25,26]. In another study using knock-out mice for the insulin receptor in liver (LIRKO), Ouaamari et al. identified SerpinB1 as a hepatocyte-derived factor that promotes compensatory β -cell response to insulin resistance [27].

So far, changes in islet cell identity in rodent models of obesity and insulin resistance have not been investigated.

Studies in human obese patients report an increase in β -cell mass and number but, surprisingly, without increased β -cell replication [28]. These findings suggest that in obesity, the increase in β -cell mass either derives from sources other than the duplication of existing β -cells, or is too small to be detected, or occurs early in response to insulin resistance/obesity. In line with the first of these hypotheses, Mezza et al. reported an increased number of cells coexpressing CK19 and insulin in subjects with insulin resistance ($1.47 \pm 0.26\%$ vs. $0.28 \pm 0.12\%$). Moreover, the authors observed an increase in the number of glucagon+/insulin+ bihormonal cells in non-diabetic insulin-resistant patients, as compared with insulin-sensitive subjects ($10.86 \pm 2.17\%$ vs. $4.51 \pm 1.07\%$). The percentage of bihormonal cells was higher inside larger islets, thus suggesting that α -to- β conversion could be an adaptive response to insulin resistance. The increase in glucagon+/insulin+ and insulin+/CK19+ coexpressing cells only partially explains the observed increase in the β -cell mass; it could be that most converting α - and ductal cells lose glucagon and CK19 expression, respectively, thus implying that the extent of reprogramming would be higher than that observed, but underestimated because of the lack of lineage tracing tools in humans [28,29]. The same group suggested that poor β -cell glucose sensitivity in obesity would lead to islet cell type interconversion, possibly α -to- β , as an attempt to cope with a higher insulin secretion demand [29].

Yet in summary, because of lineage tracing studies in humans are inviable, the possible contribution of non- β -cells to the β -cell mass in response to insulin resistance cannot be determined, and the reverse possibility, namely that the double positive cells reflect a β -to- α transdifferentiation process, cannot be excluded either.

Stress induced by surgical damage

Islet cell plasticity and the regeneration potential of pancreatic β -cells have been studied using surgical strategies aimed at inducing pancreatitis. Below we provide an overview of cell fate changes after partial pancreatectomy, ligation of the main pancreatic duct and wrapping the whole pancreas in cellophane.

Pancreatectomy

Pancreatectomy is the surgical removal of the pancreas, or a portion of it. Removal of up to 70% of the gland does not lead to diabetes [30], while 90% pancreatectomy is diabetogenic [31]. Regenerative growth upon pancreatectomy has been shown to be proportional to the amount of pancreatic mass removed, and it concerns both exocrine and endocrine cells [31]. Using an inducible *Cre/loxP* system to irreversibly trace the β -cells, Dor et al. showed that β -cell regeneration upon pancreatectomy occurs mainly through proliferation of pre-existing β -cells [4]. However, because of the low efficiency of β -cell labeling ($\approx 30\%$), this strategy would not allow detecting low levels of neogenesis or transdifferentiation, which might eventually take place together with proliferation. This is precisely what other authors claim: that islets arise in proliferating ductal regions [32,33], or that there is a conversion of glucagon-producing α -cells into β -cells. Hayashi et al. reported the presence of cells co-expressing insulin and glucagon at the periphery of islets 2-5 days post pancreatectomy in rats [34]. However, no cell lineage tracing was conducted in this study to specifically determine the contribution of islet non- β -cells to β -cell regeneration.

Whether similar mechanisms operate in humans remain to be defined. Complete pancreas regeneration following 90-95% pancreatectomy has been reported in children aged between 9 days and 2 years [35]. On the contrary, 50% pancreatectomy in adult patients aged between 39 and 72 years, followed for 1.8 ± 1.2 years, did not lead to any increase in the pancreatic volume [36]. This suggests that the regeneration capacity upon pancreatectomy could decline with age or vary depending on the extent of pancreatic mass loss, as suggested in rodents. Yet in a more recent study, the same authors show that adults who underwent 50% partial pancreatectomy recover glucose homeostasis 3.1 years after surgery, which suggests an improvement in the β -cell function even without an increase in the β -cell mass [37]. Interestingly, Yoneda et al. evaluated pancreatic tissues obtained by pancreatectomy from patients in 4 different stages of glucose tolerance: normal glucose tolerance, impaired glucose tolerance, newly diagnosed diabetes and long-standing diabetes. Cells coexpressing insulin and somatostatin or insulin and glucagon were increased in sections from patients with impaired glucose tolerance and newly diagnosed diabetes compared to patients with normal glucose tolerance. The double-positive cells were mainly scattered as single cells or in clusters within the exocrine pancreas, which is compatible with the idea that β -cell neoformation occurs after pancreatectomy in human patients with impaired glucose tolerance or newly diagnosed diabetes [38].

