Models of Regeneration of Beta Cells in Vivo
Ioana Ilie¹*, MD,PhD, Razvan Ilie², MD

Abstract - Due to the widespread increase in the number of subjects suffering from diabetes, our understanding of the principles governing the formation and function of this organ need to be greatly improved. Although in vitro generation of insulin-secreting cells from embryonic stem cells, induced-pluripotent stem cells and adult stem/progenitor-like cells has been reported, most of these cells are functionally immature and show poor glucose-responsive insulin secretion compared to that of native pancreatic b-cells. The recent evidence that cells in the adult pancreas exhibit more plasticity than previously recognized has opened up new areas of research, such as cellular reprogramming and in vivo β-cell regeneration. Increasing the understanding and finding the right strategies to generate functional b-cells or a whole organ in vivo would not only bring enormous benefits to type I diabetes patients but could also constitute a promising approach to treating type II diabetes as well as type I diabetes in paediatric patients where islet transplantation, even with its substantially improved outcome in recent years, is still not indicated.

Key words - β-cell, in vivo, regeneration

I. INTRODUCTION

Due to the widespread prevalence of debilitating diseases caused by pancreatic dysfunction, our understanding of the principles governing the formation and function of this organ need to be greatly improved [1]. There are more and more patients suffering from diabetes all over the world. For example, according to the American Diabetes Association, about 8% of the population of the United States is affected by diabetes. The prognosis of pancreatic cancer, specifically pancreatic ductal adenocarcinoma, is extremely bad, with an average survival of 6 months, and 5 year survival for only 3%–5% [2].

Our comprehension of the development, functional capacity, and cellular plasticity of the organ will greatly contribute to the understanding the etiology of these diseases, and the subsequent discovery of cures [1]. The dependence of type 1 diabetics (T1D) on exogenous insulin has been significantly decreased by whole pancreas and islet transplants using the Edmonton protocol [3]. However, a drawback is the limited availability of cadaver tissue that makes transplants only feasible for a small fraction of patients. Because of this, there have been various alternative approaches to generate β-cells that can be transplanted to ameliorate hyperglycemia, such as differentiation of embryonic stem cells, expansion of existing cells, and finally, directing liver and other pancreatic cells to β-cells [1, 4]. However, taking into account the new experimental evidence that proves the ability of adult differentiated pancreatic cells to reprogram and change their phenotype [5], exploration of the intrinsic spontaneous capacity of the adult pancreas to regenerate β-cells, particularly from heterologous origins, has lately enter the spotlight as means for the development of therapeutic treatments for diabetes [6].

This review summarizes the regenerative processes of the pancreas monitored in animal models in vivo, as well as the methods of promoting regeneration of the pancreas in vivo for the treatment of diabetes in the future.

II. MECHANISMS OF B-CELL REGENERATION IN VIVO

There are two major possible pathways to beta-cell regeneration, beta-cell replication and beta-cell neogenesis. Hence, new pancreatic b-cells could be generated by induction of self-replication (proliferation) of pre-existing islet β-cells or of adult stem/progenitor cells (neogenesis) in vivo [7] (Figure 1).

In summary, regenerative processes of the pancreas observed in animal models in vivo are composed of several complex mechanisms: replication and proliferation of preexisting β-cells in the residual islets, β-cell neogenesis in residual islets (by intraislet progenitor cells), the proliferation of duct cells and subsequent differentiation into new β-cells, which may include progenitor cells in and/or around the expanding duct, dedifferentiation of acinar cells and differentiation into β-cells or transdifferentiation of acinar cells to islet cells and transdifferentiation of alpha-cells into β-cells [1, 4].
Figure 1: Proposed regeneration routes in the adult endocrine pancreas. There are two major pathways to β-cell regeneration, β-cell replication and β-cell neogenesis.

The in vivo regenerative process in the pancreas is a great promise for a permanent cure for diabetes in the future. This can be achieved by combining favorable growth and differentiation factors. The plausible site that could enhance the regenerative process of the pancreas may be divided into several steps: (1) a triggering step for the initiation of pancreatic stem progenitor cells replication (2) the proliferation of progenitor cells for physiological demand, and (3) differentiation of the progenitors into fully functional β-cells. Though there have been insights on steps (2) and (3), further investigations of effective factors are needed in future studies [4].

