Pancreatic islet regeneration: Therapeutic potential, unknowns and controversy

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DATES:
Received: 22 Oct. 2014
Revised: 10 Nov. 2014
Accepted: 15 Nov. 2014

KEYWORDS:
diabetes; islets of Langerhans; β-cells; neogenesis; transdifferentiation

HOW TO CITE:
Cockburn IL, Ferris WF. Pancreatic islet regeneration: Therapeutic potential, unknowns and controversy. S Afr J Sci. 2015;111(7/8), Art. #2014-0369, 5 pages. http://dx.doi.org/10.17159/sajs.2015/20140369

Glucose homeostasis in mammals is primarily maintained by the insulin-secreting β-cells contained within pancreas-resident islets of Langerhans. Gross disruption of this glucose regulation as a result of pancreatic dysfunction frequently results in diabetes, which is currently a major health concern in South Africa, as well as globally. For many years, researchers have realised that the pancreas, and specifically the islets of Langerhans, have a regenerative capacity, as islet mass has frequently been shown to increase following induced pancreatic injury. Given that gross β-cell loss contributes significantly to the pathogenesis of both type 1 and type 2 diabetes, endogenous pancreatic islet regeneration has been investigated extensively as a potential β-cell replacement therapy for diabetes. From the extensive research conducted on pancreatic regeneration, opposing findings and opinions have arisen as to *how,* and more recently even *if,* pancreatic regeneration occurs following induced injury. In this review, we outline and discuss the three primary mechanisms by which pancreatic regeneration is proposed to occur: neogenesis, β-cell replication and transdifferentiation. We further explain some of the advanced techniques used in pancreatic regeneration research, and conclude that despite the technologically advanced research tools available to researchers today, the mechanisms governing pancreatic regeneration may remain elusive until more powerful techniques are developed to allow for real-time, live-cell assessment of morphology and gene expression within the pancreas.

Diabetes: Therapies and challenges

The prevalence of diabetes and its comorbidities, such as cardiovascular disease, is increasing rapidly both globally and in South Africa.[1] It is currently estimated that 347 million people worldwide suffer from the disease and the prevalence of diabetes is predicted to double between 2005 and 2030.[2] Indeed, in a recent comprehensive survey on health and nutrition in South Africa, diabetes was diagnosed in 9.6% of the survey participants (aged ≥15 years),[3] which, based on South Africa's current population, equates to ~5 million people living with the disease. Of particular concern is that the prevalence in some demographic groups far exceeds the national average: diabetes was diagnosed in as much as 30.7% of the Asian / Indian study participants.[3] This malady exerts a considerable burden of disease, which will increase with its rapidly escalating prevalence.

Diabetes is commonly subdivided into two types, with type 1 diabetes (T1D) believed to account for only 5–10% of all diabetes cases[4], although little data are currently available regarding the prevalence of T1D in South Africa[5]. The insulin deficiency associated with T1D is caused by an autoimmune destruction of insulin-producing pancreatic β-cells.[6] The hyperglycaemia and ketosis resulting from gross β-cell depletion in T1D patients are currently treated with insulin replacement,[7] and research into potential T1D therapeutics is therefore commonly focused on developing strategies to eliminate the dependence of patients on exogenous insulin. One such approach, which represents a major advancement in diabetes therapy, is islet or pancreas transplantation. Transplantation of either whole pancreata or isolated islets as a means to regain pancreatic endocrine function has been successfully used to reverse T1D; however, limitations – including an insufficient number of donor organs, poor cell viability and undesirable effects of the accompanying immunosuppressive drugs – have meant that transplantation is currently not a feasible and sustainable solution.[8,9] Although type 2 diabetes (T2D) is generally characterised by hyperglycaemia primarily resulting from insulin resistance, insufficient insulin production as a result of the loss of β-cells, as in T1D, is also important in the aetiology of the disease[10], as β-cell function and mass are known to be reduced in T2D patients[11].

To address the limitations associated with the management of diabetes, such as the exogenous insulin-dependence of patients as well as the shortcomings of islet or pancreas transplantation, possible alternative sources of β-cells for replacement therapy have been investigated. These alternatives include the stimulation of the pancreas to promote the endogenous regeneration of viable β-cells. With a view to future pharmacological or cell therapy interventions, researchers have looked to in-utero pancreatic organogenesis to ascertain which molecular pathways may be important for generating increased islet mass. In particular, the temporal expression of transcription factors involved in pancreatic cell fate determination has been investigated and characterised.