Pancreatic duct ligation

Pancreatic duct ligation (PDL) is a surgical procedure in which the main pancreatic duct is ligated between the head and tail of the organ, resulting in an obstruction of exocrine products drainage out of the body and tail regions of the pancreas. As it does not involve reduction of the pancreatic mass, it is considered as a model of tissue remodeling rather than of regeneration. PDL allows a severe lesion (pancreatitis) to be limited to the distal portion of the gland (body-tail), with little to none in the proximal part (head) [39]. Most of the studies upon PDL have focused on the origin of new islet cells from progenitors located in the ductal epithelium, which activate *Ngn3* expression and give rise to all islet cell types, including glucose-responsive β -cells [40]. In addition, differentiated ductal cells have been

proposed to be a source of β -cells upon PDL, yet this is still controversial. Indeed, using a Cre-based lineage tracing with a human carbonic anhydrase II (CAII) promoter, Inada et al. reported that pancreatic ductal cells are pancreatic progenitors and they give rise to insulin-producing β -cells upon PDL [7]. However, the total percentage of traced β -cells was not quantified before and after PDL, and therefore the validity of this finding is questionable. In addition, other studies failed to lineage-trace β -cells to a ductal origin, using either Hnf1b + [41] or Sox9 + as ductal cell markers [42,43]. Also, Rankin and colleagues concluded that there is no generation of new β -cells after PDL, even if determining the β -cell mass in such massive lesions is difficult [44].

To continue with contradictory observations, at least in appearance, acinar cells have also been proposed to give rise to insulin+ cells upon PDL. Using a tamoxifen-inducible *Ptf1aCreERTM* line, Cheng Pan et al. showed that upon PDL, Ptf1a+ acinar cells activate Sox9 and Hnf1b, thus becoming facultative progenitors and undergoing a long-term reprogramming into endocrine cells, via a ductal intermediate form. In the same study the authors reported that when PDL is combined with β -cell destruction induced by toxin administration (streptozotocin -STZ), there is induction of insulin expression in acinar cells [45].

In another example of confounding observations, PDL combined with ablation of β -cells using another drug (alloxan, ALX) was suggested to induce insulin expression in α -cells [46], yet these observations were not confirmed by others following a similar approach [47]. Both studies were inconclusive since they relied on hormone colocalization rather than on the genetic tracing of cells.

A surgical variation of mechanical stress induction is the wrapping of the pancreas. The application of a cellophane wrap around the head of the pancreas was established in hamsters. This procedure triggers ductal cell proliferation followed by differentiation of glucagon-, somatostatin- and insulin-producing cells, which form new islets, according to the authors [48]. Again, these studies were not conclusive for the same reasons mentioned above.

Stress induced by altering gene expression in islet cells

Changes in islet cell identity have been observed by altering the function of endogenous proteins playing essential roles for endocrine cell function, or upon ectopic expression of transcription factors *in vivo* using transgenic strategies.

The β -cell-specific and inducible expression of a mutated form of the ATP channel (Kir6.2-V59M) leads to hyperglycemia and has been shown to result in ≈ 20 fold increase in the number of glucagon and insulin double-positive bihormonal cells, which was reversed when glycaemia was normalized. Tracing β -cells here revealed that 7% of reporter (RFP)-positive cells contained insulin and glucagon as well as Pdx-1, Glut2 and MafA, and 8% expressed glucagon and MafB and retained expression of Pdx-1 and Glut2, thus indicating a β -to- α cell transdifferentiation [49].

β -to- α cell transdifferentiation has also been reported upon the constitutive expression of Arx in embryonic β -cells [50] as well as upon β -cell inducible inactivation of Pdx1 [51] and constitutive inactivation of DNA methyltransferase 1 [52]. Conversely, forced expression of Pax4 or Pdx1 in mouse embryonic α -cells has been shown to convert them into β -cells [53,54].

