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Evolving Function and Potential of Pancreatic Alpha Cells

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Abstract

The alpha cells that co-occupy the islets in association with beta cells have been long recognized as the source of glucagon, a hyperglycemia-producing and diabetogenic hormone. Although the mechanisms that control the functions of alpha cells, glucagon secretion, and the role of glucagon in diabetes have remained somewhat enigmatic over the fifty years since their discovery, seminal findings during the past few years have moved alpha cells into the spotlight of scientific discovery. These findings obtained largely from studies in mice are: Alpha cells have the capacity to trans-differentiate into insulin-producing beta cells. Alpha cells contain a GLP-1 generating system that produces GLP-1 locally for paracrine actions within the islets that likely promotes beta cell growth and survival and maintains beta cell mass. Impairment of glucagon signaling both prevents the occurrence of diabetes in conditions of the near absence of insulin and expands alpha cell mass. Alpha cells appear to serve as helper cells or guardians of beta cells to ensure their health and well-being. Of potential relevance to the possibility of promoting the transformation of alpha to beta cells is the observation that impairment of glucagon signaling leads to a marked increase in alpha cell mass in the islets. Such alpha cell hyperplasia provides an increased supply of alpha cells for their transdifferentiation into new beta cells. In this review we discuss these recent discoveries from the perspective of their potential relevance to the treatment of diabetes.

Keywords

Alpha cells; Diabetes; Proglucagon; Transdifferentiation; Beta cells; GLP-1

Introduction

Over nine decades ago the alpha cells were identified as the source of the hyperglycemia-producing “contaminant” factor contained in the pancreas extracts used to isolate insulin [1]. The hyperglycemic factor was named glucagon and subsequently shown to arise from alpha cells that co-occupy the islets of Langerhans in the pancreas along with the beta cells that produce insulin, and accompanying delta and PP cells that produce the hormones somatostatin and pancreatic polypeptide, respectively. The biological actions of glucagon

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are counter-regulatory to those of insulin. Insulin lowers prandial blood glucose levels by stimulating glucose uptake in peripheral tissues whereas glucagon raises post-prandial (fasting) blood glucose levels by stimulating hepatic glucose production (gluconeogenesis). As a consequence of its glucose-elevating actions, glucagon was proposed 45 years ago to be diabetogenic, an important contributor to the fasting hyperglycemia that occurs in patients with diabetes [2].

The prevalence of diabetes, both type 1 (T1D) and type 2 (T2D), is increasing throughout the world at an alarming rate [3]. The fundamental cause of both types of diabetes is a failure of the beta cells in the pancreas to produce insulin in the amounts needed to efficiently modulate nutrient utilization. Both T1D and T2D are characterized by a reduction in beta cell mass; T1D as a result of near complete destruction of beta cells by autoimmunity and T2D as a result of a gradual loss of beta cells, and loss of function of remaining beta cells due to insulin resistance, hyperglycemia, and oxidative stress (glucotoxicity). The treatment of diabetes has involved both pharmacologic and cellular approaches [4]. Pharmacologic approaches include the use of insulin in T1D and drugs that increase insulin sensitivity and improve glycemic control in T2D. Cellular approaches include pancreas transplants and the transplantation of donor islets into the livers of T1D patients. Although helpful, these exogenous treatments currently in use for the treatment of diabetes are not fully effective in their normalization of glucose homeostasis. A major effort is directed at finding the means to stimulate the endogenous formation and growth of new beta cells in the pancreas to replace those destroyed by autoimmunity and by glucolipotoxicity. Such formation of new beta cells is believed to be possible by the stimulation of beta cell neogenesis from stem/progenitor cells that exist in the pancreatic ducts, or by the differentiation of exocrine stem-like cells into beta cells [5,6]. Most remarkable has been the recent discovery that the glucagon-producing alpha cells (and somatostatin-producing delta cells) of the islets are capable of trans-differentiation into insulin-producing beta cells [7-12]. This process of beta cell neogenesis and trans-differentiation (BCNT) for the creation of new beta cells in the pancreas opens up new avenues for research into devising a lasting treatment for diabetes, and is the topic of this review. Several review articles on the biology of alpha cells are available [1, 13-20].

Proglucagon gives rise to proglucagon-derived peptides (PGDPs)

It is important to emphasize that the “glucagon” gene (Gcg) encodes a mRNA for a prohormone, proglucagon, that when translated into protein, contains several peptide hormones in addition to glucagon [21-23]. These hormones include glucagon-like peptides 1 and 2 (GLP-1, GLP-2), oxyntomodulin, glicentin, glicentin-related polypeptide (GRPP), and the intervening peptides 1 and 2. (**Figure 1**). GLPs 1 and 2, and oxyntomodulin have demonstrated biological activities in the promotion of cell growth. These peptides are formed from the proglucagon precursor by selected enzymatic cleavages mediated by specific endopeptidases. These endopeptidases include the prohormone convertases (PCs) of the subtilisin-kexin class (Pcsks) that cleave proglucagon at sites of basic residues, lysine and arginine, and thereby liberate the parent peptide hormones, glucagon, oxyntomodulin, GLP-1, and GLP-2 from the prohormone [24]. The parent peptide GLP-1, produced from the PC-mediated cleavage of proglucagon, is further post-translationally modified by the

removal of two amino-terminal amino acids by diaminopeptidyl peptidase-4 that severely reduces the receptor agonism of GLP-1. Subsequent cleavages of GLP-1 by neutral endopeptidases, such as neprilysin (NEP 24.11) generate small nona and pentapeptides from the C-terminus of GLP-1 [25]. These peptides are implicated in the regulation of energy metabolism and the reduction of oxidative stress in cells [17]. The expression of the proglucagon gene (Gcg) is somewhat restricted to the alpha cells in the pancreatic islets, the intestinal L-cells located in the distal ileum and colon, and the hindbrain. The gene encoding the GLP-1 receptor (Glp1r), a G-protein coupled receptor linked to the activation of adenylate cyclase and cAMP signaling, is expressed in several organs reflecting the sites of GLP-1 actions. These organs are the pancreas in which the receptor is enriched in the beta cells of the islets, stomach, brain (hypothalamus), heart, lung, and kidneys. Whether or not GLP-1 receptor is expressed in liver, skeletal muscle, adipose tissue, and pancreatic ducts remains controversial. The spectrum of PGDPs produced by post-translational proteolysis of proglucagon differs amongst different tissues and is determined by factors in the metabolic environment. For example, under normal homeostatic metabolic conditions the alpha cells express exclusively the prohormone convertase PC2 resulting in the production of glucagon with little or no production of GLP-1, whereas the intestinal L-cells express solely GLP-1 and GLP-2 due to the expression of PC1/3. However, in conditions of beta cell injuries, alpha cell hyperplasia, and diabetes, alpha cells turn on the expression of PC1/3 and produce GLP-1 in addition to glucagon (see below).