In-utero pancreatic development

Knowledge of the in-utero development of the mammalian pancreas, and in particular of the origin and development of the islets of Langerhans and β-cells, provides a starting point for investigations into potential regenerative processes in the adult pancreas. All exocrine and endocrine cell types of the pancreas originate from a common pool of progenitor cells in the gut endoderm of the embryo. A pancreatic bud forms from the endoderm, and subsequently expands and forms branched structures which eventually form the pancreatic ducts.[12] During the extension of these branches, clusters of endocrine cells bud off and aggregate to form the islets of Langerhans.[13] A brief overview of the in-utero development of islet cells and the transcription factors involved in this process is depicted in Figure 1.

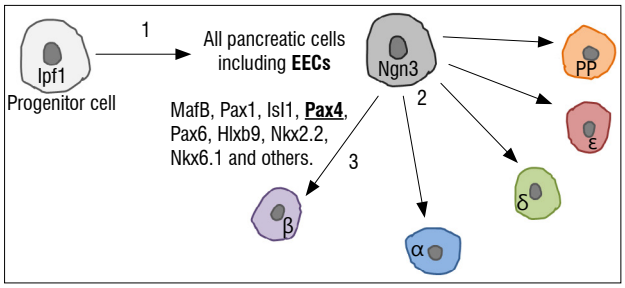

Figure 1: Brief overview of the regulation of in-utero β-cell development by transcription factors during pancreatic organogenesis. (1) Mesenchymal Ipf1-expressing cells in the gut give rise to all pancreatic cells including exocrine endothelial cells (EECs). (2) A subset of EECs which express Ngn3 then differentiate into the five cell types constituting the islets of Langerhans. (3) The development of β-cells from Ngn3+progenitor cells requires the expression of numerous transcription factors including MafB, Pax1, Isl1, Pax4, Pax6, Hlxb9, Nkx2.2 and Nkx6.1.

The transcription factor Ipf1 (insulin promoter factor 1, Pdx1 in rodents) is known to play a major role in pancreatic specification and growth in early embryonic development, and indeed, lineage-tracing experiments have shown that all pancreatic cells, both exocrine and endocrine, arise from Ipf1-expressing progenitor cells.[14] Further along in pancreatic development, the endocrine portion of the pancreas (islets of Langerhans) is formed from a subset of pancreatic duct endothelial cells expressing a second transcription factor central to islet development, namely neurogenin 3 (Ngn3): Ngn3-expressing progenitors differentiate into the five endocrine cell types (α-, β-, δ-, ε- and PP-cells), which subsequently separate from the endothelium and cluster to form the islets of Langerhans.[15,16] Of the five islet cell types, α- and β-cells are the most abundant[17]; and while murine islets consist of a β-cell core surrounded by a mantle of α- and δ-cells, the α-, β- and δ-cells are dispersed throughout human and non-human primate islets[18].

The development of β-cells, in particular from Ngn3$^+$ cells, is regulated by the Pax4 gene[19], a member of the homeobox transcription factor gene family which comprises a large and diverse group of genes that play an important role in embryonic development[20]. Pax4 expression is known to peak during in-utero β-cell development[21], and, in a knockout study, Pax4-deficient mice showed significantly diminished β-cell development[19]. The absence of Pax4 expression in mature islet cells is indicative of the importance of Pax4 expression to β-cell development, in particular, rather than β-cell function or maintenance.[21]

Pancreatic regeneration

Discovery and models

Since the 1960s, many reports have demonstrated in-vivo manipulation of the pancreas by mechanical stress (injury) to stimulate the regeneration of damaged tissue and, importantly, also an increase in islet mass in the pancreas. Ligation or partial occlusion of the main pancreatic duct[22–25] as well as incomplete pancreatectomy[26,27] have been shown to result in pancreatic regeneration – observed as increased mass of the endocrine portion (islets) of pancreata following induced injury. In the 1970 study by Boquist and Edström[22], ligation of the main pancreatic duct in rats resulted in degeneration of acinar cells, with no signs of regeneration of this exocrine moiety of the pancreas. There was, however, an increase in endocrine cells following duct ligation, specifically by endocrine clusters seen to bud off from proliferating ductules and develop into islets. Similarly, Rosenberg and colleagues[23] observed the formation of new islets from hyperplasic (proliferating) ductules after partial occlusion of the pancreatic ducts of hamsters by wrapping a thin cellophane strip around the head of the pancreas. In more significant pancreatic injury (90% pancreatectomy in rats), pronounced regeneration of both the endocrine and exocrine pancreatic tissues was observed 8 weeks subsequent to surgeries.[26] All these studies involved subjecting the pancreata of study animals to prolonged mechanical stress; however, it was later shown that even brief occlusion of the main pancreatic duct (by gently squeezing the pancreas for 60 s) in rats results in duct cell proliferation and signs of islet regeneration[24], as well as overall increased endocrine mass (by 80%) 56 days after surgery, suggestive of islet neogenesis[25]. The fact that apparent islet neogenesis was observed even after only very brief occlusion of the pancreatic duct suggested that the signalling events triggering these regenerative processes in response to pancreatic injury occur immediately at the initiation of injury.

