The Ambiguous beta-cell : On the loss of human pancreatic beta-cell identity

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CHAPTER 1

GENERAL INTRODUCTION

Diabetes mellitus is amongst the leading causes of morbidity and mortality worldwide. Insulin-producing pancreatic β-cells are central in establishing adequate glucose regulation and loss of functional β-cells results in the development of diabetes. Although it was previously thought that fully differentiated cells cannot change phenotype, recent murine studies indicated that mature β-cells can change identity into other islet cells under conditions of (metabolic) stress. It has been hypothesized that this process is associated with β-cell dysfunction and loss of β-cell mass that occurs in diabetes. Moreover, it was shown that islet cells can convert into functional β-cells, providing a possible source to obtain new β-cells. It is not known whether adult human β-cells can change identity and whether mechanisms of islet cell conversion play a role in human diabetes. The aim of this thesis is to explore the stability of adult human β-cell identity and to investigate whether loss of β-cell identity plays a role in the pathophysiology of diabetes.

DIFFERENT FORMS OF DIABETES MELLITUS

Diabetes mellitus is characterized by glycemic dysregulation, caused by an imbalance in the secretion of the pancreatic hormones insulin and glucagon in relation to blood glucose levels. Presenting clinical symptoms typically consist of polyuria, polydipsia, weight loss and sometimes polyphagia (1). Two main diabetes subtypes are distinguished based on the pathophysiology; type 1 diabetes mellitus (T1DM) and type 2 (T2DM). While glucose levels in the range of 3.5-6 mmol/l are considered normal, a fasting serum glucose level of ≥7 mmol/l is diagnostic for diabetes (2). Damage to macro- and microvasculature caused by dysregulation of glucose homeostasis, makes diabetes the leading cause of renal failure and blindness in developed countries, and increases the risk of stroke and lower-limb amputations (3-5). The number of diabetes patients increased globally from 153 million in 1980 to 347 million in 2008 (6). In the Netherlands, these numbers correspond to ~1 million diabetes patients, of which approximately 90% has T2DM.

T1DM is caused by a selective autoimmune destruction of the pancreatic β-cells, leading to insulin deficiency (7). Even though genetic predisposition and environmental factors are implied in the pathogenesis, the precise triggers eliciting the autoimmune reaction are not known. The disease predominantly occurs in younger people that are in direct need of lifelong exogenous insulin replacement therapy (8). The pathophysiology of T2DM results from a complex interplay of genetic factors and lifestyle behaviours that are associated with obesity. This interplay causes peripheral cells such as muscle or adipose tissue to respond inadequately to normal insulin levels, a process known as insulin resistance (9). Pancreatic islets will normally adapt to increased serum glucose levels by increasing the number of β-cells and increasing insulin secretion of individual β-cells (10). Failure of β-cell mass adaptation can arise from a lack of newly formed β-cells or by increased rates of apoptosis. Furthermore, when glucose concentrations are chronically elevated, first phase insulin secretion becomes blunted (11). Together, β-cell dysfunction, loss of β-cell mass and insulin resistance with increased hepatic glucose production lead to worsening of hyperglycemia.

Less often occurring diabetes subtypes include the distinct forms of Maturity-Onset Diabetes of the Young (MODY), caused by autosomal dominant inherited monogenic defects in essential β-cell genes (12). Furthermore, diabetes can be induced by pregnancy (gestational diabetes), certain drugs amongst which immunosuppressive drugs, exocrine pancreatic disorders such as cystic-fibrosis or severe pancreatitis, or in the context of syndromal disorders (1).

PANCREATIC ISLET PHYSIOLOGY

The pancreas consists of two glandular compartments that exert an exocrine and endocrine function. The exocrine gland plays a role in digestion by the secretion of digestive enzymes such as amylase or lipase and secretion of bicarbonates via the pancreatic duct system into the duodenum (13). The endocrine compartment occupies 1 to 2% of the pancreas and consists of approximately 1 million cell clusters that are scattered throughout the exocrine pancreas, so called islets of Langerhans.

Islet hormones

Pancreatic islets are highly vascularized micro-organs that secrete hormones directly in the bloodstream. The most frequent cell types in human islets are insulin-producing β-cells, representing 50-70% of islet cells, and glucagon-secreting α-cells (20-30%). The remaining islet cell types either produce somatostatin (δ-cells) or pancreatic polypeptide (PP-cells), while ghrelin-producing ε-cells can be found mainly during development and at lower frequency in adult human islets (14). The unique architecture of human islets has been emphasized by several studies (15-17). The majority of β-cells (~70%) are in direct contact with α-cells, and are in contact to the microvasculature to facilitate hormone secretion. In contrast to the human situation, mouse islets have a distinct architecture where β-cells comprise ~80% of islet cells and form the islet core surrounded by other islet cells (mainly α-cells) in the periphery (18).

Insulin protein is exclusively produced in β-cells. Upon transcription, the mRNA is translated into the protein preproinsulin. Post-translational processing in the endoplasmic reticulum includes proper folding of the protein and the formation of disulphide bonds between the A and B chains (19). Subsequently, the C chain will be cleaved off in the Golgi apparatus by prohormone convertase 1/3 (PC1/3), resulting in C-peptide and monomeric insulin (20). Mature insulin is then stored in secretory granules as stable hexamers surrounding 2 zinc atoms at a local pH 5.5. Under high glucose conditions, β-cells secrete insulin into the surrounding neutral pH leading to dissociation of the hexamers (19).

The prohormone proglucagon can be processed into different products following enzymatic cleavage. In α-cells, the abundance of PC2 results primarily in the 29 amino acid long peptide hormone glucagon. In intestinal L-cells, proglucagon is cleaved via PC1/3, resulting in incretin hormones such as glucagon-like peptide-1 (GLP-1), GLP-2 and glicentin (21).

Hormone secretion

Accurate glucose sensing and insulin secretion are essential to maintain glucose levels within a narrow physiological range. These processes are established by an intricate network of glucose transporters and enzymes in combination with the dense capillary network. During normal glucose levels, the β-cell membrane potential is negatively charged. Increased blood glucose concentrations lead to increased glucose uptake by β-cells, in animals via the glucose transporter Glut2 and in humans more likely via Glut1 (22). Glucose will be phosphorylated by glucokinase and used by mitochondria to produce ATP (23). The higher ATP/ADP ratio leads to closure of K_{ATP}-channels, which increases the membrane resistance and in turn leads to depolarization and opening of voltage-gated Ca^{2+}-channels. The increased intracellular calcium concentration triggers fusion of insulin granules with the plasma membrane inducing exocytosis (Fig. 1) (23). Circulating insulin binds the insulin receptor on its target cells and signals intracellularly via the insulin receptor substrate proteins (IRS) and phosphatidylinositol 3-kinase (PI3K) (24). Insulin signalling stimulates glucose uptake mainly in the liver, muscle and adipose tissue via activation of the glucose transporter Glut4, decreases hepatic glucose production (gluconeogenesis and glycogenolysis) and induces glycogen storage and lipid synthesis.

Glucagon secretion is generally triggered by low glucose levels, but can also be stimulated by circulating amino acids or lipids. Although the mechanisms of glucagon granule secretion are not fully understood, it is controlled by a combination of intrinsic K_{ATP}-channel dependent glucose sensing (hypoglycemia stimulates glucagon secretion), neuronal signalling (autonomic nervous system) and paracrine mechanisms (e.g. insulin, somatostatin and Zn^{2+}) (25;26). Similar to insulin secretion, glucagon exocytosis depends on electrical activity and is a Ca^{2+}-dependent process. However, using the K_{ATP}-channel stimulator diazoxide, it was shown that glucagon secretion is inhibited both when the K_{ATP}-channel activity is too low or too high and has a specific bell-shaped optimum (27). Circulating glucagon binds to the glucagon receptor, signalling inwards via cyclic AMP and protein kinase A (28). Glucagon works counterregulatory to insulin by stimulating glucose production in the liver via gluconeogenesis and glycogenolysis, but also exerts negative feedback on glucagon secretion itself and induces insulin secretion (29).

In conclusion, mature α- and β-cells are highly specialized endocrine cells that, in order to be fully functional, depend on a defined set of proteins that allow cell-specific actions such as hormone processing, glucose sensing and hormone secretion (including granule formation, transport and release). For the sake of this thesis, we will define functional islet cells as those that express and secrete hormones in a physiological manner to maintain normal glucose levels.