Topography of islet endocrine cells supports paracrine signaling of alpha and beta cells

The islets consist of four major cell types, alpha, beta, delta, and PP that produce glucagon and PGDPs, insulin, somatostatin, and pancreatic polypeptide respectively. The non-beta endocrine cells, particularly the alpha cells, lie in close contact with the insulin-producing beta cells. In rodent islets, which consist of 60-70% beta and 20% alpha cells, 30-40% of beta cells are in direct contact with alpha cells and in human islets consisting of 50% beta and 40% alpha cells, up to 70% of beta cells contact alpha cells [26]. In both mouse and human islets >90% of alpha cells are in contact with beta cells.

Remarkably, high-resolution confocal microscopy of human islets reveals that the endocrine cells are adherent to vessels and that cytoplasmic extensions of beta cells envelope alpha cells [27]. This unusual topography involving intimate cell-cell contacts bespeaks the existence of a network of intercellular paracrine signaling [28]. Based on the recent findings of a remarkable plasticity of islet endocrine cells in their capacity to transdifferentiate from one hormone expressing phenotype to another, one conjectures that the close juxtapositioning of alpha cells to beta cells in islets might to some extent reflect the interconversion of the two cell types by a dynamic process of bi-directional trans-differentiation important in the promotion of beta cell regeneration (see below).

Development of islet cells

The pancreas arises from an outcropping of epithelial cells in the foregut of the developing mouse embryo at embryo day e8.5. The primitive undifferentiated epithelium in the outcropping assumes a duct-like phenotype. The expression of the transcription factor Pdx-1 is essential for early pancreas development to occur since genetic disruption of its

expression arrests development resulting in pancreatic agenesis [29]. The subsequent lineage commitments of the cellular phenotypes that make up the mature pancreas are determined by the spatial and temporal expression of cell-specific transcription factors, so-called master regulators of tissue types. The cell lineages and their predominant transcription factors and their determining phenotypes are: Exocrine pancreas-Ptf1, Ducts-Sox9, Endocrine-Ngn3, Beta cells-Pdx-1, MafA, Pax4, Alpha cells-Arx, Irx2, MafB. See review by van der Meulen and Huising 2015 [30] for detailed information on the population of transcription factors that distinguish alpha and beta cells.

The primitive duct-like epithelium undergoes branching morphogenesis from which endocrine cells arise. These newly-formed endocrine cells express the transcription factor neurogenin-3 (Ngn3) that defines the endocrine lineage during pancreas development. The proglucagon gene, Gcg, is the earliest islet hormone expressed (e10), followed by insulin (e11), and somatostatin and pancreatic polypeptide (e12-13). Notably, lineage-tracing experiments in mice have shown that the alpha and beta cells arise independently from distinct origins [31]. Alpha cells do not appear to be progenitors that give rise to beta cells during development. The results of these lineage tracing experiments are somewhat intriguing in light of the recent discoveries that in the adult pancreas alpha cells and beta cells can transdifferentiate one cell type into the other [7,32,33], and that beta cells can reversibly dedifferentiate into stem/progenitor-like cells, and then into alpha cells [33]. These findings of the remarkable plasticity of alpha and beta cells to transdifferentiate and dedifferentiate in response to beta cell stress and injuries is discussed below.

Beta cell neogenesis and transdifferentiation (BCNT)

The weight of accumulated evidence points to the existence of facultative somatic stem cells in the adult mouse pancreas. These stem/progenitor cells appear to reside within or adjacent to the large pancreatic ducts, and possibly within acini of the exocrine tissue. Given the appropriate environmental conditions these stem/progenitor cells can give rise to new endocrine cells including beta cells in a process known as beta cell neogenesis. One stimulus for neogenesis is injury of the pancreas, or specifically, injury of existing beta cells. Multiple studies using different mouse models of pancreatic injury including duct ligation, caerulein, beta cell toxins (streptozotocin, alloxan) treatments, or metabolic stresses such as glucotoxicity promote the development of alpha cell hyperplasia [17]. These pancreatic and beta cell injuries prompt the amplification of the stem/progenitor cells and their differentiation into beta cells (**Figure 2**). A most remarkable recent discovery has been the finding that alpha cells, specifically immature alpha cells, pro-alpha cells, are precursors of beta cells [7-11]. Beta cells arise by their transdifferentiation from alpha cells. The initial breakthrough that led to the identification of this transdifferentiation of alpha to beta cells originated from the Collombat laboratory [7]. These ground-breaking, proof-of-concept studies were a culmination of a series of earlier studies in mice demonstrating a remarkable interplay between the cellular levels of the transcription factors Arx and Pax4 as critical determinants of the alpha and beta cell phenotypes, respectively [32,34-36]. In particular, a high Arx/Pax4 ratio maintains the phenotype of alpha cells. Lowering this ratio by either the reduction of Arx or the elevation of Pax4, promotes the transformation of alpha to beta cells [36,37] (**Figure 2**). Key studies in genetically engineered mice demonstrated that the

induction of Pax4 overexpression (by tamoxifen) in alpha cells resulted in the highly efficient transdifferentiation of alpha cells into beta cells. This transdifferentiation severely depleted the population of alpha cells, thereby lowering plasma levels of glucagon, resulting in the stimulation of the proliferation of duct-associated stem/progenitor cells that differentiated into immature pro-alpha cells [7] (**Figure 2**). This mouse model of alpha cell specific inducible Pax4 overexpression was further used to completely regenerate functional beta cells of mice in which the endogenous beta cells were ablated by the beta cell toxin streptozotocin. This complete regeneration of beta cells following toxin-induced ablation was observed in three consecutive temporal cycles of beta cell ablation over a period of several weeks [11].

Remarkably, studies from the Herrera laboratory found that following extreme ablation of beta cells with diphtheria toxin, the transdifferentiation of alpha cells into beta cells. These findings occurred in mice without genetic manipulations of transcription factor expression, and without the occurrence of cell proliferation. That is, the alpha cells transformed into beta cells by an epigenetic reprogramming [8]. This finding of alpha cell transdifferentiation seen in adult mice is in contrast to later findings of the transdifferentiation of somatostatin-producing delta cells, observed in pre-pubertal and not in adult mice [12]. In these studies involving the ablation of beta cells in pre-pubertal mice, the delta cells, and not the alpha cells, first dedifferentiate, proliferate, and later transform into beta cells [12]. A recent study highlights both the importance of master regulator transcription factors in determining the endocrine phenotype of islet cells and that beta cells can transdifferentiate into alpha cells [38]. Following the conditional deletion of Pdx1 specifically in beta cells of mice, insulin production decreased and the mice developed hyperglycemia. The mass of beta cells marked by yellow fluorescence protein remained intact but no longer produced insulin. Instead, these cells were found to have transformed directly into alpha cells without dedifferentiation as no Ngn3 expression was detected. The reprogrammed former beta cells expressed the proglucagon and MafB genes, a characteristic of alpha cells, indicating a de-repression of alpha cell transcriptional programs in response to the loss of Pdx1 in beta cells. These findings indicate that Pdx1, a master regulator of the beta cell phenotype, not only activates genes essential for beta cell identity but also represses those associated with alpha cell identity. Earlier studies have also demonstrated beta to alpha cell transformation in response to genetic alterations in the balance of master regulator transcription factors [39,40]. These findings further underscore the importance of master regulator proteins in the determinations of alpha and beta cell fates and phenotype. The important findings of Bramswig [41] demonstrate that the epigenetic chromatin signature of alpha cells closely resembles that of stem cells. These recent findings of the extraordinary plasticity of alpha cells have opened a new avenue for the treatment of diabetes in which a diminished beta cell mass contributes to the pathogenesis of the disease.