Proposed mechanisms of regeneration

The observations described above lead into key and currently unresolved questions regarding the mechanisms and processes governing islet regeneration. Islet regeneration, and specifically regeneration of β-cells following pancreatic injury, has generally been attributed to one of three mechanisms: transdifferentiation of non-endocrine cells into β-cells, neogenesis of β-cells from progenitor cells, or replication of existing β-cells (Figure 2); however, contradictory opinions and data surrounding these theories are plentiful.

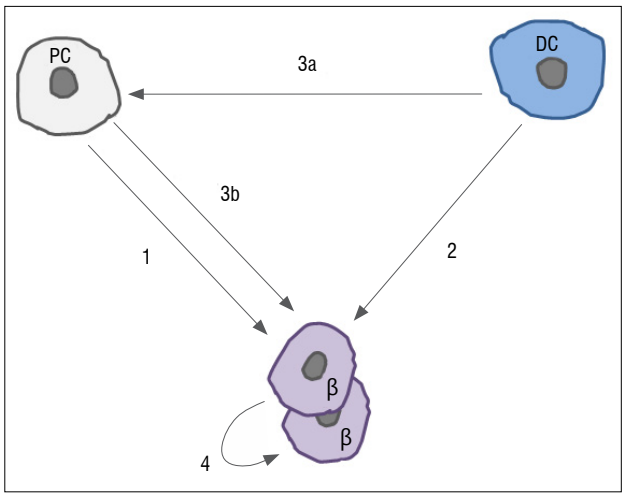

Figure 2: Proposed mechanisms of β-cell regeneration. β-Cells (β) have been proposed to regenerate via one of several mechanisms: (1) neogenesis via differentiation of specialised progenitor cells (PC); (2) transdifferentiation of differentiated non-β-cells (DC) to β-cells, in which DCs are either exocrine (acinar or ductal) or endocrine (α-cells) non-β-cells undergoing either exocrine-to-endocrine or inter-endocrine transdifferentiation, respectively; (3) de-differentiation of differentiated non-β-cells to progenitor-type cells (3a) and subsequent re-differentiation of these progenitor-type cells to β-cells (3b); or (4) replication or self-renewal of existing, differentiated β-cells.

Transdifferentiation

As islet cells originate from a subset of duct cells during pancreatic organogenesis (as described above), transdifferentiation has been investigated as a possible mechanism by which islet regeneration occurs in the adult pancreas. In adult rats subjected to pancreatic duct ligation, transdifferentiation has been identified in the pancreas in the form of cells co-expressing markers from more than one terminally differentiated cell type, indicating a mixed lineage. These cells include those expressing both epithelial and β-cell markers; cells co-expressing epithelial and α-cell markers; duct cells expressing GLUT-2, the β-cell-specific glucose transporter protein[28]; or intra- and extra-islet cells co-expressing acinar cell and β-cell markers[29]. In these and other studies,[30–32] increased islet cell number following pancreatic injury was thus attributed mainly to the transdifferentiation of non-endocrine (acinar or duct) cells to endocrine (α- or β-) cells. In an in-vitro study, AR42J acinar-derived amylase-secreting cells were converted to insulin-secreting cells by treatment with the growth factors betacellulin

and activin A,[33] and although insulin secretion by these cells does not confirm their identity as β-cells, the observed conversion is suggestive of exocrine-to-endocrine transdifferentiation. In a more recent lineage-tracing study on transgenic mice subjected to dipthera toxin-induced β-cell ablation, inter-endocrine transdifferentiation was also suggested to occur: new β-cells were identified as arising from α-cells.[34]