EMBRYONIC DEVELOPMENT OF THE PANCREAS

Pancreas development is first noticeable by the formation of endodermal outgrowths on the foregut endoderm, called the dorsal and ventral pancreatic buds. These branching epithelial structures start to arise at embryonic day (E) 9.5-10 in mice, comparable to

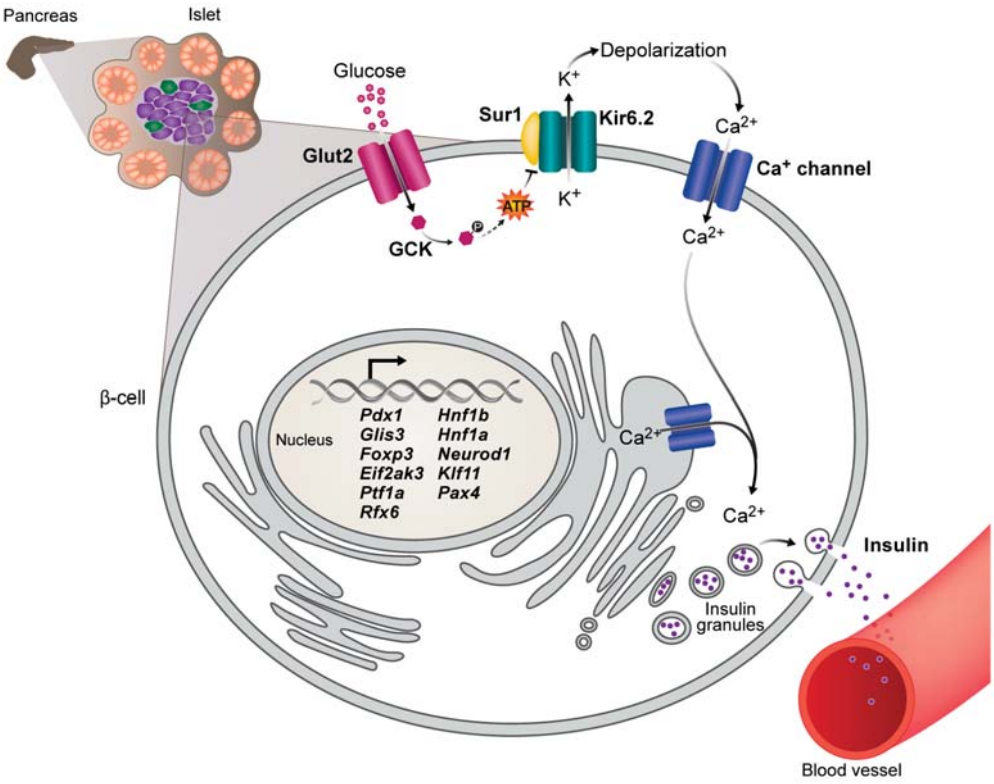

Figure 1. Glucose stimulated insulin secretion. In pancreatic β-cells, glucose stimulated insulin secretion starts by transportation of glucose into the cell via glucose transporters (Glut2). Glucose is phosphorylated by glucokinase (GCK) into glucose-6-phosphate and eventually converted into ATP. The increased ATP/ADP-ratio will trigger the closure of ATP-dependent potassium channels (subunits Sur1 and Kir6.2) which will lead to membrane depolarization and opening of calcium channels. The rise in intracellular calcium levels stimulates the exocytosis of insulin-containing granules into the bloodstream. Transcription factors that are essential for proper β-cell function are noted in the nucleus of the cell. *Figure adapted from Pagliuca and Melton with permission (113).*

a human embryonic age of 2-3 weeks (30;31). The early transition from definitive endoderm to pancreatic endoderm is mainly initiated by the transcription factor pancreatic duodenal homeobox-1 (Pdx1) (32), and null mutations in this gene result in pancreatic agenesis (33). Epithelial proliferation and remodelling follows a tip-trunk segregation. Acinar structures will derive from the cells in the tip of the proliferating epithelium, while ductal and endocrine cells are formed from bipotent progenitors in the trunk region starting at E11.5 (34). Expression of neurogenin3 (Ngn3) indicates commitment of trunk cells to the endocrine lineage (35). These precursor cells subsequently delaminate from the epithelium to form islet-like structures (36). In humans, vascularised structures in the parenchyme containing small islet aggregations appear from week 14 to 15, whereas adult-like islets containing all four endocrine cell types and a fine capillary network are observed by the beginning of the second trimester (31).

Lineage-specification within the endocrine compartment is controlled by the expression of specific transcription factors (Fig. 2) (35). Studies on genetically mutant mice have shown that the presence or absence of single transcription factors can skew endocrine progenitors towards a specific hormonal fate. For example, Nkx2.2 mutant mice show an increased number of ghrelin-containing ε-cells and a reduction in α-, β- and PP-cells (37). Deletion of Pax4 results in the loss of β-cells and an increased proportion of both α-cells and ε-cells, while the opposite holds true for Arx null mutant mice, in which the number of β-cells and δ-cells increases at the expense of α-cells (38;39). Finally, Arx/Pax4 double mutant mice show a massive number of δ-cells, but no α- or β-cells (40).

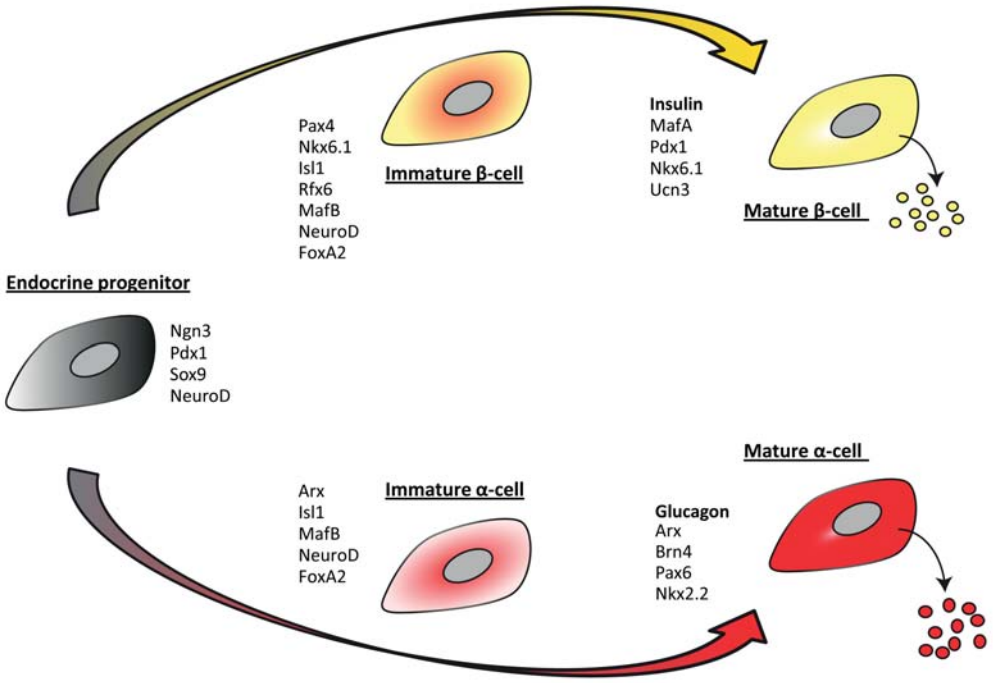

Figure 2. Lineage differentiation of α-cells and β-cells. Development of α- and β-cells is regulated by a complex interplay of specific transcription factors enabling functional maturation. Note that some factors (such as Pdx1) play a role in early cell development as well as in mature β-cells. Furthermore, despite their opposing functions in glucose metabolism, α- and β-cells share the expression of many transcription factors during development (such as NeuroD, FoxA2 and MafB).

Further maturation to form functional endocrine cells takes place after lineage specification. Although fetal human islets already secrete insulin during the first half of gestation (~week 17-20), implying the development of glucose sensing and hormone processing, the typical biphasic insulin secretion occurs in the early postnatal period (41;42). The developmental processes enabling glucose induced insulin secretion (GSIS) are not fully elucidated, but important roles have been identified for transcription factors

MafA, MafB, Pdx1, FoxA2 and NeuroD using genetically modified mouse models. High expression of MafA appears critical for the maturation of functional islet cells, also when the insulin-producing cells are derived from human embryonic stem cells (43). Accordingly, the loss of MafA during mouse development did not reduce the numbers of endocrine cells, but rendered them glucose-intolerant after birth (44). MafB is expressed both in α- and β-cells during development. During postnatal maturation in mice, MafB becomes restricted to α-cells, while MafA remains restricted to β-cells (45;46). While Pdx1 plays an essential role in the development of the early pancreas, its role is equally essential during further development and β-cell maturation. Both MafA and Pdx1 recognize specific binding sites that are present on promoters of essential β-cell genes such as *Insulin, Nkx6.1, Glut2*, *Glucokinase*, the zinc transporter *Slc30a8* and K_{ATP}-channels such as Kir6.2 (*Kcnj11)* (47;48). Accordingly, mice that lack Pdx1 specifically in β-cells become diabetic during adulthood and show impaired expression of both Glut2 and insulin (49). Foxa2 is essential in the maturation of both α- and β-cells. Initial islet cell specification is not perturbed in conditional *Foxa2*$^{-/-}$ mice (unconditional homozygous *Foxa2* null mice die during midgestation), but a 90% reduction in glucagon expression occurs in α-cell specific KO mice with a complete lack of the prohormone convertase PC2 (50). β-cell specific FoxA2 KO mice die shortly after birth because of (amongst others) the lack of K_{ATP}-channel expression (Kir6.2) and sulfonylurea receptor 1 (SUR1), resulting in hyperinsulinemic hypoglycemia (51;52). Finally, inactivation of NeuroD in β-cells during development resulted in severe glucose intolerance because of impaired GSIS, even though the islets still had half of the amount of insulin present (53).

Altogether, the development of mature islet cells depends on the complex interaction of specific transcription factors at specific time points. These factors will eventually activate the genes that constitute functional hormone secreting cells.