Reversible dedifferentiation of beta cells to stem-like cells in response to metabolic stress

A remarkable recent unanticipated discovery is that in response to metabolic stress beta cells in the adult mouse pancreas dedifferentiate into stem-like cells [33, 42]. These cells cease their expression of insulin and instead express Ngn3, the master regulator of endocrine transcriptional programs during lineage commitment along with embryonic stem cell marker

proteins. In one study a substantial fraction of the dedifferentiated stem-like cells transformed into alpha cells [33]. In another study hyperglycemia-induced glucotoxic stress dedifferentiated mouse beta cells into Ngn3 positive cells that no longer express insulin [42]. Remarkably, upon normalization of plasma glucose levels and resulting alleviation of glucotoxic stress, these same undifferentiated Ngn3 positive cells redifferentiate back into functioning beta cells that produce insulin and thereafter maintain euglycemia [42]. These findings of reversible dedifferentiation and redifferentiation of beta cells demonstrate the remarkable plasticity of islet endocrine cells. It is tempting to speculate on the purpose of such dedifferentiation in response to metabolic stress. Beta cells are noted for their unusually low levels of antioxidant proteins rendering them highly susceptible to oxidative stress by free radicals, damage of essential mitochondrial proteins leading to the activation of programmed death pathways [43]. The source of free radicals such as reactive oxygen and nitrogen species is mitochondrial oxidative phosphorylation. Beta cells have high levels of oxidative phosphorylation owing to the metabolism of glucose and the high rates of protein synthesis. A characteristic of undifferentiated cells, such as rapidly proliferating stem-like cells is a switch in bioenergetics from oxidative phosphorylation to aerobic glycolysis (Warburg shift) [44]. Undifferentiated cells metabolize glucose predominantly by aerobic glycolysis rather than by oxidative phosphorylation. Even though the generation of ATP by aerobic glycolysis (2 ATPs per mole of glucose) is much less efficient than it is by oxidative phosphorylation (32 ATPs per mole of glucose), aerobic glycolysis does not generate reactive oxygen and nitrogen species that can lead to cell death. Thusly, the process of dedifferentiation of beta cells in response to oxidative stress might represent a mechanism for the cells to escape programmed cell death. In essence, the beta cells remain beta cells by genotype, but change their phenotype as a defense against oxidative damage. This process is reversible as when the oxidative stress is alleviated the dedifferentiated cells redifferentiate back into insulin producing beta cells.

Production of GLP-1 by hyperplastic alpha cells

That alpha cells are a source of GLP-1 production is a relatively new finding. It has been generally believed that alpha cells produce glucagon exclusively and produce very little if any GLP-1 derived from the cleavages of proglucagon. This situation is a consequence of the exclusive expression in mature alpha cells of the prohormone convertase PC2, which gives rise to glucagon and not GLP-1 from the cleavages of proglucagon. The formation of GLP-1 requires cleavage of proglucagon by PC1/3, which is not expressed in mature alpha cells. However, recent studies indicate that under certain circumstances alpha cells can produce GLP-1. These studies reveal that hyperplasia of alpha cells results in the induction of the expression of PC1/3 and their production of GLP-1 in large amounts resulting in marked elevations in the circulating plasma levels of both GLP-1 and glucagon. Two conditions that lead to marked alpha cell hyperplasia have been extensively described. One is injury of beta cells of diverse types and the other is impairment of glucagon receptor signaling [Reviewed in 16,17].

Alpha cell hyperplasia and GLP-1 production in response to beta cell injuries

Historically, it has been recognized that injury of beta cells by various causes such as beta cell toxins (streptozotocin, alloxan), metabolic stress, autoimmunity promotes the expansion

of alpha cell mass [17, 45-47]. An example of marked alpha cell hyperplasia resulting from beta cell injury is the recent study showing that complete ablation of beta cells by streptozotocin in normal wild type mice results in a ten-fold increase in the alpha cell mass of the islets [48]. Notably, the increase in alpha cell mass after streptozotocin treatment of glucagon receptor null mice is 39-fold [48].

The production of GLP-1 by alpha cells in response to beta cell stress and injury appears to be due to the induction of the expression of PC1/3 in alpha cells [46,47, 49-53]. Moreover, the induction of the production of GLP-1 by alpha cells is not restricted to rats and mice. Islets from obese, insulin resistant [54] and type 2 diabetic [55] human donors generate GLP-1 whereas control islets from normal human subjects do not [54,55]. Alpha cell hyperplasia is also seen in type 1 autoimmune diabetes in which autoreactive T cells attack and destroy beta cells [56-58]. In the NOD autoimmunity mouse, alpha cell mass is increased. Plasma GLP-1 levels in these mice are increased by 5 to 200-fold [58]. A demonstration of the development of alpha cell hyperplasia in diabetic human subjects in response to diabetes is provided in the report by Butler et al [59]. Although these findings are controversial with regard to effects of GLP-1 therapy, the fact remains that a marked expansion of alpha cell mass is seen in histologic sections of pancreases obtained from diabetic individuals. These and multiple other images of pancreases from both T1D and T2D patients are available at the JDRF sponsored network for pancreatic organ donors with diabetes (nPOD) [<http://www.jdrfnpod.org>].

It is tempting to speculate on the mechanism(s) responsible for the alpha cell hyperplasia that occurs in response to beta cell injury. It seems reasonable to propose that injured beta cells release paracrine regeneration factors (RegenFs), such as stromal cell-derived factor-1 (SDF-1/CXCL12) Liu et al [53] and IL6 [60] (**Figure 3**). These factors act on alpha cells adjacent to the beta cells and promote their de-differentiation to immature pro-alpha cells that express prohormone convertase PC1/3 resulting in the production of GLP-1 from proglucagon. This mechanism is akin to the fate of alpha cells during the embryonic development of the islets. During development pro-alpha cells proliferate, express PC1/3 [61], produce GLP-1, and express the GLP-1 receptor [62], not seen in fully differentiated mature alpha cells. The production of GLP-1 within islets by alpha cells juxtaposed to beta cells is proposed to trigger the regeneration of beta cells by promoting their growth and survival (**Figure 3**).