Desai and colleagues[35] refuted the hypothesis that exocrine acinar cells transdifferentiate into endocrine β-cells based on the findings of their in vivo lineage-tracing experiments in which the acinar cells of transgenic mice were genetically labelled. This labelling strategy allowed for the progeny of these cells to be identified, thereby enabling the investigators to identify cells of acinar origin. Various models of pancreatic injury were used to induce regeneration, after which a lack of labelled endocrine cells was observed, leading the authors to conclude that 'acinar cells do not normally transdifferentiate into islet beta cells in vivo in adult mice'[35]. The findings of another genetic labelling-based study further countered the notion of transdifferentiation being the mechanism by which islet regeneration occurs by indicating that β-cells only arise from duct epithelial cells during embryogenesis, and that these epithelial cells do not significantly contribute to endocrine or acinar cell populations after birth.[36]

The findings described here clearly demonstrate that reports regarding transdifferentiation as a mechanism by which pancreatic regeneration occurs are as contradictory as they are plentiful.

Neogenesis from specialised progenitors

A population of specialised progenitor cells in the pancreas that can give rise to new β-cells would be extremely valuable for the development of endogenous cell therapies for the treatment of diabetes-associated β-cell depletion. Such a cell population could conceivably either be isolated, expanded ex vivo and used for transplantation or alternatively be stimulated to produce new β-cells in vivo. Thus far, however, whether or not such a specialised progenitor cell population exists in the pancreas remains unclear despite a number of reports in support of the existence of such cells. In corroboration with the existence of progenitor cells within the pancreas, flow cytometry has been used to identify a side population of cells in the murine pancreas which has been described as a putative stem cell population. These cells, identified as stem cells based on their ability to expel the Hoechst 33342 DNA-binding dye, were shown to undergo hyperplasia in vivo after β-cell or pancreas injury and, upon induction, the cells proliferated and differentiated in vitro giving rise to endocrine cells that exhibited glucose-stimulated insulin secretion.[37] These findings led the authors to conclude that progenitor cells that give rise to endocrine cells exist within the pancreas, a conclusion also reached by other researchers who identified endogenous β-cell progenitors on the basis of the expression of the islet cell-specific transcription factor Ngn3: Ngn3-expressing cells located in the ductal lining of the pancreas were shown to be multi-potent progenitor cells with the ability to give rise to new glucose-responsive β-cells both in situ and in vitro.[38] Very small embryonic-like stem cells (VSELs), a novel type of pluripotent stem cell reported to exist in various adult murine organs including the pancreas,[39] were recently reported to mobilise to the pancreas following partial pancreatectomy in mice. These VSELs reportedly differentiated into progenitor cells expressing Pdx1 (the transcription factor expressed by progenitor cells that differentiate into all pancreatic cell types during organogenesis), potentially giving rise to new acinar and islet cells.[40]

Based on the findings of another study using a partial pancreatectomy model in rats, islet regeneration has been suggested to occur via initial de-differentiation of duct cells to progenitor-type cells, followed by re-differentiation of these cells, which 'recapitulate aspects of embryonic pancreas differentiation' to facilitate pancreatic regeneration[41] – a mechanism which can be classed as neogenesis or transdifferentiation, or indeed a combination of the two.

A report opposing the hypothesis that β-cell regeneration following pancreatic injury occurs via neogenesis was recently published: the results of lineage-tracing experiments carried out using various models of β-cell loss in the adult murine pancreas led the authors to conclude that little to no β-cell neogenesis occurs in the adult pancreas under normal and pathological (partial pancreatectomy, duct ligation or treatment with β-cell-specific toxins) conditions.[42]

β-cell replication

The controversies and conflicting opinions within the field of islet regeneration are clearly demonstrated by the literature on β-cell replication as the mechanism driving β-cell maintenance and/or regeneration: 'Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation'[43]; 'β-cell replication is the primary mechanism subserving the postnatal expansion of β-cell mass in humans'[44] and 'β-cell growth and regeneration: Replication is only part of the story'[45] are the titles of just some of these publications.