Selective constitutive inactivation of FoxO1 (forkhead transcription factor acting as a critical regulator of hepatic glucose and lipid metabolism) in β -cells causes the loss of insulin, Pdx1 and MafA expression, but only in conditions of physiological stress [55,56]. This was coupled to the dedifferentiation into progenitor-like stages, with the expression of the progenitor markers Oct4, Ngn3, Nanog and L-Myc [55]. A fraction of dedifferentiated β -cells subsequently re-differentiate into α , δ and PP cells [55]. The concept of β -cell dedifferentiation was also postulated in a more recent study by genetically inducing a K_{ATP} channel gain-of-function mutation, leading to diabetes [57]. Interestingly, upon restoration of glycemia by administration of insulin, β -cells re-acquired their fate, thus re-expressing insulin [57]. Despite the clear interest of these observations, the dedifferentiation concept remains poorly characterized. Additional experiments are needed to determine whether in these models β -cells reverse to a multipotent progenitor state or they just lose their differentiation markers resulting in cells that resemble immature β -cells.

Evidence of β -cell dedifferentiation has been recently reported in humans by looking at the expression of progenitor markers in endocrine cells, and it has been proposed to be the cause of the β -cell deficit in T2D. In a first study, Cinti et al. defined a dedifferentiated cell as synaptophysin-positive (*i.e.* of endocrine nature) but hormone-negative. By comparing pancreas sections of diabetic and non-diabetic donors, the authors showed an increase in the number of cells co-expressing insulin and synaptophysin, or glucagon-somatostatin-PP and synaptophysin [58]. The number of cells expressing ALDH1A3, a progenitor cell marker, that were hormone negative, was 3-fold higher in diabetics compared to healthy donors. Moreover, FoxO1 and Nkx6.1, normally expressed in the cytoplasm and nucleus of β -cells, respectively, were mislocalized or decreased in the patients: cells contained insulin or not, with cytoplasmic NKX6.1 and lacking FOXO1. The authors suggested that these could be dedifferentiating β -cells. Interestingly, the 2 factors were ectopically expressed in some cells containing either glucagon or somatostatin. All these observations suggest that the dedifferentiation observed in rodents might also occur in human diabetic patients [58]. Yet this interpretation was challenged by others proposing that the cells with mixed identity in diabetics are indeed newly-formed β -cells, rather than derived from dedifferentiation [59]. In the absence of lineage tracing studies in humans, the identity of these cells will remain undetermined.

Recent evidence re-launches the idea that there is a resident pluripotent cell population in the pancreatic ducts of transgenic mice or, more likely, that adult ductal cells can somewhat “easily” reprogram to become endocrine cells. Inactivation of Fbw7 in adult pancreatic ducts, a SCF-type E3 ubiquitin ligase substrate recognition component, induces the reprogramming of some ductal cells into the production of glucagon, somatostatin or insulin [60].

Stress induced by directed selective β -cell destruction

The *in vivo* ablation of a given cell type is one of the most powerful approaches to investigate organ homeostasis and regeneration. This is possible by using either i) *toxins* (f.i. diphtheria toxin, DT) given to transgenic mice carrying their specific receptor on a given cell type, ii) *TAM* or *DOX* administrated to transgenic mice carrying inactive lox P-flanked genes encoding for toxins or proteins inducing β -cell apoptosis, and iii) *drugs* selectively toxic for specific cell types (e.g. STZ, ALX).

Genetic β -cell ablation

Islet cell plasticity has been extensively studied thanks to the development of strategies to selectively ablate β -cells in different experimental models. In some of these studies, proliferation of escaping β -cells was proposed to be the main mechanism leading to diabetes recovery [61,62]. Moreover, interconversion of islet cells was also observed and described as an attempt to regenerate lost β -cells. One of the first pieces of evidence suggesting the existence of islet cell interconversion events upon β -cell loss was reported in 2007, when Pisharath et al. developed a transgenic zebrafish to ablate the β -cells, which become sensitive to metronidazole administration through the expression of nitroreductase under the control of an insulin promoter. Interestingly, by using photo-convertible Kaede to lineage-trace β -cells, the authors showed that insulin+ cells of non- β -cell origin appeared at the islet periphery upon β -cell death, and contributed to β -cell regeneration together with surviving proliferating β -cells [63]. In the same study, by using antisense morpholinos to prevent exocrine pancreas development, the authors showed that β -cell regeneration was independent of exocrine cells, thus implying the possibility that other islet endocrine cells started producing insulin upon injury [63]. Subsequently, using the same system to ablate β -cells together with lineage-tracing δ - and α -cells, the authors showed that α -to- β cell conversion was the main process contributing to islet reconstitution. This was further confirmed by using morpholinos to knock-down the *gcgn* gene or deplete α -cells: both resulted in a significant reduction in regeneration. Interestingly, in this situation the authors noticed an increased number of cells co-expressing insulin and somatostatin, suggesting that δ -cells might acquire plasticity in absence of α -cells [64].