Alpha cell hyperplasia and GLP-1 production in response impaired glucagon receptor signaling

Alpha cell hyperplasia occurs in response to impaired glucagon receptor-mediated signaling in rodents and in humans. The hallmark mouse model is the glucagon receptor null mouse (*Gcgr*^{-/-}) that has been extensively studied [17]. Alpha cell mass in *Gcgr* knockout [63,64] and knockdown [65] mice is increased many-fold and plasma levels of both glucagon and GLP-1 are elevated 10 to 100-fold. Similar phenotypes are seen in proglucagon null mice (*Gcg*^{-/-}) [66], PC2 null mice (*Pcsk2*^{-/-}) [67], mice with immune blockade of the glucagon receptor [68], and mice with knock out of liver *Gsalpha* [69] or the liver glucagon receptor [70]. Humans with inactivating mutations in the glucagon receptor develop alpha cell

hyperplasia with markedly elevated plasma levels of glucagon and GLP-1 [71-73]. One important caveat regarding the alpha cell hyperplasia associated with impaired glucagon receptor signaling is the propensity to develop pancreatic neuroendocrine tumors (pNETs). A description of the development of these tumors in mice was first reported by Yu et al (72) in the glucagon receptor knockout mouse. The development of pNETs is age-related with 100% penetrance by 10 to 12 months of age. Recently, Hayashi [74] has reported the development of pNETs in 100% of glucagon and GLP-1 deficient Gcg null mice by 15 months of age. >90% of these tumors have metastasized to liver and brain of the mice. Metastases of pNETs also occurs in Gcgr null mice [72]. Humans with inactivating Gcgr mutations have severe alpha cell hyperplasia, nesidioblastosis, and pNETs with markedly elevated plasma glucagon levels [71,73]. This syndrome of alpha cell hyperplasia and pNETs caused by mutation(s) in the coding exons of the glucagon receptor gene has been designated Mahvash disease [73]. The index case of Mahvash disease was an individual who was homozygous (autosomal recessive) for a P86S mutation in the receptor. Six cases of Mahvash disease have been reported as of 2013 [73]. The prevalence of inactivating mutations in the glucagon receptor is estimated to be ~0.1%, or ~4 per million individuals [73]. Since only six cases of Mahvash disease have been identified the penetrance of the mutation and disease manifestation might be variable.

Impaired glucagon receptor signaling ameliorates insulin deficient diabetes

During the 1970's Unger and associates proposed the notion that diabetes is a bi-hormonal disease caused by a combination of insulin deficiency and glucagon excess [75]. Indeed this idea of the pathogenesis of diabetes makes sense since the actions of glucagon are counter-regulatory to those of insulin. Uncontrolled hepatic glucose production is a major cause of fasting hyperglycemia in diabetic individuals [76]. Hepatic glucose production is controlled in large part by glucagon-mediated stimulation of gluconeogenesis in the liver. Therefore, glucagon can be considered a potential diabetogenic hormone. Recent findings by Unger and coworkers have underscored the importance of glucagon in the worsening of diabetes. They find that mice with no glucagon receptor, the Gcgr $-/-$ mice, do not develop diabetes in conditions of complete ablation of pancreatic beta cells [48]. After double-dose ablation of beta cells with streptozotocin, proinsulin in the islets is undetectable. However, of note, plasma insulin remains detectable at levels of 10% those of normal mice, leaving open the possibility that glucagon deficiency might increase insulin sensitivity owing to the elevated levels of GLP-1 in these mice. Further, this same group found that the insulin deficient type 1 diabetic NOD mice achieve glycemic control by a loss of glucagon signaling in response to immune-blockade of the glucagon receptor [77]. These findings in mice support the contention that excessive glucagon is indeed diabetogenic in humans. Efforts to develop effective therapies to reduce glucagon levels or to inhibit glucagon action in diabetic individuals seem highly worthwhile, although caution is indicated due to the development of alpha cell hyperplasia and transition to neuroendocrine neoplasia.

Alpha cells as guardians of beta cells

A summation of the information gathered on the functions of alpha cells supports an important role for these cells in the protection and regeneration of beta cells. It is tempting to conjecture that because of the critical importance of insulin produced by beta cells both in

development and in adult life, alpha cells have evolved to function as helper cells to maintain the health of beta cells. The findings of intimate cell-cell contacts between alpha and beta cells in the islets suggest the existence of intercellular paracrine communication between these two cell types. One function of alpha cells is to induce the production of GLP-1 in response to injury signals emanating from stressed beta cells. GLP-1 is known to provide cytoprotection for and to stimulate the proliferation of beta cells. Further, in conditions of severe depletion of beta cells alpha cells are prompted to transdifferentiate into new beta cells to replace those that are lost.

Alpha cell enigmas remain

Although much information has accumulated indicating important functions for alpha cells in providing protection for beta cells via the production of GLP-1, and for producing glucagon to maintain plasma glucose levels in the post-absorptive state, several enigmas remain. Importantly, the ablation of the alpha cell lineage in mice by disruption of the *Arx* gene does not affect the development, growth, or health of mice. (78). These mice tend to have hypoglycemia, but remain healthy throughout their life span. Clearly, alpha cells, unlike beta cells, are not essential for life and health in mice. Another unanticipated finding has been the essentially normal developmental and metabolic phenotype of mice with a knockout of the proglucagon gene (*Gcg*). The mice have no proglucagon or proglucagon-derived peptides, such as glucagon or GLP-1 and are born fully developed without metabolic abnormalities other than a tendency towards hypoglycemia (66). However, the *Gcg* null and *Gcgr* null mice develop massive alpha cell hyperplasia. As the *Gcg* null mice age, 100% of them develop pancreatic neuroendocrine tumors that are malignant and metastasize to liver and brain (74). The mechanism(s) involved in alpha cell hyperplasia in response to the absence of glucagon receptor signaling remains unknown. A mechanism that might contribute to the hyperplasia of alpha cells in response to reduced glucagon signaling is the stimulation of the expansion of stem/progenitor cells in the pancreas and their differentiation into alpha cells. Based on the studies of Collombat et al (7) a plausible model for the marked increase in beta cells via their transdifferentiation from alpha cells is the loss of glucagon signaling due to a severe depletion of alpha cells as they efficiently transform into beta cells. The depletion of alpha cells lowers plasma levels of glucagon, which relieves the suppression of the expansion of stem/progenitor cells that become alpha cells. One aspect of these findings in mice is the possibility that adaptive compensation occurs in genetically modified mice when mutations are introduced into the germ line. It is unknown what the consequences of the absence of alpha cells or proglucagon-derived peptides might have in humans. Although, as mentioned earlier, inactivating mutations in the glucagon receptor in humans appears to promote the growth of alpha cells, and in at least one case, the development of pancreatic neuroendocrine neoplasia (71).

Future Directions

The discoveries of the past several years on the role of alpha cells in the promotion of the regeneration of beta cells opens new avenues to approaches for the treatment of diabetes. One such approach involving alpha cells might be to identify a means to coax alpha cells to produce GLP-1, perhaps by the stimulation of the prohormone convertase PC1/3 in alpha cells. GLP-1 can then act locally on beta cells within islets to provide cytoprotection against

stress and to stimulate their proliferation. Another approach is to find a means to stimulate the transformation of alpha cells into beta cells. For example, identify a factor(s) that suppresses the expression of the transcription factor Arx in alpha cells. This approach could be combined with the judicious use of glucagon receptor antagonists that decrease glucagon actions and promote the expansion of alpha cell mass thereby increasing the numbers of alpha cells available for their transdifferentiation into beta cells. Reducing glucagon receptor signaling also offers the possibility of increasing the formation and expansion of stem/progenitor cells and their differentiation into alpha cells, as indicated by the findings of Collombat 2009. Attempts to develop effective glucagon receptor antagonists are ongoing as reducing glucagon signaling has anti-diabetogenic effects (see above). However, caution is indicated in the use of glucagon receptor antagonists to avoid extreme alpha cell hyperplasia because of the findings in mice of the development of pancreatic neuroendocrine tumors (see above).