THE ROLE OF α- AND β-CELLS IN THE PATHOPHYSIOLOGY OF DIABETES

Whereas the architecture of pancreatic islet cells and cellular processes is delicately organized during normal development, it becomes disturbed in diseased state. In T1DM, islet pathophysiology is generally characterized by the autoimmune destruction of β-cells (Fig. 3) (54). However, recent studies indicate that T1DM is not a static disease after initial β-cell destruction. Pathological features of T1DM such as insulitis and HLA class I hyperexpression can still be present in patients with longstanding disease and usually occur in a multifocal pattern throughout the pancreas (55). Keenan et al showed that 67.4% of patients with longstanding diabetes (>50 years) have residual β-cell function defined as measurable serum C-peptide levels (56). Besides the primary profile of β-cell pathology in T1DM subjects, patients also have defects in α-cell regulation. Already in recent-onset patients, impairment in the suppression of glucagon levels in response to hyperglycemia is apparent, even though fasting plasma glucagon levels are similar to healthy controls (57). Moreover, the glucagon response to hypoglycemia can become blunted early in the course of type 1 diabetes (58). Possible explanations are that the local (intraislet)

insulin-mediated α-cell suppression is lost in T1DM because of the insulin deficiency or that the autoimmune process may change the microenvironment so that α-cells become unresponsive to paracrine insulin signalling (59).

Though less extreme than in T1DM, changes in islet architecture are distinct in T2DM (Fig. 3). An initial phase of β-cell mass compensation occurs following chronic exposure to high glucose load and insulin resistance, but final β-cell decompensation will result in glycemic dysregulation (11). Although β-cell dysfunction (disturbed GSIS) is central in the decompensation, marked changes in islet architecture can also be found. First, several studies on post-mortem sections or isolated islets of T2DM donors have shown a decreased β-cell mass and increased rates of β-cell apoptosis compared to non-diabetic controls (60-63). Although the importance in the etiology of T2DM is since long debated, the fact that β-cell mass is decreased in T2DM is now generally accepted (64;65).

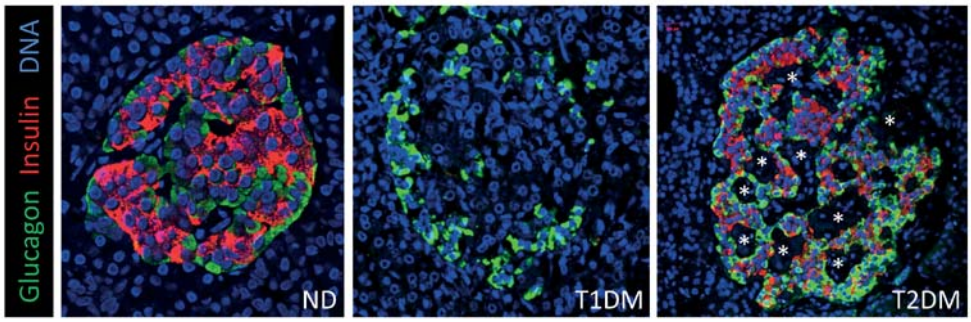

Figure 3. Changes in islet architecture related to diabetes. Representative images showing the islet architecture in a pancreatic biopsy of a non-diabetic donor (ND) and a donor with T1DM or T2DM stained for the hormones insulin (red) and glucagon (green). ND islets (left panel) display a mixed architecture of α- and β-cells containing a higher proportion of β-cells. Islets in T1DM (middle panel) are characterized by destruction of β-cells while α-cells remain. Islets in T2DM display a reduced number of β-cells and may show degradation of islet architecture by the formation of amyloid plaques (indicated by asterisks).

Glucagon dysregulation plays an important role in T2DM as well. On the islet level, although a relative increase in α-cells has been reported in T2DM (15;63;66;67), a recent study measuring absolute α-cell mass did not find this difference (29). Functionally, glucagon regulation is clearly abnormal under hyperglycemic conditions, and recent studies support that fasting plasma glucagon levels are elevated as well (68-71). The paradoxically elevated glucagon levels during hyperglycemia contribute to increased hepatic glucose production, thereby worsening hyperglycemia (72). Furthermore, the formation of islet amyloidosis is a hallmark of T2DM islets. While islet amyloid polypeptide (IAPP) is normally stored in insulin granules and co-secreted, its aggregation into β-sheets leads to the formation of islet amyloid plaques (73). Even though it is controversial whether amyloid is a cause or a consequence of β-cell dysfunction and apoptosis, there is a clear association with T2DM (66;74;75). Moreover, it has been shown that IAPP oligomers can be toxic *in vitro* and can elicit an inflammatory response (76;77).

In summary, though the pathophysiology of T1DM and T2DM are clearly different, islet architecture and islet cell function are affected in both types of diabetes. Moreover, both α-cell and β-cell function are impaired, resulting in hyperglycemia and a poor response to hypoglycemia.

DIABETES TREATMENT USING INSULIN REPLACEMENT OR β-CELL REPLACEMENT THERAPY

Since both types of diabetes are characterized by a failure of β-cells leading to hyperglycemia, current therapy is mainly focused on the replacement of β-cell function. The common goal for both T1DM and T2DM is to replace the (relative) insulin shortage to prevent shortterm dysregulation and longterm complications (78).

T1DM requires lifelong insulin replacement therapy. This can either be achieved by manual injection of long- and short-acting insulin or by insulin pump therapy. New developments that combine continuous glucose monitoring with smart algorithms can automatically titrate the required amount of insulin, thereby alleviating the burden of repeated glucose measurements (79).

Since the pathogenesis of T2DM reaches beyond the pancreatic islets, therapy is aimed at several targets altogether resulting in preservation of β-cell function and action. The action of clinically approved drugs include the inhibition of hepatic glucose production (biguanides), ameliorating peripheral insulin sensitivity (biguanides, thiazolidinediones), increasing insulin levels either irrespective of plasma glucose (sulphonylurea derivatives, exogenous insulin) or glucose-dependent (incretin-based therapy), or increasing glucose excretion (SGLT2 inhibitors) and often a combination of these modes of action (80).

Tight glucose regulation using sulphonylurea derivatives or insulin injections has the down side of an increased risk of hypoglycemia. The combination of attenuated glucagon and epinephrine responses causes the clinical syndrome of defective glucose counterregulation (81). Strict glycemic control therefore significantly increases the risk of hypoglycemic events both in patients with T1DM and T2DM that are under insulin therapy (78;82). The accompanying fear of hypoglycemia provides a psychological barrier that can have further negative impact on diabetes management (83).

Current β-cell replacement therapy; pancreas or islet transplantation

For patients that suffer from labile glucose regulation and its long-term complications despite optimization of insulin therapy, β-cell mass replacement is a therapeutical option. The concept of transplanting pancreas grafts or extracts is already under investigation since 1894 (84). Currently, β-cell replacement is achieved by transplantation of a whole donor pancreas or of isolated islets. In the latter case, the pancreas is first enzymatically digested and islets are separated from the non-islet tissue using a density gradient. The purified islet preparation can then be cultured and transplanted, commonly by infusion in the portal vein (85).

Whole pancreas transplantation can be performed as a simultaneous pancreas and kidney transplant (SPK), pancreas after kidney transplantation (PAK) or pancreas transplantation

alone (PTA). Graft survival rates after 3 years currently range from 75-85% with SPK showing the most favourable results (86). Besides replenishing the functional β-cell mass, pancreas transplantation normalizes glycemic control by restoring glucagon secretion, restoring the epinephrine response to hypoglycemia and by normalizing hepatic glucose production (87-89). Pancreas transplantation is an invasive procedure and the associated complications increase recipient morbidity and mortality. Amongst the most common complications requiring relaparotomy belong pancreas graft thrombosis, intraabdominal bleeding and deep wound infections (90).

For islet transplantation until the year 2000, only 12% of transplantations resulted in insulin independence for a time period of more than a week. It was only since the so-called Edmonton protocol was developed that intraportal islet transplantation was considered to be a successful and promising experimental therapy (91). The main reasons for the success of this protocol were found in the greater number of islets transplanted and the new immunosuppressive regimen that was applied. While high doses of glucocorticoids were commonly used (known to be diabetogenic), the Edmonton study developed a glucocorticoid-free regimen containing sirolimus, low-dose tacrolimus and daclizumab (a monoclonal antibody against the interleukin-2 receptor), inhibiting T-cell proliferation. After one year, insulin independence was achieved in 44% of patients, while 28% had partial graft function (92). More than 750 patients worldwide received an intraportal islet transplantation ever since, either as islet transplantation alone or following kidney transplantation (93). Criteria for patient eligibility include glycemic lability despite treatment optimization, recurrent hypoglycemic episodes and hypoglycemia unawareness. Follow-up data from the Edmonton cohort recently showed that 15% of transplanted patients was insulin independent after nine years (93). In >70% of these patients persistent C-peptide secretion and complete protection from hypoglycemic episodes was apparent, usually through the use of multiple (two or three) donors (93). A cohort from France showed similar results using the Edmonton protocol, reporting 57% insulin independence after 3.3 years (94). Furthermore, a recent islet-after-kidney transplantation cohort from Leiden showed the presence of C-peptide in 92% of patients after a 2-year follow-up period (95). Islet transplantation improves insulin sensitivity, is associated with restoration of hepatic glucose production and some but not all reports describe an improved counterregulatory reaction (96-99). The relatively uninvasive nature of percutaneous intraportal islet transplantation is associated with few procedure-related complications which include partial portal vein thrombosis and liver bleedings (92).