Another approach would be represented by a gene delivery method which has attempted to promote in vivo regeneration. Taniguchi et al. [8] performed adenoviral vector mediated gene delivery of transcription factors PDX-1 and ngn3 in the mouse pancreas by retrograde intracommmon bile ductal injection. The gene delivery of PDX-1 in the duct induced the proliferation of pancreatic ductal cells and neogenesis of insulin-producing cells. The treatment of diabetes may greatly benefit from this new approach [1, 4].

Proof of the In Vivo Regeneration Capacity of β-Cells

B-cell expansion takes place through neogenesis from progenitors within the pancreatic epithelium during embryonic pancreas development. However, in postnatal life, the turnover rate of pancreatic β-cells in adult is generally low and under normal conditions, β-cell proliferation through the cyclinD2-CDK complex is the main method of adjusting for changes in insulin demand, e.g., during pregnancy or development of obesity. With age, however, accumulation of tumor suppressors p16INK4a/p19ARF triggers Bmi-1 and Ezh2 [2, 9].

The in situ regenerating pancreatic β-cells have been proven promising factors by the significant proliferation capacity of postnatal rodent β-cells in situations of increased metabolic demand [6, 10] as well as by the normal expansion of the β-cell mass that takes place during the neonatal period [11] or with obesity in adult cells in humans [12, 13].

Moreover, evidence of cell regeneration has been supported by the identification of residual β-cells in T1D patients after onset [14] or even many years after diagnosis [15-17]. However, an interesting equivalent of the “honeymoon period” has been described in T1D patients during pregnancy with measurable C-peptide levels and transient reduction of the insulin requirements [18]. This phenomenon has been initially accounted for by replication [19], but a recent autopsy study on pancreases during or after pregnancy suggested neogenesis by identifying increased in the relative cells volume, in the small islets proportion, as well as in the number of insulin cells in the ducts but no change in β-cell replication, cell size, or apoptosis frequency [20]. Therefore, the possibility of using cell regeneration for therapy is still debatable.

Neogenesis has recently been under intense debate, with many lineage tracing studies in mice showing contradictory results [21-23]. The type and extent of pancreatic injury influences neogenesis from ducts and this has been shown by a recent study that demonstrated different regeneration depending on the affected cell type and the extent of diphtheria toxin-induced apoptosis[24]. When both acinar and islet cells were massively killed by diphtheria toxin expressed under the Pdx1 promoter, duct cells gave rise to both acinar and endocrine cells, recovery of 60% of the -cell mass, and reversal of hyperglycemia. However, when only acinar cells were killed by elastase-driven toxin, duct cells only gave rise to new acinar cells. Moreover, there is evidence that neogenesis from the ducts takes place by recapitulating pancreatic embryonic development as proved by using a pancreatic duct ligation (PDL) model in the mouse [25] and partial pancreatectomy in the rat [26, 27].

In the human pancreas, cells coexpressing cytokeratin and insulin indirectly suggest neogenesis [28]. Many studies have identified insulin-containing cells within the ducts, either at autopsy [11] or in biopsy from organ donors [28, 29]. Currently, it has been generally accepted that after birth, neogenesis from ducts occurs mostly in the neonatal period and that it can be stimulated in the post-injury regeneration (as shown in rodents) [21].

Stimulation of In Vivo Generation of New β-Cells
In rodents, growth factors such as betacellulin (BTC) [30] or combinations of glucagon-like peptide (GLP)-1/gastrin [31] or epidermal growth factor/gastrin[32, 33], have proved to be potential factors in the cell mass growth and diabetes reversal by stimulating β-cell replication and neogenesis, though this has not been demonstrated in humans, too [10, 34].

It has been reported that BTC, acting in coordination with activin A, converted pancreatic AR42J cells into insulin-producing cells [4, 35]. Moreover, several in vivo studies indicated that BTC can promote the regeneration of β -cells by acting in multiple steps, and the combination of activin A and BTC may be a more effective treatment [4]. Islet neogenesis associated protein-pentadecapeptide (INGAPP) was shown to stimulate neogenesis, reversing diabetes in streptozotocin (STZ)- treated mice [36]. However, when studied in humans, the results of INGAP-PP were unclear - only isolated parameters being very slightly improved (arginine-stimulated C-peptide secretion in the T1D trial, HbA1C levels in the T2D trial)[37, 38]. Incretin therapy or GLP-1 receptor agonist and then biological half-life, long acting analogues (namely, exenatide and liraglutide) and inhibitors of the degrading enzyme DPPIV have proved great potential in diabetes therapy, after proof-of-concept studies were performed in T2D patients to whom GLP-1 was administered [39-41].