Islet cell plasticity upon injury has also been documented in rodents. Ablation of 70-80% of the β -cells upon DOX administration to *Insulin-rtTA;TetO-DTA* mice results in the appearance of few insulin and glucagon co-expressing cells, yet their origin was not investigated by performing lineage tracing. However, in this model, enhanced proliferation of the abundant surviving β -cells played the major role in regeneration, leading to normalization of blood glucose and islet architecture [62].

The first studies combining extreme genetic β -cell loss together with non- β -cell lineage tracing in rodents were performed by our group [8,9]. Upon near-total apoptotic β -cell ablation induced by DT administration to 2-month-old mice expressing the diphtheria toxin receptor (DTR) at the surface of β -cells, we showed the spontaneous engagement of 1-2% of α -cells into insulin production [9]. One month after DT-mediated injury, between 50-80% or all insulin-expressing cells were in fact reprogrammed α -cells, i.e. were lineage-traced to an α -cell origin. This fate-switch occurs by direct transdifferentiation, reprogramming or

conversion, without transient de-differentiation or proliferation. The reprogrammed α -cells expressed β -cell-specific transcription factors such as Pdx1 and Nkx6.1 together with insulin. Interestingly, a fraction of the converted α -cells retained glucagon expression, implying that without a lineage tracing the number of converted cells would be underestimated. In another study using the same mouse model in combination with δ -cell lineage tracing, we observed that together with α -cells, a small fraction of δ -cells also undergo a direct conversion upon massive β -cell death in adult mice [8]. Indeed, 15-20% of the insulin+ cells found 30 days after DT treatment, were reprogrammed δ -cells. Interestingly, α -cells are progressively recruited into insulin production with time [8], thus allowing the restoration of near normal glycemic values in the long-term, in a sub cohort of diabetic mice.

Callout n°3: “ α - and δ -cells can spontaneously reprogram into insulin production.”

In a more recent study, Shamsi et al. investigated β -cell regeneration in a model mimicking the slow progression and extent of β -cell loss seen in T1D. The authors specifically inactivated TIF-1A in β -cells with TAM administration, thus leading to cell cycle arrest and p53-mediated progressive apoptosis. Adaptive β -cell proliferation was the main mechanism occurring in this model, which led to diabetes recovery. But interestingly, a fraction of bihormonal cells, coexpressing insulin and glucagon, as well as Pdx1 and Arx, also arose in regenerating islets [61].

The islet cell plasticity capacity changes with age. Indeed, when β -cells are ablated in 2-week-old *RIP-DTR* mice, there is no α -to- β cell conversion, since the newly formed insulin+ cells are not lineage-traced to α -cells [8]. Yet there is massive reprogramming of δ -cells in β -cell-ablated juveniles, leading to β -cell reconstitution and diabetes recovery by 4 months of age. Interestingly, the adaptive fate change of δ -cells in juveniles occurs through a mechanism that differs from the one observed in adults: δ -cells undergo dedifferentiation, characterized by somatostatin loss, then proliferate and redifferentiate into β -cells [8].

Pharmacologically-induced β -cell ablation

STZ and ALX are drugs frequently used to induce experimental diabetes in rodents. They both enter the β -cells through the glucose transporter, GLUT 2. STZ is known to be a highly genotoxic alkylating agent, producing DNA strand breaks. ALX induces intracellular ROS formation in presence of intracellular thiols, through a cyclic redox reaction with its reduction product, dialuroic acid. Both drugs trigger morphological and glycemic features characteristic of necrotic cell death [65].

Upon STZ and ALX administration, β -cell loss is followed by regeneration through proliferation of escaping β -cells [66,67] but, based on hormone colocalization, some authors suggest that α -cells could give rise to new insulin-expressing cells. For instance, in a recent paper Cheng et al. showed the reappearance of insulin-producing cells within 48 hours after STZ injection [68]. These newly-generated insulin+ cells could be derived from α - or some mesenchymal cells through the transient expression of vimentin and MafB. Yet, again, a cell lineage tracing analysis to define their origin was missing.