A current drawback of islet transplantation is the limited number of surviving islet cells directly following transplantation, estimations referring to 20-40% of the infused islet cell mass (100). The majority of this loss likely occurs directly following infusion in the portal vein due to hypoxia in the venous system, hyperglycemia and instant blood-mediated inflammatory reactions (101). Moreover, even though immunosuppressive regimens have improved, many of the drugs used are still harmful to islets. To improve survival of transplanted islets, several research groups focus on the production of encapsulation devices that serve as an impermeable barrier to immune cells while permitting nutrient diffusion and actively recruiting vascularization (102;103).

1 PRODUCING DE NOVO β-CELLS FROM STEM OR PROGENITOR CELLS

Considering the ~1 million diabetes patients, even the most promising scenario in which every offered donor organ in the Netherlands would be used to transplant one patient, the number of transplantations would not exceed ~250 per year. Clearly, the shortage of donor organs limits the widespread use of β-cell replacement therapy, and calls for novel sources of β-cells.

New β-cells could either be derived by directed differentiation of (pluripotent) stem cells or progenitor cells, transdifferentiation or lineage conversion of other cell types, or by replication of pre-existing β-cells (Fig. 4) (104). While β-cell replication is a potent mechanism in murine models (105), very low levels are present in mature human β-cells (60;106;107). For β-cell replication to become a promising therapeutic approach, mechanisms that prevent human β-cells from proliferating should first be uncovered and circumvented in a safe manner (108;109).

Pluripotent stem cells (PSC) have the potency to self-renew indefinitely and to differentiate into all 3 embryonic germ layers (endoderm, ectoderm and mesoderm). Commonly used PSC include human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC, reprogrammed adult somatic cells such as skin fibroblasts) (110;111). Since the differentiated state of PSC is comparable to that of the early embryo, differentiation protocols to obtain β-cells recapitulate embryonic development by the addition of specific growth factors and extracellular matrices (112;113). Standardized protocols initially lead to the formation of immature insulin-producing cells *in vitro* that become further maturated (i.e. glucose-responsive) within 3-4 months following transplantation into immunodeficient mice (43;114). Recently, extended differentiation protocols for hESC and hiPSC obtained cells that secreted insulin in comparable amount to adult β-cells in response to multiple glucose challenges *in vitro* and prevented or reversed hyperglycemia in mice (115;116). Remaining hurdles for clinical application include the ethical discussion (mainly on the use of hESC), the development of robustly defined protocols and safety issues concerning genetic stability and the risk of teratoma formation (117).

Adult stem cells or organ-specific progenitor cells are committed to differentiate into the cell types of the organ in which they reside. Typical examples of active adult stem cells reside in the intestine and the skin, where ongoing tissue renewal takes places (118). Several reports have used lineage tracing methods to show that cells from the pancreatic ductal compartment contribute to the replacement of functional β-cells following injury in adult mice (119;120). Upon injury by partial duct ligation, the adult stem cell marker *Lgr5* was expressed in regenerating pancreatic ducts and isolated cells could be expanded *ex vivo* (121). Since these studies were questioned using alternative lineage tracing strategies, the debate on the actual presence and location of pancreatic progenitor cells in adult pancreas is ongoing (34;122;123). However, studies on human cadaveric donor organs have noted more insulin-expressing cells in the ductal epithelium of pancreas from obese donors and pregnant women compared to controls (60;124). Moreover, culture of human islet-depleted tissue remaining after islet isolation formed islet-like structures that showed insulin expression (125-127).

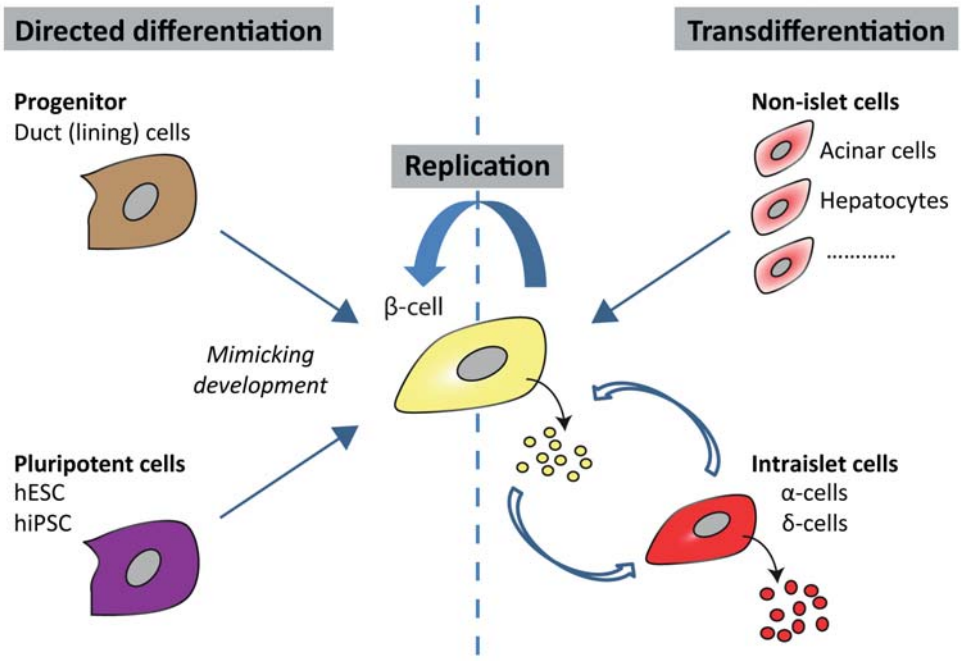

Figure 4. Strategies to generate new β-cells. Directions to generate new β-cells are divided as directed differentiation, replication or transdifferentiation. Directed differentiation makes use of pluripotent stem cells or adult progenitor cells. Differentiation protocols aim to mimic normal development by using combinations of growth factors, small molecules and matrix components to obtain new β-cells. Replication of pre-existing β-cells aims to identify small molecules that trigger proliferation of endogenous β-cell mass. Transdifferentiation relates to the reprogramming or conversion of terminally differentiated cell types. Conversion into insulin-producing cells has been achieved from related non-islet cells (acinar cells and hepatocytes) and from islet-derived cells (α- and δ-cells). hESC, human embryonic stem cells; hiPSC, human induced pluripotent stem cells.

Other groups reported the finding of rare stem cells that were isolated from adult mouse and human pancreas and differentiated into β-cells and neurons (128). Genetic lineage tracing revealed that these cells were derived from initially insulin-expressing cells (129), it is therefore not clear whether these cells represent true stem cells *in vivo* or β-cells that were dedifferentiated *in vitro* as shown previously (108;130).

Finally, mesenchymal stem cells (MSC) have been investigated to develop new β-cells. MSC are easily retrievable from bone marrow, adipose tissue or umbilical cord blood, can be expanded for multiple passages and can differentiate into blood, bone and adipose tissue cells (131). Initial reports described that MSC derived from bone marrow or splenocytes had the potential to differentiate into β-cells (132-134). Unfortunately, these studies could not be confirmed by several other groups (135;136). Current studies using MSC are focused on their angiogenic potential and evident role in immunomodulation in combination with allotransplantation (137;138).

Altogether, the application of stem cell technology holds great promise for future clinical application and important steps are being made especially in the differentiation of

pluripotent cells. Remaining hurdles including reproducibility of differentiation protocols and safety issues will need to be addressed in the near future.

DEDIFFERENTIATION AND TRANSDIFFERENTIATION

Stem cell differentiation has commonly been viewed as the ongoing commitment of a progenitor towards a terminally differentiated (unipotent) cell (139). This model is challenged by the finding that a fully differentiated cell can convert into another, a process called transdifferentiation or direct lineage conversion, or can take a step back to a less differentiated state, called dedifferentiation (140;141).

Dedifferentiation has been described in cardiac regeneration in zebrafish. After 20% removal of the zebrafish cardiac ventricle, regeneration occurred by dedifferentiation of cardiomyocytes, thereby enabling these cells to proliferate again before redifferentiating and restoring the healthy cardiomyocyte pool (142). A similar process takes place after Schwann cell damage in peripheral nerves. Dedifferentiation into a precursor cell type allows proliferation while subsequent redifferentiation into mature Schwann cells provide remyelination (143). Intriguingly, the salamander Ambystoma mexicanum (or axolotl) can reconstitute a fully functional limb after injury or amputation. Cells that are adjacent to the wound dedifferentiate and form a blastema that consists of tissue specific progenitor cells that proliferate and eventually redifferentiate to create a regenerated limb (144).

In these examples, the injured cell type itself provides regeneration via dedifferentiation and proliferation. Transdifferentiation or direct lineage conversion describes the process by which another differentiated cell type takes on the function of the injured cell type, either directly or via a dedifferentiation step (Fig. 5). Natural transdifferentiation has been described in the process of lens regeneration in adult newts. Damage to the lens results in dedifferentiation of pigment epithelial cells that normally reside in the dorsal iris, and subsequent transdifferentiation of these pigment cells regenerates the lens (145). In experimental models, transdifferentiation usually requires the genetic introduction of specific transcription factors or miRNAs in cells that are (in lineage) closely related to the desired cell type (146). In the pancreas, studies on transdifferentiation are either focused on obtaining new β-cells or understanding how β-cells maintain a stable identity.