T2D patients might greatly benefit from these agents through insulin secretion stimulation (incretin effect), inhibition of glucagon release, delay of gastric emptying, and decrease of food intake. However, although GLP-1 receptor agonist has been shown to increase β-cell mass in rodents [42, 43], long-term data have yet to provide evidence for such increase in T2D patients [10, 44].

β cell self-renewal

The major mechanism leading to regeneration of insulin producing cells in the adult pancreas is self-renewal of preexisting β -cells. Preexisting insulin-producing cells have been widely agreed to possess the ability of undergoing self-renewal under normal physiological condition[45, 46] and following injury [47],—including pancreatectomy, diphtheria-toxin-induced β-cell ablation, and pancreatic duct ligation [45, 48-50]. Hence, the increase of β -cell mass in obese individuals and during pregnancy seems to be driven by the amplification of preexisting β -cells [47, 51].

Most studies on the regeneration of pancreatic b-cells in vivo have been performed in rodents using pancreatic injury models. Nicotinamide, an inhibitor of poly(adenosinediphosphate-ribose)synthethase/polymerase, prevents diabetes development in experimental animals after administration of the β -cell toxins, streptozotocin and alloxan [52]. Though by an yet unknown mechanism, the proliferation and differentiation of pancreatic endocrine cells have been proved to benefit from this agent, as demonstrated by many in vitro studies [53, 54]. Exendin-4, an analog of GLP-1, has been reported to enhance both proliferation and neogenesis of pancreatic b-cells in rats with 90% pancreatectomy [42]. BTC, a growth factor belonging to the epidermal growth factor (EGF) family, has been shown to promote neogenesis of β -cells and ameliorate glucose intolerance in mice with selective alloxan perfusion [7, 30] and is also reported to enhance proliferation of β -cells in 90% pancreatectomized rats [7, 55].

Neogenesis

• Stem/progenitor cells in the adult pancreas

Though stem/progenitor cells presence in the adult pancreas and their possible location are still under intense debate [7], at least two populations of pancreatic stem cells have been possibly identified in the pancreas. The first group of stem cells consists of ductal cells and acinar cells. These cells are pancreatic ductal epithelium cells that express the ductal marker CK-19 and PDX1 [56]and have, thus, the ability to expand and differentiate into endocrine cells [57]. The second group of stem cells consists of islet derived stem cells. The existence of an islet-derived population of stem cells or intraislet progenitors which can lay an important role in islet neogenesis is supported by studies that show a population of insulin-containing cells reappearing in the islets after total destruction of the b-cell mass with streptozotocin [47, 58-61] (Figure 1).

Moreover, researchers have been striving to identify the precise origin of adult endocrine endogenous progenitors that may originate from the ductal epithelium [62, 63]. However, it has not been yet clearly stated whether just a subset or all cells from the duct epithelium contain a progenitor potential or if other pancreatic cell types, (e.g., acinar cells) could function as endocrine stem cell/ precursors or transdifferentiate into β-cells [64].

Thus, acinar AR42J cells proved to generate insulin- and glucagon-producing cells, when treated with BTC, activin, or glucagonlike peptide, although several lineage tracing experiments demonstrated that preexisting acinar cells do not contribute to endocrine cells [35, 65]. Additionally, it has been showed that centroacinar/terminal duct cells express stem cell markers and may, therefore, generate progenitor cells engaged in islet regeneration, as shown by others [66, 67] (Figure 1). Furthermore, ectopic combined expression of three factors, Pdx1, Ngn3, and MafA can lead to the transdifferentiation of acinar cells into functional beta-cells in mice, and clearly sustain the inherent ability of the acinar compartment to generate islet cells [68].

Moreover, various studies of pancreatic injury models, and transgenic mice showed that progenitor/stem cells reside in the duct epithelium, where cells expressing the proendocrine marker Ngn3 were spotted. It is extremely important to demonstrate the robust
regeneration capacity of alpha-cells, triggered by alterations in the glucagon signaling pathway [47, 69].