Unpublished evidence from our laboratory reveals that injection of a single high dose of STZ to 2-month-old C57BL/6 mice triggers the appearance of glucagon+/insulin+ bihormonal cells, as detected 1 month after β -cell destruction (Fig.1). These double positive cells could either be i) α -cells that start producing insulin, ii) β -cells that start expressing glucagon, or iii) an undefined cell that starts making the two hormones upon β -cell loss. We lineage-traced them to an α -cell origin, as follows. We treated 2-month-old *Glucagon-rtTA;TetO-Cre;R26-YFP* mice with DOX to irreversibly label α -cells through YFP expression, and then with either multiple low-dose or single high-dose STZ (STZ Low and High respectively), or ALX, or DT (Fig.2A) to induce β -cell ablation. The number of β -cells per islet section dropped by 77.4%, 89.8%, 92.3% and 98.7% in STZ Low, STZ High, ALX and DT respectively (Fig.2B). Interestingly, a small fraction of YFP-traced α -cells was insulin+ 30 days after STZ, indicating that α -cells convert to insulin production after suboptimal β -cell loss (Fig.2C,D). Bihormonal glucagon+/insulin+ cells were never lineage-traced to δ - or β -cells using *Somatostatin-Cre;R26-YFP* and *RIP-CreER;R26-YFP* mice, respectively (Fig.3A,B). As previously shown upon DT [9], about 40% of the reprogrammed α -cells retained glucagon expression (Fig.2B upper lane and Fig.3C). Moreover, the β -cell-specific transcription factors Pdx1 and Nkx6.1 were expressed in a fraction of α -cells after STZ, as we previously reported after DT treatment in *RIP-DTR* mice (Fig. 3D,E) [9].

We performed similar studies tracing δ -cells in 2-month-old *SomatostatinCre;R26-YFP* mice (Fig.4). Preliminary data show that the percentage of YFP-traced δ -cells expressing insulin after STZ-mediated β -cell loss is not increased compared to control animals. However, in STZ-treated animals we detected an increase in the number of YFP+/insulin+ cells co-expressing somatostatin, supporting the occurrence of low-level δ -to- β cell transdifferentiation also in this model (Fig.4). Of note, cells coexpressing Pdx1+ and somatostatin+, with or without insulin, were previously described by others shortly after high-dose STZ treatment [69]; however, because of the lack of any cell tracing analysis, the origin of such cells remained unexplained in those studies.

We previously reported that, like in *RIP-DTR* mice, the δ -to- β cell conversion appears to be age-dependent after STZ-mediated β -cell killing: STZ administration to 15-day-old pups led to the appearance of insulin+ cells, which were in fact reprogrammed δ -cells [8].

Conclusions

The type of injury appears to determine the cellular response for recovery in the pancreas (summarized in Fig.5). The conditions triggering cell plasticity have not been elucidated yet, since this is a recent discovery. We had previously reported α - and δ -cell plasticity, leading to insulin production, in conditions of extreme β -cell death. Here we provide evidence showing that conditions of less severe β -cell demise, such as after STZ or ALX administration to wild-type animals, also lead to insulin expression in α - and δ -cells. These results suggest that α - and δ -cell plasticity does not depend on the mode of β -cell death, being this apoptotic upon DT treatment and mainly necrotic upon STZ or ALX administration. Yet β -cell numbers have to be reduced below a certain threshold in order to unleash the α - and δ -cell plasticity: we previously reported that there is no cell conversion

when 50% or more β -cells remain [9]. This minimal amount of β -cell loss required for triggering non- β -cell conversion events is currently under investigation.

Callout n°4: “ α - and δ -cell reprogramming does not require near-total β -cell loss, and is independent of the β -cell death mode (apoptosis or necrosis).”

The factors and signals controlling adaptive changes in fate among the different pancreatic islet cells are largely unknown. Their identification will help define the molecular mechanisms involved in reprogramming, thus providing the proper niche for its modulation. Reprogramming and reprogrammed cells need to be characterized: a clear evaluation of the maturation/differentiation degree after the change of identity is of particular interest, since partially reprogrammed cells might undergo long-term malignant transformation or revert to the original cellular identity.

Whether cell plasticity is also a feature of human islets will remain unknown until appropriate lineage tracing analyses are performed. Yet, bihormonal cells have been found on human pancreatic sections [70,71], and additional evidences suggesting plasticity in human islet cells come from *in vitro* experiments. Indeed, a recent study with dissociated human islets reports that some small-molecular compounds, such as BRD7389 and GW8510, can up-regulate insulin expression in α -cells, thus promoting the α -to- β -like conversion [72,73]. Similarly, manipulation of the histone methylation signature might be exploited to promote α -to- β cell fate conversion in human cells [74].