It was initially believed that significant β-cell proliferation does not occur after the initial periods of β-cell mass growth during the neonatal and infancy stages of development, and that β-cell mass is not replenished or maintained by β-cell proliferation.[46-48] Studies on β-cell replication have thus generally been carried out to assess not only β-cell replenishment following pancreatic injury, but also increases and maintenance of β-cell mass under normal physiological conditions. Similar to the lineage-tracing experiments carried out by Desai and colleagues[35] (described previously), Dor et al.[43] made use of a transgenic mouse model and a tamoxifen-inducible Cre/lox system to integrate genes into the genome by which β-cells were specifically labelled with a histochemically detectable, heritable label. Subsequent to removal of the initial 'pulse' (tamoxifen treatment), only pre-labelled β-cells and progeny of such cells would carry the detectable label, allowing newly formed β-cells to be identified as being β-cell-derived or non-β-cell-derived. These genetic labelling experiments led the authors to conclude that, during normal adult life or following pancreatectomy in the murine pancreas, adult β-cells are formed or replenished by replication rather than by islet neogenesis or differentiation of stem cells.[43] In agreement with the conclusion reached by Dor and colleagues, a later human study in which β-cell mass was assessed using computer tomography techniques revealed that β-cell mass expansion during infancy, the period during which β-cell growth rates were found to be highest, occurs primarily via β-cell replication[44]; and in a transgenic mouse model, near-complete β-cell ablation was followed by full recovery of the pancreas, which was described to occur via β-cell replication within existing islets rather than islet neogenesis[49]. In an extensive review by Bonner-Weir and colleagues[45], β-cell replication and neogenesis as mechanisms of pancreatic regeneration or postnatal islet growth are described as not being mutually exclusive. The authors go on to review the many reports on pancreatic regeneration and conclude that both neogenesis and β-cell replication contribute to β-cell mass maintenance, and that both these mechanisms have the potential to be harnessed for therapeutic applications.[45]

Doubt shed on regeneration

Controversy surrounding β-cell regeneration has recently extended from differing opinions on the mechanisms allowing for regeneration, to doubts being raised as to whether regeneration following pancreatic injury does in fact occur at all: Rankin et al.[50] recently reported on extensive experiments carried out in an adult mouse model which, in agreement with the report by Xiao and colleagues[42], show that adult β-cells do not develop from specialised pancreas-resident progenitor cells following pancreatic injury and, importantly, also that new β-cells are not generated following pancreatic duct ligation.[50] The authors of this report attribute the apparent regeneration that has frequently been described for similar pancreatic injury models to quantitative artefacts and variable recovery of pancreatic tissue; an extensive morphometric assessment of the entire murine pancreas in Rankin et al.'s study indicates that β-cell mass is unaltered in the ligated pancreas compared with sham-operated tissue and therefore that injury of the adult murine pancreas does not induce β-cell regeneration.[50]

Both Xiao et al.[42] and Rankin et al.[50] conclude that β-cell neogenesis does not occur following pancreatic injury; however, in both these studies, the expression of the islet-specific transcription factor Ngn3 was shown to be increased following pancreatic injury. Kushner and colleagues attributed the observed induction of Ngn3 expression by ligation in their study to an 'artefact due to differences in RNA recovery from injured compared with uninjured pancreas'[50], while damage to the injured portion of the pancreas (specifically exocrine cell contents and various inflammatory factors) was proposed to induce an up-regulation of Ngn3 expression in existing β-cells by Xiao and colleagues[42].

Perspective

In this brief review we have presented the diverse and often contradictory findings of some of the many investigations into pancreatic regeneration carried out over the last five decades. It is clear from these studies that putative islet regeneration is a complex, poorly understood and controversial research area, but the potential benefits of understanding and possibly harnessing the processes involved are immense. Despite recent reports contesting the existence of β-cell regeneration, a lot of unknowns in this field remain to be clarified: Why do different studies obtain vastly different results when investigating the same models and systems? If pancreatic regeneration does not take place, why is Ngn3 up-regulated following pancreatic injury?

Although sophisticated techniques available to us today, such as genetic lineage-tracing technology, are extremely powerful, they too are limited. In the case of investigations into pancreatic injury-induced events, the ideal scenario would be one in which cells can be monitored in real time within the pancreas, simultaneously assessing both morphological changes and gene expression. Although three-dimensional microscopy and the culturing of whole or partial fragments of tissues have advanced considerably in recent years, this ideal is still beyond our capabilities. Until such time that technological advances will allow for such assessments to be carried out, definitive mechanisms that potentially stimulate an increase in β-cell mass triggered by pancreatic injury remain elusive.

Acknowledgements

We thank the Harry Crossley Foundation for funding our research on pancreatic islet neogenesis.

Authors' contributions

I.C. researched and wrote the review; W.F. was involved in planning the review outline and edited drafts of the manuscript.

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