Summary

The alpha cells of the pancreatic islets, long recognized for their production of glucagon, a diabetogenic hormone that regulates hepatic glucose production to maintain plasma glucose levels during fasting, has become a focus of attention as a potential target for the treatment of diabetes. Alpha cells are now known to increase in numbers (alpha cell hyperplasia) and to produce GLP-1, in response to beta cell injuries. As GLP-1 increases the proliferation and cytoprotection of beta cells, intra-islet paracrine signaling between injured beta cells and alpha cells might be a process designed to repair and regenerate beta cells that are reduced in diabetes. In response to extreme injury of beta cells alpha cells can transform (transdifferentiate) into functioning beta cells. Genetic manipulation studies in mice show that bidirectional transformation of alpha and beta cells is modulated by the cellular ratios of the transcription factors Arx and Pax4. High and low ratios of Arx/Pax4 determine the alpha and beta cell phenotypes, respectively. Thusly, alpha cells play important roles in beta cell neogenesis and transdifferentiation. Alpha cell hyperplasia also occurs in response to the impairment of glucagon signaling, both in mice and humans. The loss of glucagon actions in genetically manipulated mice prevents the development of diabetes in the complete absence of pancreatic insulin, a convincing demonstration of the potent diabetogenic actions of glucagon. These recent findings on the biology of alpha cells provide a compelling rationale for exploiting alpha cells as a means to generate new beta cells. Therapeutic strategies might involve treatment of diabetic individuals with a combination of glucagon receptor antagonists and small molecules that target alpha cells and suppress the expression of the transcription factor Arx, and/or increase the expression of Pax4. Antagonism of glucagon actions would reduce hepatic gluconeogenesis and resulting hyperglycemia, and also increase the target mass of alpha cells for transformation into beta cells by pharmacological manipulation of the Arx/Pax4 ratio in alpha cells.

Reference List

1. Ahrén B. Glucagon--Early breakthroughs and recent discoveries. *Peptides*. 2015; 67:74–81. [PubMed: 25814364]
2. Unger RH. Glucagon physiology and pathophysiology. *N Engl J Med*. 1971; 285:443–449. [PubMed: 4997492]

3. Kharroubi AT, Darwish HM. Diabetes mellitus: The epidemic of the century. *World J Diabetes*. 2015; 6:850–867. [PubMed: 26131326]
4. Cornell S. Continual evolution of type 2 diabetes: an update on pathophysiology and emerging treatment options. *Ther Clin Risk Manag*. 2015; 11:621–632. [PubMed: 25931824]
5. Lysy PA, Weir GC, Bonner-Weir S. Making β cells from adult cells within the pancreas. *Curr Diab Rep*. 2013; 13:695–703. [PubMed: 23925431]
6. Bouwens L, Houbracken I, Mfopou JK. The use of stem cells for pancreatic regeneration in diabetes mellitus. *Nat Rev Endocrinol*. 2013; 9:598–606. [PubMed: 23877422]
- 7*. Collombat P, Xu X, Ravassard P, Sosa-Pineda B, Dussaud S, Billestrup N, Madsen OD, Serup P, Heimberg H, Mansouri A. The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into alpha and subsequently beta cells. *Cell*. 2009; 138:449–462. [PubMed: 19665969]
8. Thorel F, Népote V, Avril I, Kohno K, Desgraz R, Chera S, Herrera PL. Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. *Nature*. 2010; 464:1149–1154. [PubMed: 20364121]
9. Chung CH, Hao E, Piran R, Keinan E, Levine F. Pancreatic beta cell neogenesis by direct conversion from mature alpha cells. *Stem Cells*. 2010; 28:1630–1638. [PubMed: 20653050]
10. Yang YP, Thorel F, Boyer DF, Herrera PL, Wright CV. Context-specific α - to- β -cell reprogramming by forced Pdx1 expression. *Genes Dev*. 2011; 25:1680–1685. [PubMed: 21852533]
- 11*. Al-Hasani K, Pfeifer A, Courtney M, Ben-Othman N, Gjernes E, Vieira A, Druelle N, Avolio F, Ravassard P, Leuckx G, Lacas-Gervais S, Ambrosetti D, Benizri E, Hecksher-Sorensen J, Gounon P, Ferrer J, Gradwohl G, Heimberg H, Mansouri A, Collombat P. Adult duct-lining cells can reprogram into β -like cells able to counter repeated cycles of toxin-induced diabetes. *Dev Cell*. 2013; 26:86–100. [PubMed: 23810513]
12. Chera S, Baronnier D, Ghila L, Cigliola V, Jensen JN, Gu G, Furuyama K, Thorel F, Gribble FM, Reimann F, Herrera PL. Diabetes recovery by age-dependent conversion of pancreatic δ -cells into insulin producers. *Nature*. 2014; 514:503–507. [PubMed: 25141178]
13. Unger RH. Glucagon physiology and pathophysiology in the light of new advances. *Diabetologia*. 1985; 28:574–578. [PubMed: 3902546]
14. Gromada J, Franklin I, Wollheim CB. Alpha-cells of the endocrine pancreas: 35 years of research but the enigma remains. *Endocr Rev*. 2007; 28:84–116. [PubMed: 17261637]
- 15*. Unger RH, Cherrington AD. Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. *J Clin Invest*. 2012; 122:4–12. [PubMed: 22214853]
16. Habener JF, Stanojevic V. α -cell role in β -cell generation and regeneration. *Islets*. 2012; 4:188–198. [PubMed: 22847495]
- 17*. Habener JF, Stanojevic V. Alpha cells come of age. *Trends Endocrinol Metab*. 2013; 24:153–163. [PubMed: 23260869]
18. Yu R. Pancreatic α -cell hyperplasia: facts and myths. *J Clin Endocrinol Metab*. 2014; 99:748–756. [PubMed: 24285676]
19. Charron MJ, Vuguin PM. Lack of glucagon receptor signaling and its implications beyond glucose homeostasis. *J Endocrinol*. 2015; 224:R123–30. [PubMed: 25568163]
20. Lund A, Bagger JI, Christensen M, Knop FK, Vilsbøll T. Glucagon and type 2 diabetes: the return of the alpha cell. *Curr Diab Rep*. 2014; 14:555. [PubMed: 25344790]
21. Kieffer TJ, Habener JF. The glucagon-like peptides. *Endocr Revs*. 1999; 20:876–913. [PubMed: 10605628]
- 22*. Sandoval DA, D'Alessio DA. Physiology of proglucagon peptides: role of glucagon and GLP-1 in health and disease. *Physiol Rev*. 2015; 95:513–548. [PubMed: 25834231]
23. Campbell JE, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. *Cell Metab*. 2013; 17:819–837. [PubMed: 23684623]
24. Bataille D. Pro-protein convertases in intermediary metabolism: islet hormones, brain/gut hormones and integrated physiology. *J Mol Med (Berl)*. 2007; 85:673–684. [PubMed: 17356847]