In summary, the adaptive changes of cell identity described in the murine pancreas represent an unexpected discovery, still at an early phase. A big amount of basic knowledge must be gathered before *in vivo* lineage reprogramming becomes clinically exploitable. Still out of sight, this perspective is nevertheless realistic, and should in any case spark scientific research that will certainly lead to exciting discoveries. Perhaps, and hopefully, even to new treatments for diabetes.

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Take-home messages(Callouts)

- 1- “Cell lineage tracing is a powerful method to irreversibly and selectively mark a given cell type in vivo, allowing to follow its fate.”
- 2- “In conditions of physiologic or induced β -cell death or dysfunction, the pancreas displays an unsuspected degree of cellular plasticity.”
- 3- “ α - and δ -cells can spontaneously reprogram into insulin production.”
- 4- “ α - and δ -cell reprogramming does not require near-total β -cell loss, and is independent of the β -cell death mode (apoptosis or necrosis).”

Figure 1. Bihormonal cells in islets of wild-type mice after STZ-induced β -cell ablation
A, Experimental design for STZ-induced diabetes in C57BL/6 nontransgenic mice. **B**, Bihormonal cells expressing insulin and glucagon are absent in islets of control mice (left panel). After STZ-mediated β -cell loss, insulin+/glucagon+ bihormonal cells appear in the islets (right panel). Scale Bar is 20 μ m. **C**, Percentage of bihormonal cells in C57BL/6 mice. (control group: 0%, STZ-treated group: 0.312 ± 0.043). * $p < 0.05$.

Figure 2. Insulin production in α-cells after STZ- and ALX-induced diabetes

A, Experimental design for tracing α-cells through YFP expression and ablating β-cells with either DT (3 injections of 120 ng), ALX (1 injection of 60 mg/kg), STZ high-dose (1 injection of 200 mg/kg), or STZ low-dose (5 injections of 60 mg/kg). **B**, Number of β-cells per islet section upon STZ, ALX or STZ induced β-cell ablation. *P 0.05 **P 0.01. **C**, YFP-traced α-cells expressing insulin, upon STZ-mediated β-cell loss. A fraction of the converted α-cells retain glucagon expression (upper panel). Scale Bar is 20 μm. **D**, % of YFP-traced α-cells expressing insulin 30 days after DT-, ALX- and STZ-mediated β-cell ablation (control 0.3±0.1%, DT 1.1±0.3%, ALX 0.8±0.09%, STZ high 0.9±0.2%, STZ low 0.6±0.2%). *P 0.05 **P 0.01.

Figure 3. Characterization of insulin-expressing α-cells after STZ treatment

A, Experimental design for α-, β-, or δ-cell tracing and STZ-induced β-cell ablation. **B**, α-, β-, and δ-cells are efficiently traced with YFP in the 3 transgenic lines (black bars). Cells co-expressing glucagon and insulin are YFP-labelled only in the transgenic line allowing α-cell lineage tracing (red bars), confirming their α-cell origin. **C**, Percentage of YFP+/insulin+ cells retaining glucagon expression 1 month after DT-, ALX- and STZ-mediated β-cell loss. **D**, Immunofluorescence staining of the β-cell-specific markers Pdx1 (upper panel) and Nkx6.1 (bottom panel) in YFP+ α-cells 30 days after STZ-induced β-cell loss. **E**, quantification of YFP+ α-cells expressing Pdx1 and Nkx6.1 (Pdx1: control $0.4 \pm 0.3\%$ STZ $3.7 \pm 0.2\%$; Nkx6.1: cnt 0.34 ± 0.3 & STZ $6.2 \pm 2\%$). Scale Bar is 20 μm . *P < 0.05

Figure 4. Insulin production in δ -cells after STZ administration

A, Experimental design for lineage-tracing δ -cells and ablating β -cells with STZ (1 injection of 200 mg/kg). **B**, YFP-traced δ -cells expressing insulin 45 days after STZ treatment. A fraction of the converted δ -cells retains somatostatin expression (upper panel). **C**, percentage of YFP-traced δ -cells expressing insulin 45 days after STZ-mediated β -cell ablation (control $1.3 \pm 0.7\%$, STZ high $1 \pm 0.7\%$). **D**, Percentage of YFP+/insulin+ cells retaining somatostatin expression 45 days after STZ-mediated β -cell loss. Scale Bar is 20 μm . * $P < 0.05$

Figure 5. Overview of the adaptive cell identity changes in the pancreas in response to a variety of stress / injury conditions.