Direct lineage conversion to obtain new β-cells

Several cell types have been used to study direct lineage conversion into β-cells. In mouse and isolated human hepatocytes, genetic activation of Pdx1 induced their conversion into insulin-producing cells that were functional *in vivo* (147;148). Zhou et al. accomplished to transdifferentiate murine pancreatic acinar cells *in vivo* into β-like cells using adenoviral delivery of 3 essential transcription factors; Ngn3, Pdx1 and MafA (141). More recently, this same cocktail of transcription factors was used to induce functional insulin-secreting cells from antral stomach cells (149). Also, rodent acinar cells were shown to differentiate into insulin-containing β-cells *in vitro* following cytokine treatment (150-152). Recent work added that a transient cytokine treatment *in vivo* could induce β-like cells from acinar cells and thereby reverse alloxan-induced hyperglycemia (153).

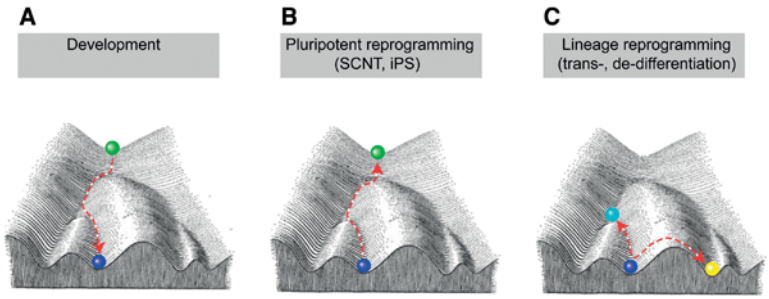

Figure 5. A landscape of development and reprogramming. *A:* In development, a pluripotent stem cell (green ball), rolls down bifurcating valleys, which represent all possible developmental paths. The cell differentiates into a mature cell (blue ball) at the bottom of the valley. *B:* During pluripotent reprogramming, including somatic cell nuclear transfer (SCNT) and formation of induced pluripotent stem (iPS) cells, the entire developmental process is reversed, and a differentiated cell is returned to a pluripotent state. This is represented by the ball rolling from the bottom of the valley backward to the top. *C:* Lineage reprogramming includes dedifferentiation and transdifferentiation, where a mature cell takes a step backward to a progenitor stage (cyan ball) or converts directly to another mature cell (yellow ball). *Figure adapted from Zhou and Melton with permission (141) .*

Intraislet cell conversion into β-cells has been observed in several lineage tracing studies in mice. Collombat et al. showed that overexpression of Pax4 in α-cells induced α-cell hyperplasia and conversion into β-cells, both during development and in adult mice (154;155). The ongoing α-cell to β-cell conversion resulted in a shortage of α-cells, that was in turn replenished by $Ngn3^{+}$ progenitor cells derived from the ductal epithelium, indicating that transdifferentiation and progenitor cell differentiation may act in concert (154). In addition, forced expression of Pdx1 in Ngn3-positive endocrine progenitors induced α- to β-cell reprogramming postnatally resulting in absence of α-cells (156). A report from Thorel et al. showed α-cell to β-cell transdifferentiation in a model of near-total β-cell ablation (157). In this study, the diphtheria toxin receptor was specifically overexpressed in β-cells so that administration of the diphtheria toxin lead to targeted β-cell destruction (>99%). Using α-cell lineage tracing, a large but variable fraction (~30-80|%) of the newly formed insulin-positive cells appeared to originate from a small subset (~5%) of α-cells (157). Using the same experimental model, it was recently shown that juvenile mice undergo massive reprogramming of δ-cells, but not α-cells, upon near total β-cell ablation, indicating that the transdifferentiation mechanism may differ with the age of the organism (158). Finally, partial α- to β-cell reprogramming was observed following treatment with a histone methyl transferases inhibitor, broadly affecting the epigenetic methylation signature (159).

These studies indicate that direct lineage conversion provides an opportunity to obtain new β-cells and can be achieved by shifting the balance of transcription factors in developmentally related cells. While all these studies are based on animal models, it is not known whether human islet cells have similar characteristics.

1 MAINTENANCE OF MATURE β-CELL IDENTITY

As β-cells are liable to loss of function under conditions of metabolic or inflammatory stress, it is essential for these cells to actively maintain functionality. Whereas embryonic β-cell development is a dynamic process that requires specific signals at specific time points, mature β-cells depend on a stable transcriptional program and supportive signalling from the microenvironment (113). The exact mechanisms that β-cells employ to maintain their differentiated identity are not fully understood, but recent studies have shed light on this issue.

Several experimental studies indicate that β-cell function becomes vulnerable when the genetic makeup is changed in the postnatal setting. Genetic loss of Pdx1 in β-cells from birth results in the development of diabetes with age, accompanied by impaired expression of the glucose transporter Glut2 and insulin, resembling MODY-4 (49). Moreover, it was shown that β-cell specific removal of Pdx1 during adulthood leads to derepression of MafB resulting in rapid conversion into α-cells (160). Deletion of Foxa1 and Foxa2 in mature β-cells affected both β-cell metabolism and insulin secretory mechanisms, leading to hyperinsulinemic hypoglycemia (161). On the post-transcriptional level, both RNA-binding proteins and several miRNAs have been shown to influence the turnover and translation of insulin synthesis and secretion (162;163). For example, miR-124a was shown to affect the expression of FoxA2 and Pdx1 (164). These studies show that subtle genetic changes may affect β-cell functionality.

Illustrating that active maintenance of β-cell identity is necessary to prevent complete transdifferentiation, several studies reported on the role of transcriptional repression of the α-cell transcription factor Arx in mature β-cells. These studies either used Arx overexpression or eliminated the transcriptional repression of Arx via (epi)genetic modifications, resulting in β-cell to α-cell conversion (38;165-167). Taken together, these studies strongly support that β-cell identity must be actively maintained and that alternative lineage repression is essential in this process.

That mature human β-cells depend on their microenvironment is illustrated by the process of epithelial-to-mesenchymal transition (EMT) that occurs within days following β-cell culture on plastic, resulting in a complete loss of the mature phenotype (168). This may in part be due to the lack of appropriate matrix and signalling molecules. Laminin 511 was previously identified in the vascular niche to promote insulin gene expression, likely via integrin β1 (169;170). Moreover, culture of purified human β-cells on laminin 511 partially blocked dedifferentiation via EMT (109). Mice with a mutated receptor for Bone Morphogenetic Protein-4 showed decreased expression of genes involved in glucose sensing, insulin processing and insulin secretion, and thereby developed diabetes (171). Although little is yet known in this field, these studies suggest that local signalling molecules and direct interaction between β-cells and the ECM are important substrates to sustain β-cell functional maturity.

Besides genetic alterations, pathological changes in the microenvironment can influence β-cell identity. Early studies in rats showed that chronic hyperglycemia following hemipancreatectomy was accompanied by a loss of β-cell transcription factors, which

likely lead to the loss of insulin expression and β-cell dysfunction (172). Similarly, β-cells that are cultured under conditions of oxidative stress lose expression of several essential transcription factors such as MafA and Pdx1 (173). Talchai et al. showed that mouse β-cells that genetically lack FoxO1 can dedifferentiate and convert into α-cells *in vivo*, but only under conditions of metabolic stress (induced by aging or multiparity) (174). The role of glucotoxicity or other stress factors on the stability of human β-cell identity is unknown.

The hypothesis that a differentiated cell encompasses a continuously active maintenance process, rather than being in a locked passive state, has been around for more than twenty years (175). Active maintenance of functional β-cell identity is under attention since rodent models suggest that β-cells can lose their identity which may result in diabetes. Much is still unknown about the exact triggers and mechanisms that influence the phenotype of mature β-cells and their relevance to human pathology has yet to become clear. However, it appears that β-cells are not passively locked cells and 'merely' produce insulin at the right moment, but are rather continuously active in a process to maintain their differentiated function.

AIM AND STRUCTURE OF THIS THESIS

Recent literature has highlighted the potential of intraislet cell conversion. However, most mechanistic studies are based on animal models while primary human islet material is scarce and studies on pancreatic tissue biopsies remain observational. The aim of this thesis is to explore the stability of the human β-cell phenotype and investigate whether loss of β-cell identity has a role in the pathophysiology of diabetes.

We first describe a case of intraportal islet transplantation in a patient with cystic fibrosis related diabetes in **Chapter 2,** illustrating the clinical benefit of β-cell replacement therapy. In **Chapter 3**, we present a novel agarose based microwell culture system that can be used for aggregate formation of human or rodent (islet) cells. We show that this platform provides reproducible results to study aggregation of primary human islet cells. In **Chapter 4**, we study the stability of human β-cells following islet cell reaggregration in the microwell culture system, using β-cell specific lineage tracing. We report that β-cells can spontaneously lose their identity and convert into glucagon-containing α-cells. In **Chapter 5**, we make use of human pancreatic tissue from donors with T2DM and matched controls to explore loss of β-cell identity in T2DM. We report that cells indicative of loss of β-cell identity are found more frequently in tissue samples from donors with a history of T2DM. In **Chapter 6**, we aim to inhibit the conversion process by studying the effects of Pax4 and GLP-1 receptor agonists in our model of β-cell conversion. We show that both factors can partially prevent loss of β-cell identity. **Chapter 7** provides a general discussion of the results described in this thesis and draws a model for the role of β-to-α cell conversion in diabetes.