25. Tomas E, Habener JF. Insulin-like actions of glucagon-like peptide-1: a dual receptor hypothesis. *Trends Endocrinol Metab.* 2010; 21:59–67. [PubMed: 20018525]
26. Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren PO, Caicedo A. The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. *Proc Natl Acad Sci U S A.* 2006; 103:2334–2339. [PubMed: 16461897]
27. Bosco D, Armanet M, Morel P, Niclauss N, Sgroi A, Muller YD, Giovannoni L, Parnaud G, Berney T. Unique arrangement of alpha- and beta-cells in human islets of Langerhans. *Diabetes.* 2010; 59:1202–1210. [PubMed: 20185817]
28. Unger RH, Orci L. Paracrinology of islets and the paracrinopathy of diabetes. *Proc Natl Acad Sci U S A.* 2010; 107:16009–16012. [PubMed: 20798346]
29. Jonsson J, Carlsson L, Edlund T, Edlund H. Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature.* 1994; 371:606–609. [PubMed: 7935793]
30. van der Meulen T, Huising MO. Role of transcription factors in the transdifferentiation of pancreatic islet cells. *J Mol Endocrinol.* 2015; 54:R103–117. [PubMed: 25791577]
31. Herrera PL. Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages. *Development.* 2000; 127:2317–2322. [PubMed: 10804174]
32. Collombat P, Xu X, Heimberg H, Mansouri A. Pancreatic beta-cells: from generation to regeneration. *Semin Cell Dev Biol.* 2010; 21:838–844. [PubMed: 20688184]
- 33*. Talchai C, Xuan S, Lin HV, Sussel L, Accili D. Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure. *Cell.* 2012; 150:1223–1234. [PubMed: 22980982]
34. Courtney M, Pfeifer A, Al-Hasani K, Gjernes E, Vieira A, Ben-Othman N, Collombat P. In vivo conversion of adult α -cells into β -like cells: a new research avenue in the context of type 1 diabetes. *Diabetes Obes Metab.* 2011; 13(Suppl 1):47–52. [PubMed: 21824256]
35. Courtney M, Gjernes E, Druelle N, Ravaut C, Vieira A, Ben-Othman N, Pfeifer A, Avolio F, Leuckx G, Lacas-Gervais S, Burel-Vandenbos F, Ambrosetti D, Hecksher-Sorensen J, Ravassard P, Heimberg H, Mansouri A, Collombat P. The inactivation of Arx in pancreatic α -cells triggers their neogenesis and conversion into functional β -like cells. *PLoS Genet.* 2013; 9:e1003934. [PubMed: 24204325]
36. Collombat P, Mansouri A, Hecksher-Sorensen J, Serup P, Krull J, et al. Opposing actions of Arx and Pax4 in endocrine pancreas development. *Genes Dev.* 2003; 17:2591–2603. (2003). [PubMed: 14561778]
37. Wilcox CL, Terry NA, Walp ER, Lee RA, May CL. Pancreatic α -cell specific deletion of mouse Arx leads to α -cell identity loss. *PLoS One.* 2013; 8:e66214. [PubMed: 23785486]
38. Gao T, McKenna B, Li C, Reichert M, Nguyen J, Singh T, Yang C, Pannikar A, Doliba N, Zhang T, Stoffers DA, Edlund H, Matschinsky F, Stein R, Stanger BZ. Pdx1 maintains β cell identity and function by repressing an α cell program. *Cell Metab.* 2014; 19:259–271. [PubMed: 24506867]
39. Collombat P, Hecksher-Sorensen J, Krull J, Berger J, Riedel D, Herrera PL, Serup P, Mansouri A. Embryonic endocrine pancreas and mature beta cells acquire alpha and PP cell phenotypes upon Arx misexpression. *J Clin Invest.* 2007; 117:961–970. [PubMed: 17404619]
40. Dhawan S, Georgia S, Tschen SI, Fan G, Bhushan A. Pancreatic beta cell identity is maintained by DNA methylation-mediated repression of Arx. *Dev Cell.* 2011; 20:419–429. [PubMed: 21497756]
41. Bramswig NC, Everett LJ, Schug J, Dorrell C, Liu C, Luo Y, Streeter PR, Naji A, Grompe M, Kaestner KH. Epigenomic plasticity enables human pancreatic α to β cell reprogramming. *J Clin Invest.* 2013; 123:1275–1284. [PubMed: 23434589]
- 42*. Wang Z, York NW, Nichols CG, Remedi MS. Pancreatic β cell dedifferentiation in diabetes and redifferentiation following insulin therapy. *Cell Metab.* 2014; 19:872–882. [PubMed: 24746806]
43. Robertson RP, Harmon J, Tran PO, Poitout V. Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. *Diabetes.* 2004; 53(Suppl 1):S119–24. [PubMed: 14749276]
44. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science.* 2009; 324:1029–1033. [PubMed: 19460998]
45. Li Z, Karlsson FA, Sandler S. Islet loss and alpha cell expansion in type 1 diabetes induced by multiple low-dose streptozotocin administration in mice. *J Endocrinol.* 2000; 165:93–99. [PubMed: 10750039]