- **Pancreatic duct cells and pancreatic acinar cells as facultative progenitors in adult pancreas**

There are several lines of studies suggesting the cell origin of regenerated pancreatic β-cells. Studies on transgenic mice expressing interferon-gamma specifically in pancreatic β-cells demonstrated a dramatic proliferation of pancreatic ductal cells, and the appearance of endocrine cells and their subsequent differentiation into endocrine cells [70]. Therefore, the authors consider these pancreatic duct cells possible facultative progenitors in adult pancreas, at the same time suggesting that pancreatic acinar cells give rise to intermediate cells that have characteristics of pancreatic duct cells, and then differentiate into endocrine cells.

Overexpression of transforming growth factor-α has been reported to induce expansion of pancreatic and duodenal homeobox 1 (Pdx1)-expressing ductal epithelium in the pancreas, with identification of focal areas of islet neogenesis [71]. Due to the fact that pancreatic acinar cells isolated from transforming growth factor-α transgenic mice convert into ductal cells [72, 73], it is highly likely that the expanded pancreatic ductal cells expressing Pdx1 derive from pancreatic acinar cells. In addition, some pancreatic injury models have been shown to exhibit pancreas regeneration. Ligation of the pancreatic duct in rats is followed by replacement of exocrine acini by duct-like structures [74]. This acinoductal metaplasia seems to partially derive from the transdifferentiation of amylase-positive pancreatic acinar cells into amylasenegative and cytokeratin-positive duct-like cells. The treatment of the rats with dexamethasone to inhibit loss of amylase expression was associated with the identification of transitional cells co-expressing amylase and cytokeratin [74], supporting the notion of acinar-to-ductal transdifferentiation. Furthermore, insulin-positive cells that also express amylase have been found, indicating acinar-to-endocrine transdifferentiation.

In spite of the fact that the histological analysis has proved that neogenesis or regeneration of pancreatic β-cells takes place in certain conditions, the cellular origin of the new b-cells has not been shown. Following genetic cell lineage tracing [45] Dor et al. demonstrated that self-replication of pre-existing β-cells is at the basis of adult pancreatic β-cells maintenance in mice. They concluded that terminally differentiated β-cells retain proliferative capacity, and brings uncertainty to the significance of the role of adult stem cells in β-cell replenishment [45]. As previously stated, cyclin D2, which is required for β-cell replication, but not for ductal and exocrine cell proliferation might be the primary mechanism for maintaining postnatal β-cell mass [46].

Moreover, it seems that, different from gastrointestinal and skin epithelia, specialized progenitors do not contribute to adult β-cell mass and that adult b-cells exhibit equal proliferation potential, and expand from within a vast and uniform pool of mature β-cells [75]. Thus, pancreatic β-cell mass is more than likely maintained primarily by self-replication of pre-existing β-cells in adult mice, though we cannot exclude the existence of pancreatic tissue-specific stem/progenitor cells (Fig 1). It has been recently shown that non-β-cells play an important role in the mass expansion growth of pancreatic b-cells from neonate through to youth [76]. Although the signals leading to proliferation of pancreatic β-cells are still to be fully characterized, several recent studies have supported the implication of EGF-receptor signaling, prolactin and placental lactogen in the expansion of β-cells in pregnancy [7, 77, 78].

In addition, it has been also demonstrated that STAT5, growth hormones, prolactin, and foxM1 promote β-cell self-renewal during pregnancy [47, 51] and hepatic activation of extracellular regulated kinase signaling induced proliferation of pancreatic β-cells through a neuronal mediated relay of metabolic signals [7, 78].

Furthermore, is should also be noted that, following streptozotocin mediated islet injury, and during aging in mice, a lineage-tracing study that employed a similar approach as described by Dor et al. [45] offered proof that, beside β-cell self-renewal, intraislet progenitors is likely to take part in β-cell regeneration [47, 59] (Fig 1).