1

REFERENCE LIST

1. Diagnosis and classification of diabetes mellitus. Diabetes Care 2009;32 Suppl 1:S62-S67
2. Chamberlain JJ, Rhinehart AS, Shaefer CF, Jr., Neuman A. Diagnosis and Management of Diabetes: Synopsis of the 2016 American Diabetes Association Standards of Medical Care in Diabetes. Ann Intern Med 2016;
3. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, Saran R, Wang AY, Yang CW. Chronic kidney disease: global dimension and perspectives. Lancet 2013;382:260-272
4. Boden-Albala B, Cammack S, Chong J, Wang C, Wright C, Rundek T, Elkind MS, Paik MC, Sacco RL. Diabetes, fasting glucose levels, and risk of ischemic stroke and vascular events: findings from the Northern Manhattan Study (NOMAS). Diabetes Care 2008;31:1132-1137
5. Icks A, Haastert B, Trautner C, Giani G, Glaeske G, Hoffmann F. Incidence of lower-limb amputations in the diabetic compared to the non-diabetic population. findings from nationwide insurance data, Germany, 2005-2007. Exp Clin Endocrinol Diabetes 2009;117:500-504
6. Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, Lin JK, Farzadfar F, Khang YH, Stevens GA, Rao M, Ali MK, Riley LM, Robinson CA, Ezzati M. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. Lancet 2011;378:31-40
7. van Belle TL, Coppieters KT, von Herrath MG. Type 1 diabetes: etiology, immunology, and therapeutic strategies. Physiol Rev 2011;91:79-118
8. Atkinson MA. The pathogenesis and natural history of type 1 diabetes. Cold Spring Harb Perspect Med 2012;2:
9. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet 2005;365:1333-1346
10. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 2006;444:840-846
11. Weir GC, Bonner-Weir S. Islet beta cell mass in diabetes and how it relates to function, birth, and death. Ann N Y Acad Sci 2013;1281:92-105
12. Fajans SS, Bell GI. MODY: history, genetics, pathophysiology, and clinical decision making. Diabetes Care 2011;34:1878-1884
13. Lee MG, Ohana E, Park HW, Yang D, Muallem S. Molecular mechanism of pancreatic and salivary gland fluid and HCO3 secretion. Physiol Rev 2012;92:39-74
14. Wierup N, Sundler F, Heller RS. The islet ghrelin cell. J Mol Endocrinol 2014;52:R35-R49
15. 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
16. 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
17. Brissova M, Fowler MJ, Nicholson WE, Chu A, Hirshberg B, Harlan DM, Powers AC. Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. J Histochem Cytochem 2005;53:1087-1097
18. Steiner DJ, Kim A, Miller K, Hara M. Pancreatic islet plasticity: interspecies comparison of islet architecture and composition. Islets 2010;2:135-145

19. Fu Z, Gilbert ER, Liu D. Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes. Curr Diabetes Rev 2013;9:25-53
20. Marzban L, Trigo-Gonzalez G, Zhu X, Rhodes CJ, Halban PA, Steiner DF, Verchere CB. Role of beta-cell prohormone convertase (PC)1/3 in processing of pro-islet amyloid polypeptide. Diabetes 2004;53:141-148
21. 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
22. McCulloch LJ, van de Bunt M, Braun M, Frayn KN, Clark A, Gloyn AL. GLUT2 (SLC2A2) is not the principal glucose transporter in human pancreatic beta cells: implications for understanding genetic association signals at this locus. Mol Genet Metab 2011;104:648-653
23. Rorsman P, Braun M. Regulation of insulin secretion in human pancreatic islets. Annu Rev Physiol 2013;75:155-179
24. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. Nat Rev Mol Cell Biol 2006;7:85-96
25. 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
26. Rorsman P, Salehi SA, Abdulkader F, Braun M, MacDonald PE. K(ATP)-channels and glucose-regulated glucagon secretion. Trends Endocrinol Metab 2008;19:277-284
27. MacDonald PE, De Marinis YZ, Ramracheya R, Salehi A, Ma X, Johnson PR, Cox R, Eliasson L, Rorsman P. A K ATP channel-dependent pathway within alpha cells regulates glucagon release from both rodent and human islets of Langerhans. PLoS Biol 2007;5:e143
28. Quesada I, Tuduri E, Ripoll C, Nadal A. Physiology of the pancreatic alpha-cell and glucagon secretion: role in glucose homeostasis and diabetes. J Endocrinol 2008;199:5-19
29. Henquin JC, Rahier J. Pancreatic alpha cell mass in European subjects with type 2 diabetes. Diabetologia 2011;54:1720-1725
30. Seymour PA, Sander M. Historical perspective: beginnings of the beta-cell: current perspectives in beta-cell development. Diabetes 2011;60:364-376
31. Lyttle BM, Li J, Krishnamurthy M, Fellows F, Wheeler MB, Goodyer CG, Wang R. Transcription factor expression in the developing human fetal endocrine pancreas. Diabetologia 2008;51:1169-1180
32. Gao N, LeLay J, Vatamaniuk MZ, Rieck S, Friedman JR, Kaestner KH. Dynamic regulation of Pdx1 enhancers by Foxa1 and Foxa2 is essential for pancreas development. Genes Dev 2008;22:3435-3448
33. Jonsson J, Carlsson L, Edlund T, Edlund H. Insulin-promoter-factor 1 is required for pancreas development in mice. Nature 1994;371:606-609
34. Solar M, Cardalda C, Houbracken I, Martin M, Maestro MA, De MN, Xu X, Grau V, Heimberg H, Bouwens L, Ferrer J. Pancreatic exocrine duct cells give rise to insulin-producing beta cells during embryogenesis but not after birth. Dev Cell 2009;17:849-860
35. Pan FC, Wright C. Pancreas organogenesis: from bud to plexus to gland. Dev Dyn 2011;240:530-565
36. Gittes GK. Developmental biology of the pancreas: a comprehensive review. Dev Biol 2009;326:4-35
37. Prado CL, Pugh-Bernard AE, Elghazi L, Sosa-Pineda B, Sussel L. Ghrelin cells replace insulin-producing beta cells in two mouse models of pancreas development. Proc Natl Acad Sci U S A 2004;101:2924-2929
38. 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

39. Courtney M, Pfeifer A, Al-Hasani K, Gjernes E, Vieira A, Ben-Othman N, Collombat P. In vivo conversion of adult alpha-cells into beta-like cells: a new research avenue in the context of type 1 diabetes. Diabetes Obes Metab 2011;13 Suppl 1:47-52

40. Collombat P, Hecksher-Sorensen J, Broccoli V, Krull J, Ponte I, Mundiger T, Smith J, Gruss P, Serup P, Mansouri A. The simultaneous loss of Arx and Pax4 genes promotes a somatostatin-producing cell fate specification at the expense of the alpha- and beta-cell lineages in the mouse endocrine pancreas. Development 2005;132:2969-2980

41. Otonkoski T, Andersson S, Knip M, Simell O. Maturation of insulin response to glucose during human fetal and neonatal development. Studies with perifusion of pancreatic isletlike cell clusters. Diabetes 1988;37:286-291

42. Rozzo A, Meneghel-Rozzo T, Delakorda SL, Yang SB, Rupnik M. Exocytosis of insulin: in vivo maturation of mouse endocrine pancreas. Ann N Y Acad Sci 2009;1152:53-62

43. Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S, Young H, Richardson M, Smart NG, Cunningham J, Agulnick AD, D'Amour KA, Carpenter MK, Baetge EE. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol 2008;26:443-452

44. Zhang C, Moriguchi T, Kajihara M, Esaki R, Harada A, Shimohata H, Oishi H, Hamada M, Morito N, Hasegawa K, Kudo T, Engel JD, Yamamoto M, Takahashi S. MafA is a key regulator of glucose-stimulated insulin secretion. Mol Cell Biol 2005;25:4969-4976

45. Nishimura W, Kondo T, Salameh T, El K, I, Dodge R, Bonner-Weir S, Sharma A. A switch from MafB to MafA expression accompanies differentiation to pancreatic beta-cells. Dev Biol 2006;293:526-539

46. Artner I, Blanchi B, Raum JC, Guo M, Kaneko T, Cordes S, Sieweke M, Stein R. MafB is required for islet beta cell maturation. Proc Natl Acad Sci U S A 2007;104:3853-3858

47. Hang Y, Stein R. MafA and MafB activity in pancreatic beta cells. Trends Endocrinol Metab 2011;22:364-373

48. Bernardo AS, Hay CW, Docherty K. Pancreatic transcription factors and their role in the birth, life and survival of the pancreatic beta cell. Mol Cell Endocrinol 2008;294:1-9

49. Ahlgren U, Jonsson J, Jonsson L, Simu K, Edlund H. beta-cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. Genes Dev 1998;12:1763-1768

50. Lee CS, Sund NJ, Behr R, Herrera PL, Kaestner KH. Foxa2 is required for the differentiation of pancreatic alpha-cells. Dev Biol 2005;278:484-495

51. Sund NJ, Vatamaniuk MZ, Casey M, Ang SL, Magnuson MA, Stoffers DA, Matschinsky FM, Kaestner KH. Tissue-specific deletion of Foxa2 in pancreatic beta cells results in hyperinsulinemic hypoglycemia. Genes Dev 2001;15:1706-1715