46. Nie Y, Nakashima M, Brubaker PL, Li QL, Perfetti R, Jansen E, Zambre Y, Pipeleers D, Friedman TC. Regulation of pancreatic PC1 and PC2 associated with increased glucagon-like peptide 1 in diabetic rats. *J Clin Invest*. 2000; 105:955–965. [PubMed: 10749575]
47. Thyssen S, Arany F, Hill DJ. Ontogeny of regeneration of beta cells in the neonatal rat after treatment with streptozotocin. *Endocrinology*. 2006; 147:2346–2356. [PubMed: 16484329]
48. Lee Y, Wang MY, Du XQ, Charron MJ, Unger RH. Glucagon receptor knockout prevents insulin-deficient type 1 diabetes in mice. *Diabetes*. 2011; 60:391–397. [PubMed: 21270251]
49. Wideman RD, Yu IL, Webber TD, Verchere CB, Johnson JD, Cheung AT, Kieffer TJ. Improving function and survival of pancreatic islets by endogenous production of glucagon-like peptide 1. *Proc Natl Acad Sci USA*. 2006; 103:13468–13473. [PubMed: 16938896]
50. Kilimnik G, Kim A, Steiner DF, Friedman TC, Hara M. Intra-islet production of GLP-1 by activation of prohormone convertase 1/3 in pancreatic α -cells in mouse models of β -cell regeneration. *Islets*. 2010; 2:149–155. [PubMed: 20657753]
51. Hansen AM, Bödvarsdóttir TB, Nordestgaard DN, Heller RS, Gotfredsen CF, Maedler K, Fels JJ, Holst JJ, Karlén AE. Upregulation of alpha cell glucagon-like peptide 1 (GLP-1) in Psammomys obesus—an adaptive response to hyperglycaemia? *Diabetologia*. 2011; 54:1379–1387. [PubMed: 21347622]
52. Whalley NM, Pritchard LE, Smith DM, White A. Processing of proglucagon to GLP-1 in pancreatic alpha-cells: is this a paracrine mechanism enabling GLP-1 to act on beta cells? *J Endocrinol*. 2011; 211:99–106. [PubMed: 21795304]
53. Liu Z, Stanojevic V, Avadhani S, Yano T, Habener JF. Stromal cell-derived factor-1 (SDF-1)/chemokine (C-X-C motif) receptor 4 (CXCR4) axis activation induces intra-islet glucagon-like peptide-1 (GLP-1) production and enhances beta cell survival. *Diabetologia*. 2011; 54:2067–2076. [PubMed: 21567300]
54. Linnemann AK, Neuman JC, Battiola TJ, Wisinski JA, Kimple ME, Davis DB. Glucagon-Like Peptide-1 Regulates Cholecystokinin Production in β -Cells to Protect From Apoptosis. *Mol Endocrinol*. 2015; 29:978–987. [PubMed: 25984632]
- 55*. Marchetti P, Lupi R, Bugliani M, Kirkpatrick CL, Sebastiani G, Grieco FA, Del Guerra S, D'Aleo V, Piro S, Marselli L, Boggi U, Filipponi F, Tinti L, Salvini L, Wollheim CB, Purrello F, Dotta F. A local glucagon-like peptide 1 (GLP-1) system in human pancreatic islets. *Diabetologia*. 2012; 55:3262–3272. [PubMed: 22965295]
56. O'Reilly LA, Gu D, Sarvetnick N, Edlund H, Phillips JM, Fulford T, Cooke A. alpha-Cell neogenesis in an animal model of IDDM. *Diabetes*. 1997; 46:599–606. [PubMed: 9075799]
57. Ogawa N, List JF, Habener JF, Maki T. Cure of overt diabetes in NOD mice by transient treatment with anti-lymphocyte serum and exendin-4. *Diabetes*. 2004; 53:1700–1705. [PubMed: 15220193]
58. Rydgren T, Börjesson A, Carlsson A, Sandler S. Elevated glucagon-like peptide-1 plasma levels, as a possible adaptive response, in diabetic NOD mice. *Biochem Biophys Res Commun*. 2012; 423:583–587. [PubMed: 22695119]
59. Butler AE, Campbell-Thompson M, Gurlo T, Dawson DW, Atkinson M, Butler PC. Marked expansion of exocrine and endocrine pancreas with incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine tumors. *Diabetes*. 2013; 62:2595–2604. [PubMed: 23524641]
60. Ellingsgaard H, Hauselmann I, Schuler B, Habib AM, Baggio LL, Meier DT, Eppler E, Bouzakri K, Wuest S, Muller YD, Hansen AM, Reinecke M, Konrad D, Gassmann M, Reimann F, Halban PA, Gromada J, Drucker DJ, Gribble FM, Ehses JA, Donath MY. Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. *Nat Med*. 2011; 17:1481–1489. [PubMed: 22037645]
61. Wilson ME, Kalamaras JA, German MS. Expression pattern of IAPP and prohormone convertase 1/3 reveals a distinctive set of endocrine cells in the embryonic pancreas. *Mech Dev*. 2002; 115:171–176. [PubMed: 12049785]
62. Kedees MH, Guz Y, Vuguin PM, Vargas C, Cui L, Steiner DF, Charron MJ, Teitelman G. Nestin expression in pancreatic endocrine and exocrine cells of mice lacking glucagon signaling. *Dev Dyn*. 2007; 236:1126–1133. [PubMed: 17366624]

- 63*. Gelling RW, Du XQ, Dichmann DS, Romer J, Huang H, Cui L, Obici S, Tang B, Holst JJ, Fledelius C, Johansen PB, Rossetti L, Jelicks LA, Serup P, Nishimura E, Charron MJ. Lower blood glucose, hyperglucagonemia, and pancreatic alpha cell hyperplasia in glucagon receptor knockout mice. *Proc Natl Acad Sci U S A*. 2003; 100:1438–1443. [PubMed: 12552113]
64. Ali S, Lamont BJ, Charron MJ, Drucker DJ. Dual elimination of the glucagon and GLP-1 receptors in mice reveals plasticity in the incretin axis. *J Clin Invest*. 2011; 121:1917–1929. [PubMed: 21540554]
65. Sloop KW, Cao JX, Siesky AM, Zhang HY, Bodenmiller DM, Cox AL, Jacobs SJ, Moyers JS, Owens RA, Showalter AD, Brenner MB, Raap A, Gromada J, Berridge BR, Monteith DK, Porksen N, McKay RA, Monia BP, Bhanot S, Watts LM, Michael MD. Hepatic and glucagon-like peptide-1-mediated reversal of diabetes by glucagon receptor antisense oligonucleotide inhibitors. *J Clin Invest*. 2004; 113:1571–1581. [PubMed: 15173883]
66. Hayashi Y, Yamamoto M, Mizoguchi H, Watanabe C, Ito R, Yamamoto S, Sun XY, Murata Y. Mice deficient for glucagon gene-derived peptides display normoglycemia and hyperplasia of islet {alpha}-cells but not of intestinal L-cells. *Mol Endocrinol*. 2009; 23:1990–1999. [PubMed: 19819987]
67. Furuta M, Yano H, Zhou A, Rouillé Y, Holst JJ, Carroll R, Ravazzola M, Orci L, Furuta H, Steiner DF. Defective prohormone processing and altered pancreatic islet morphology in mice lacking active SPC2. *Proc Natl Acad Sci U S A*. 1997; 94:6646–6651. [PubMed: 9192619]
68. Gu W, Yan H, Winters KA, Komorowski R, Vonderfecht S, Atangan L, Sivits G, Hill D, Yang J, Bi V, Shen Y, Hu S, Boone T, Lindberg RA, Véniant MM. Long-term inhibition of the glucagon receptor with a monoclonal antibody in mice causes sustained improvement in glycemic control, with reversible alpha-cell hyperplasia and hyperglucagonemia. *J Pharmacol Exp Ther*. 2009; 331:871–881. [PubMed: 19720878]
69. Chen M, Gavrilova O, Zhao WQ, Nguyen A, Lorenzo J, Shen L, Nackers L, Pack S, Jou W, Weinstein LS. Increased glucose tolerance and reduced adiposity in the absence of fasting hypoglycemia in mice with liver-specific Gs alpha deficiency. *J Clin Invest*. 2005; 115:3217–3227. [PubMed: 16239968]
70. Longuet C, Robledo AM, Dean ED, Dai C, Ali S, McGuinness I, de Chavez V, Vuguin PM, Charron MJ, Powers AC, Drucker DJ. Liver-specific disruption of the murine glucagon receptor produces α -cell hyperplasia: evidence for a circulating α -cell growth factor. *Diabetes*. 2013; 62:1196–1205. [PubMed: 23160527]
71. Zhou C, Dhall D, Nissen NN, Chen CR, Yu R. Homozygous P86S mutation of the human glucagon receptor is associated with hyperglucagonemia, alpha cell hyperplasia, and islet cell tumor. *Pancreas*. 2009; 38:941–946. [PubMed: 19657311]
72. Yu R, Wawrowsky K, Zhou C. A natural inactivating mutant of human glucagon receptor exhibits multiple abnormalities in processing and signaling. *Endocrinol Nutr*. 2011; 58:258–266. [PubMed: 21680267]
- 73*. Lucas MB, Yu VE, Yu R. Mahvash Disease: Pancreatic Neuroendocrine Tumor Syndrome Caused by Inactivating Glucagon Receptor Mutation. *J Mol Genet Med*. 2013; 7:84.
- 74*. Takano Y, Kasai K, Takagishi Y, Kikumori T, Imai T, Murata Y, Hayashi Y. Pancreatic Neuroendocrine Tumors in Mice Deficient in Proglucagon-Derived Peptides. *PLoS One*. 2015; 10:e0133812. [PubMed: 26192435]
75. Unger RH, Raskin P, Srikant CB, Orci L. Glucagon and the A cells. *Recent Prog Horm Res*. 1976; 33:477–517. [PubMed: 801196]
76. Beberitz GR, Schuster HF. The impact of fatty acid oxidation on energy utilization: targets and therapy. *Curr Pharm Des*. 2002; 8:1199–1227. [PubMed: 12052217]
77. Wang MY, Yan H, Shi Z, Evans MR, Yu X, Lee Y, Chen S, Williams A, Philippe J, Roth MG, Unger RH. Glucagon receptor antibody completely suppresses type 1 diabetes phenotype without insulin by disrupting a novel diabetogenic pathway. *Proc Natl Acad Sci U S A*. 2015; 112:2503–2508. [PubMed: 25675519]
78. Hancock AS, Du A, Liu J, Miller M, May CL. Glucagon deficiency reduces hepatic glucose production and improves glucose tolerance in adult mice. *Mol Endocrinol*. 2010; 24:1605–1614. [PubMed: 20592160]