Thus, several sources of progenitor/stem cells in the adult pancreas were proposed to contribute to islet cell neogenesis and give rise to functional beta-cells in vivo. There are reports showing that progenitors for β-cells are activated in injured adult mouse pancreas, and are located in the ductal lining. Differentiation of the adult progenitors depends on the Ngn3, and produces all islet cell types both in situ and cultured in embryonic pancreas explants [25]. Using several models of pancreas injury, and including transgenic animals, endocrine cells expressing insulin, glucagon, but also Ngn3, as well as Pdx1 labeled cells were detected in the ductal epithelium. All these provide strong evidence that indeed ductal lining structures may comprise multipotent facultative stem/progenitor cells [6, 26, 69, 79]. In fact, PDL in the pancreas was accompanied by the emergence of Ngn3-labeled duct cells that are able to differentiate in all endocrine cells [25], suggesting that adult β-cells can be generated not only from pre-existing β-cells, but also from non-β-cells [25]. This is corroborated by a study performed by Inada et al.[63] using carbonic anhydrase II promoter to genetically mark duct derivatives in PDL injury model and demonstrating that duct cells have indeed the capacity to give rise to endocrine as well as exocrine cells. However, on the contrary, Solar et al.[80] showed that the duct cells (HNF 1b as the marker) lose potential for differentiation into β-cells after birth. Thus, the origin of newly formed b-cells in the adult pancreas remains to be identified [7].

The finding that duct cells may contain facultative stem cells [79] implicates that cells has to delaminate...
from these epithelial structures and migrate to populate the islets of Langerhans [47].

- **Glucagon for Use as Potential Stem Cell of Endocrine Cell Regeneration**

The combination of PDL with the ablation of β-cells through alloxan also triggered significant β-cell regeneration. However, the newly formed β-cells do not seem to originate from the duct epithelium but through direct conversion of alpha-cells [61]. Hence, it has been suggested that β-cell neogenesis occurs from intrinsalet progenitors [61]. On the other hand, another mouse model, where alpha-cells were directly transformed into functional β-cells by the conditional misexpression of the transcription factor Pax4 in glucagon-producing cells, were followed by glucagon neogenesis through the activation of duct-derived facultative stem cells [81, 82]. Transgenic mice ectopically expressing Pax4 in glucagon-producing cells develop an age-dependent increase in islet size and β-cell mass. Remarkably, the so induced transdifferentiation of alpha-cells showed that, to compensate the thereby compromised glucagon signaling, alpha-cell neogenesis took place in these mice. Consequently, a permanent cycle of alpha-cell/regeneration and conversion into β-cell, was suggested, eventually playing a role in increasing islet size [81, 82]. Remarkably, spontaneous transdifferentiation of alpha-cells into β-cells was reported to occur in mice with global ablation of insulin-producing β-cells by diphtheria toxin [5]. It has also been reported that in the islets of Type I diabetes patients, an increase in alpha-cell number was identified [83]. Moreover, this also applies to the islets of type I diabetes induced in mice by streptozotocin depletion of β-cells [84]. All these studies may suggest that glucagon-producing alpha-cells could potentially be employed as a source for the generation of insulin-producing cells to replace depleted β-cells in diabetic patients. Although during normal endocrine pancreas genesis adult insulin- and glucagon-producing cells differentiate from two independent cell lineages, alpha-cells may still represent a stem cell pool in the adult pancreas. As stated earlier glucagon has been shown to affect the first wave of differentiation of insulin expressing cells in the developing mouse pancreas [85]. Interestingly, alpha-cells have been recently suggested as being more plastic and more capable to dedifferentiate to acquire a Pro-alpha-cell state and give rise to β-cells [86].

**Conclusion**

In spite of the controversies they still create, most of the studies greatly demonstrate the capacity of the adult endocrine pancreas to regenerate. Moreover, the three major epithelial cell types within the pancreas have been lately proved to exhibit a substantial amount of plasticity [47]. No matter the case, the research for the cure for diabetes has entered new, promising grounds. Increasing the understanding of the mechanisms that would trigger endogenous β-cell regeneration, finding the right stimulus or the favorable growth and differentiation factors to turn a quiescent endocrine progenitor into an activated one would not only bring enormous benefits to type I diabetes patients, who have few or no residual β-cells that could be stimulated to replicate, but could also constitute a promising approach to treating type II diabetes. Furthermore, it may also represent a promising direction for finding a cure, particularly for paediatric patients where islet transplantation, even with its substantially improved outcome in recent years, is still not indicated.

**Disclosure:**

The authors report no conflicts of interest.

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