52. Lantz KA, Vatamaniuk MZ, Brestelli JE, Friedman JR, Matschinsky FM, Kaestner KH. Foxa2 regulates multiple pathways of insulin secretion. J Clin Invest 2004;114:512-520

53. Gu C, Stein GH, Pan N, Goebbels S, Hornberg H, Nave KA, Herrera P, White P, Kaestner KH, Sussel L, Lee JE. Pancreatic beta cells require NeuroD to achieve and maintain functional maturity. Cell Metab 2010;11:298-310

54. Bluestone JA, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature 2010;464:1293-1300

55. Coppieters KT, Dotta F, Amirian N, Campbell PD, Kay TW, Atkinson MA, Roep BO, von Herrath MG. Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. J Exp Med 2012;209:51-60

56. Keenan HA, Sun JK, Levine J, Doria A, Aiello LP, Eisenbarth G, Bonner-Weir S, King GL. Residual insulin production and pancreatic ss-cell turnover after 50 years of diabetes: Joslin Medalist Study. Diabetes 2010;59:2846-2853

57. Greenbaum CJ, Prigeon RL, D'Alessio DA. Impaired beta-cell function, incretin effect, and glucagon suppression in patients with type 1 diabetes who have normal fasting glucose. Diabetes 2002;51:951-957

58. Siafarikas A, Johnston RJ, Bulsara MK, O'Leary P, Jones TW, Davis EA. Early loss of the glucagon response to hypoglycemia in adolescents with type 1 diabetes. Diabetes Care 2012;35:1757-1762

59. Unger RH, Cherrington AD. Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. J Clin Invest 2012;122:4-12

60. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 2003;52:102-110

61. Rahier J, Guiot Y, Goebbels RM, Sempoux C, Henquin JC. Pancreatic beta-cell mass in European subjects with type 2 diabetes. Diabetes Obes Metab 2008;10 Suppl 4:32-42

62. Marchetti P, Del GS, Marselli L, Lupi R, Masini M, Pollera M, Bugliani M, Boggi U, Vistoli F, Mosca F, Del PS. Pancreatic islets from type 2 diabetic patients have functional defects and increased apoptosis that are ameliorated by metformin. J Clin Endocrinol Metab 2004;89:5535-5541

63. Yoon KH, Ko SH, Cho JH, Lee JM, Ahn YB, Song KH, Yoo SJ, Kang MI, Cha BY, Lee KW, Son HY, Kang SK, Kim HS, Lee IK, Bonner-Weir S. Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea. J Clin Endocrinol Metab 2003;88:2300-2308

64. Bonner-Weir S, O'Brien TD. Islets in type 2 diabetes: in honor of Dr. Robert C. Turner. Diabetes 2008;57:2899-2904

65. Ashcroft FM, Rorsman P. Diabetes mellitus and the beta cell: the last ten years. Cell 2012;148:1160-1171

66. Clark A, Wells CA, Buley ID, Cruickshank JK, Vanhegan RI, Matthews DR, Cooper GJ, Holman RR, Turner RC. Islet amyloid, increased A-cells, reduced B-cells and exocrine fibrosis: quantitative changes in the pancreas in type 2 diabetes. Diabetes Res 1988;9:151-159

67. Iki K, Pour PM. Distribution of pancreatic endocrine cells including IAPP-expressing cells in non-diabetic and type 2 diabetic cases. J Histochem Cytochem 2007;55:111-118

68. Unger RH, Aguilar-Parada E, Muller WA, Eisentraut AM. Studies of pancreatic alpha cell function in normal and diabetic subjects. J Clin Invest 1970;49:837-848

69. Woerle HJ, Szoke E, Meyer C, Dostou JM, Wittlin SD, Gosmanov NR, Welle SL, Gerich JE. Mechanisms for abnormal postprandial glucose metabolism in type 2 diabetes. Am J Physiol Endocrinol Metab 2006;290:E67-E77

70. Basu R, Schwenk WF, Rizza RA. Both fasting glucose production and disappearance are abnormal in people with "mild" and "severe" type 2 diabetes. Am J Physiol Endocrinol Metab 2004;287:E55-E62

71. Manell H, Staaf J, Manukyan L, Kristinsson H, Cen J, Stenlid R, Ciba I, Forslund A, Bergsten P. Altered Plasma Levels of Glucagon, GLP-1 and Glicentin During OGTT in Adolescents With Obesity and Type 2 Diabetes. J Clin Endocrinol Metab 2016;101:1181-1189

72. Dunning BE, Gerich JE. The role of alpha-cell dysregulation in fasting and postprandial hyperglycemia in type 2 diabetes and therapeutic implications. Endocr Rev 2007;28:253-283

73. Westermark P, Andersson A, Westermark GT. Islet amyloid polypeptide, islet amyloid, and diabetes mellitus. Physiol Rev 2011;91:795-826

74. Rocken C, Linke RP, Saeger W. Immunohistology of islet amyloid polypeptide in diabetes mellitus: semi-quantitative studies in a post-mortem series. Virchows Arch A Pathol Anat Histopathol 1992;421:339-344

75. Jurgens CA, Toukatly MN, Fligner CL, Udayasankar J, Subramanian SL, Zraika S, Aston-Mourney K, Carr DB, Westermark P, Westermark GT, Kahn SE, Hull RL. beta-cell loss and beta-cell apoptosis in human type 2 diabetes are related to islet amyloid deposition. Am J Pathol 2011;178:2632-2640

76. Lorenzo A, Razzaboni B, Weir GC, Yankner BA. Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus. Nature 1994;368:756-760

77. Masters SL, Dunne A, Subramanian SL, Hull RL, Tannahill GM, Sharp FA, Becker C, Franchi L, Yoshihara E, Chen Z, Mullooly N, Mielke LA, Harris J, Coll RC, Mills KH, Mok KH, Newsholme P, Nunez G, Yodoi J, Kahn SE, Lavelle EC, O'Neill LA. Activation of the NLRP3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1beta in type 2 diabetes. Nat Immunol 2010;11:897-904

78. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998;352:837-853

79. Pickup JC. Management of diabetes mellitus: is the pump mightier than the pen? Nat Rev Endocrinol 2012;8:425-433

80. de Koning EJ, Bonner-Weir S, Rabelink TJ. Preservation of beta-cell function by targeting beta-cell mass. Trends Pharmacol Sci 2008;29:218-227

81. Cryer PE, Davis SN, Shamoon H. Hypoglycemia in diabetes. Diabetes Care 2003;26:1902-1912

82. Hypoglycemia in the Diabetes Control and Complications Trial. The Diabetes Control and Complications Trial Research Group. Diabetes 1997;46:271-286

83. Wild D, von MR, Brohan E, Christensen T, Clauson P, Gonder-Frederick L. A critical review of the literature on fear of hypoglycemia in diabetes: Implications for diabetes management and patient education. Patient Educ Couns 2007;68:10-15

84. Williams, PW. Notes on Diabetes Treated with Extract and by Graft Of Sheep'sPancreas. BMJ 2, 1303-1304. 1894.

85. Robertson RP. Islet transplantation as a treatment for diabetes - a work in progress. N Engl J Med 2004;350:694-705

86. White SA, Shaw JA, Sutherland DE. Pancreas transplantation. Lancet 2009;373:1808-1817

87. Barrou Z, Seaquist ER, Robertson RP. Pancreas transplantation in diabetic humans normalizes hepatic glucose production during hypoglycemia. Diabetes 1994;43:661-666

88. Kendall DM, Rooney DP, Smets YF, Salazar BL, Robertson RP. Pancreas transplantation restores epinephrine response and symptom recognition during hypoglycemia in patients with long-standing type I diabetes and autonomic neuropathy. Diabetes 1997;46:249-257

89. Diem P, Redmon JB, Abid M, Moran A, Sutherland DE, Halter JB, Robertson RP. Glucagon, catecholamine and pancreatic polypeptide secretion in type I diabetic recipients of pancreas allografts. J Clin Invest 1990;86:2008-2013

90. Troppmann C. Complications after pancreas transplantation. Curr Opin Organ Transplant 2010;15:112-118

91. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 2000;343:230-238

92. Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, Secchi A, Brendel MD, Berney T, Brennan DC, Cagliero E, Alejandro R, Ryan EA, DiMercurio B, Morel P, Polonsky KS, Reems JA, Bretzel RG, Bertuzzi F, Froud T, Kandaswamy R, Sutherland DE, Eisenbarth G, Segal M, Preiksaitis J, Korbutt GS, Barton FB, Viviano L, Seyfert-Margolis V, Bluestone J,

Lakey JR. International trial of the Edmonton protocol for islet transplantation. N Engl J Med 2006;355:1318-1330