Practice Points

- Studies in genetically manipulated mouse models have identified a transcription factor called aristas (Arx) that when absent or severely reduced, prompts alpha cells to convert into functioning beta cells. Likewise, the transcription factor paired homeobox 4 (Pax4) is capable of regenerating ablated beta cells when conditionally expressed in alpha cells in mice. To date it remains unknown whether these two transcription factors contribute to the genetic background of humans who develop diabetes.
- Recent studies in mice suggest that excessive glucagon signaling might be more diabetogenic than has heretofore been appreciated. In the complete absence of glucagon receptor signaling in mice diabetes does not develop even in the near absence of insulin.
- The absence or severe impairment of glucagon signaling in mice results in alpha cell hyperplasia that with age progresses to alpha cell nesidioblastosis and to the formation of pancreatic neuroendocrine tumors (pNETs) with complete penetrance and distant metastases. An inactivating genetic mutation (P86S) in the glucagon receptor of six individuals has been described and termed Mahvash Disease. These individuals develop severe alpha cell hyperplasia and pNETs with metastatic potential. Although Mavash Disease is autosomal recessive and as such is a rare disease, consideration of the disease. Manifestations of Mahvash Disease include the absence of glucagonoma syndrome, abdominal pain, occasional hypoglycemia, extremely elevated plasma glucagon levels, pancreatic hypertrophy, and the identification of alpha cell nesidioblastosis upon endoscopic biopsy of the panceas.

Research Agenda

- Discover a means to specifically stimulate the production of GLP-1 in alpha cells. For example, activation of the expression of the prohormone convertase PC1/3 so as to preferentially form GLP-1 by the cleavage of proglucagon thereby promoting beta cell regeneration.
- Identify factor(s) that would stimulate repression of the expression of the transcription factor Aristaless (Arx) in alpha cells and thereby promote the transdifferentiation of the alpha cells into beta cells.
- Develop effective antagonists of glucagon actions so as to increase the numbers of alpha cells in islets to serve as substrates for their conversion into beta cells.
- Explore approaches to reduce, but not eliminate, glucagon signaling, which is counter-regulatory to insulin signaling, leading to improved glycemic control in individuals with type 2 diabetes.

Figure 1.

Proglucagon cleavages by prohormone convertases generate multiple proglucagon-derived peptides. In the pancreatic islets (left) proglucagon in mature alpha cells is cleaved by prohormone convertase 2 (PC2) producing glucagon (Gluca) as the major bioactive peptide, leaving the glucagon-like peptides (GLPs) within the inactive major proglucagon fragment (MPGF). In conditions of beta cell injuries, paracrine signaling from beta cells activates the prohormone convertase 1/3 (PC1/3) in immature pro-alpha cells resulting in the production of glucagon-like peptide-1 (GLP-1). GLP-1 is conjectured to promote the regeneration of injured beta cells. In the intestine (right) the bioactive peptides derived from cleavage of proglucagon by PC1/3 in the intestinal L-cells are the GLPs. Glucagon remains in the inactive proglucagon fragment glicentin. After its secretion GLP-1 is cleaved by the diaminopeptidyl peptidase-4 (Dpp4), which inactivates insulin-releasing (insulinotropic) actions resulting in the formation of an amino-terminally truncated peptide with insulin-like actions (insulinomimetic).

Figure 2.

Pathway depicting neogenesis of beta cells in the adult pancreas.

A reduction in beta cell mass owing to injury promotes the expansion of facultative stem/progenitor (progen) cells that express the transcription factor Pdx1, a master determinant of the pancreas lineage. A subpopulation of stem/progen cells transition into endocrine progenitor (endocrine progen) cells that express the endocrine lineage-specific transcription factor Ngn3. The undifferentiated endocrine cells proceed to differentiate into immature alpha cells, pro-alpha cells. The fate of pro-alpha cells is determined by the relative cellular levels of the transcription factors Arx and Pax4. High Arx/Pax4 determines the mature alpha cell fate, whereas a high Pax4/Arx ratio leads to differentiation into beta cells. Alpha cells can directly transdifferentiate into beta cells in conditions of severe beta cell ablation and in genetically manipulated mice in which Arx expression is suppressed or Pax4 is over-expressed. Distinguishing features between pro-alpha cells and mature alpha cells are that pro-alpha cells express PC1/3, produce GLP-1, and express the GLP-1 receptor, whereas mature alpha cells do not.

Lineage tracing experiments in which Pax4 is over-expressed in the pro-alpha cell lineage have shown that glucagon suppresses the expansion of stem/progenitor cells, as severe depletion of mature alpha cells by their transdifferentiation into beta cells drastically lowers glucagon levels and stimulates the expansion of stem/progenitor cells.

Figure 3.

Model of proposed paracrine interactions between beta and alpha cells in response to injury of beta cells. Regeneration factors (RegenFs) produced by injured beta cells act on adjacent alpha cells to induce the expression of prohormone convertase 1/3 (PC1/3) and the formation of GLP-1 from the cleavage of proglucagon. GLP-1 acts via paracrine signaling on beta cells to promote their regeneration (proliferation and survival). RegenFs, such as stromal cell-derived factor-1 (SDF-1), act on beta cells via autocrine signaling to promote survival. Since immature alpha cells express GLP-1 receptors feedback of GLP-1 might stimulate the proliferation of the cells and be involved in the development of the alpha cell hyperplasia that is seen in conditions of beta cell injury.