93. McCall M, Shapiro AM. Update on islet transplantation. Cold Spring Harb Perspect Med 2012;2:a007823
94. Vantyghem MC, Kerr-Conte J, Arnalsteen L, Sergent G, Defrance F, Gmyr V, Declerck N, Raverdy V, Vandewalle B, Pigny P, Noel C, Pattou F. Primary graft function, metabolic control, and graft survival after islet transplantation. Diabetes Care 2009;32:1473-1478
95. Nijhoff MF, Engelse MA, Dubbeld J, Braat AE, Ringers J, Roelen DL, van Erkel AR, Spijker HS, Bouwsma H, van der Boog PJ, de Fijter JW, Rabelink TJ, de Koning EJ. Glycemic Stability Through Islet-After-Kidney Transplantation Using an Alemtuzumab-Based Induction Regimen and Long-Term Triple-Maintenance Immunosuppression. Am J Transplant 2016;16:246-253
96. Rickels MR, Schutta MH, Mueller R, Markmann JF, Barker CF, Naji A, Teff KL. Islet cell hormonal responses to hypoglycemia after human islet transplantation for type 1 diabetes. Diabetes 2005;54:3205-3211
97. Rickels MR, Kong SM, Fuller C, Dalton-Bakes C, Ferguson JF, Reilly MP, Teff KL, Naji A. Improvement in insulin sensitivity after human islet transplantation for type 1 diabetes. J Clin Endocrinol Metab 2013;98:E1780-E1785
98. Paty BW, Ryan EA, Shapiro AM, Lakey JR, Robertson RP. Intrahepatic islet transplantation in type 1 diabetic patients does not restore hypoglycemic hormonal counterregulation or symptom recognition after insulin independence. Diabetes 2002;51:3428-3434
99. Rickels MR. Recovery of endocrine function after islet and pancreas transplantation. Curr Diab Rep 2012;12:587-596
100. Korsgren O, Lundgren T, Felldin M, Foss A, Isaksson B, Permert J, Persson NH, Rafael E, Ryden M, Salmela K, Tibell A, Tufveson G, Nilsson B. Optimising islet engraftment is critical for successful clinical islet transplantation. Diabetologia 2008;51:227-232
101. Carlsson PO. Influence of microenvironment on engraftment of transplanted beta-cells. Ups J Med Sci 2011;116:1-7
102. Buitinga M, Truckenmuller R, Engelse MA, Moroni L, Ten Hoopen HW, Van Blitterswijk CA, de Koning EJ, van Apeldoorn AA, Karperien M. Microwell scaffolds for the extrahepatic transplantation of islets of Langerhans. PLoS One 2013;8:e64772
103. O'Sullivan ES, Vegas A, Anderson DG, Weir GC. Islets transplanted in immunoisolation devices: a review of the progress and the challenges that remain. Endocr Rev 2011;32:827-844
104. Carlotti F, Zaldumbide A, Ellenbroek JH, Spijker HS, Hoeben RC, de Koning EJ. beta-Cell Generation: Can Rodent Studies Be Translated to Humans? J Transplant 2011;2011:892453
105. Dor Y, Brown J, Martinez OI, Melton DA. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature 2004;429:41-46
106. Parnaud G, Bosco D, Berney T, Pattou F, Kerr-Conte J, Donath MY, Bruun C, Mandrup-Poulsen T, Billestrup N, Halban PA. Proliferation of sorted human and rat beta cells. Diabetologia 2008;51:91-100
107. Rutti S, Sauter NS, Bouzakri K, Prazak R, Halban PA, Donath MY. In vitro proliferation of adult human beta-cells. PLoS One 2012;7:e35801
108. Russ HA, Bar Y, Ravassard P, Efrat S. In vitro proliferation of cells derived from adult human beta-cells revealed by cell-lineage tracing. Diabetes 2008;57:1575-1583
109. Banerjee M, Virtanen I, Palgi J, Korsgren O, Otonkoski T. Proliferation and plasticity of human beta cells on physiologically occurring laminin isoforms. Mol Cell Endocrinol 2012;355:78-86

110. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663-676

111. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861-872

112. Van HD, D'Amour KA, German MS. Derivation of insulin-producing cells from human embryonic stem cells. Stem Cell Res 2009;3:73-87

113. Pagliuca FW, Melton DA. How to make a functional beta-cell. Development 2013;140:2472-2483

114. Rezania A, Bruin JE, Riedel MJ, Mojibian M, Asadi A, Xu J, Gauvin R, Narayan K, Karanu F, O'Neil JJ, Ao Z, Warnock GL, Kieffer TJ. Maturation of human embryonic stem cell-derived pancreatic progenitors into functional islets capable of treating pre-existing diabetes in mice. Diabetes 2012;61:2016-2029

115. Pagliuca FW, Millman JR, Gurtler M, Segel M, Van DA, Ryu JH, Peterson QP, Greiner D, Melton DA. Generation of Functional Human Pancreatic beta Cells In Vitro. Cell 2014;159:428-439

116. Rezania A, Bruin JE, Arora P, Rubin A, Batushansky I, Asadi A, O'Dwyer S, Quiskamp N, Mojibian M, Albrecht T, Yang YH, Johnson JD, Kieffer TJ. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. Nat Biotechnol 2014;32:1121-1133

117. Mikkers HM, Freund C, Mummery CL, Hoeben RC. Cell replacement therapies: is it time to reprogram? Hum Gene Ther 2014;25:866-874

118. Barker N, Bartfeld S, Clevers H. Tissue-resident adult stem cell populations of rapidly self-renewing organs. Cell Stem Cell 2010;7:656-670

119. Inada A, Nienaber C, Katsuta H, Fujitani Y, Levine J, Morita R, Sharma A, Bonner-Weir S. Carbonic anhydrase II-positive pancreatic cells are progenitors for both endocrine and exocrine pancreas after birth. Proc Natl Acad Sci U S A 2008;105:19915-19919

120. Xu X, D'Hoker J, Stange G, Bonne S, De LN, Xiao X, Van de Casteele M, Mellitzer G, Ling Z, Pipeleers D, Bouwens L, Scharfmann R, Gradwohl G, Heimberg H. Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 2008;132:197-207

121. Huch M, Bonfanti P, Boj SF, Sato T, Loomans CJ, van de Wetering M, Sojoodi M, Li VS, Schuijers J, Gracanin A, Ringnalda F, Begthel H, Hamer K, Mulder J, van Es JH, de KE, Vries RG, Heimberg H, Clevers H. Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. EMBO J 2013;32:2708-2721

122. Kushner JA, Weir GC, Bonner-Weir S. Ductal origin hypothesis of pancreatic regeneration under attack. Cell Metab 2010;11:2-3

123. Kopp JL, Dubois CL, Schaffer AE, Hao E, Shih HP, Seymour PA, Ma J, Sander M. Sox9+ ductal cells are multipotent progenitors throughout development but do not produce new endocrine cells in the normal or injured adult pancreas. Development 2011;138:653-665

124. Butler AE, Cao-Minh L, Galasso R, Rizza RA, Corradin A, Cobelli C, Butler PC. Adaptive changes in pancreatic beta cell fractional area and beta cell turnover in human pregnancy. Diabetologia 2010;53:2167-2176

125. Yatoh S, Dodge R, Akashi T, Omer A, Sharma A, Weir GC, Bonner-Weir S. Differentiation of affinity-purified human pancreatic duct cells to beta-cells. Diabetes 2007;56:1802-1809

126. Gao R, Ustinov J, Korsgren O, Otonkoski T. In vitro neogenesis of human islets reflects the plasticity of differentiated human pancreatic cells. Diabetologia 2005;48:2296-2304

127. Lee J, Sugiyama T, Liu Y, Wang J, Gu X, Lei J, Markmann JF, Miyazaki S, Miyazaki J, Szot GL, Bottino R, Kim SK. Expansion and conversion of human pancreatic ductal cells into insulin-secreting endocrine cells. Elife 2013;2:

128. Seaberg RM, Smukler SR, Kieffer TJ, Enikolopov G, Asghar Z, Wheeler MB, Korbutt G, van der Kooy D. Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. Nat Biotechnol 2004;22:1115-1124

129. Smukler SR, Arntfield ME, Razavi R, Bikopoulos G, Karpowicz P, Seaberg R, Dai F, Lee S, Ahrens R, Fraser PE, Wheeler MB, van der Kooy D. The adult mouse and human pancreas contain rare multipotent stem cells that express insulin. Cell Stem Cell 2011;8:281-293

130. Bouwens L, Houbracken I, Mfopou JK. The use of stem cells for pancreatic regeneration in diabetes mellitus. Nat Rev Endocrinol 2013;9:598-606

131. Carlotti F, Zaldumbide A, Loomans CJ, van RE, Engelse M, de Koning EJ, Hoeben RC. Isolated human islets contain a distinct population of mesenchymal stem cells. Islets 2010;2:164-173

132. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41-49

133. Ianus A, Holz GG, Theise ND, Hussain MA. In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. J Clin Invest 2003;111:843-850

134. Kodama S, Kuhtreiber W, Fujimura S, Dale EA, Faustman DL. Islet regeneration during the reversal of autoimmune diabetes in NOD mice. Science 2003;302:1223-1227

135. Couzin J. Immunology. Diabetes studies conflict on power of spleen cells. Science 2006;311:1694

136. Taneera J, Rosengren A, Renstrom E, Nygren JM, Serup P, Rorsman P, Jacobsen SE. Failure of transplanted bone marrow cells to adopt a pancreatic beta-cell fate. Diabetes 2006;55:290-296

137. Dominguez-Bendala J, Lanzoni G, Inverardi L, Ricordi C. Concise review: mesenchymal stem cells for diabetes. Stem Cells Transl Med 2012;1:59-63

138. Abdi R, Fiorina P, Adra CN, Atkinson M, Sayegh MH. Immunomodulation by mesenchymal stem cells: a potential therapeutic strategy for type 1 diabetes. Diabetes 2008;57:1759-1767

139. Trucco M. Regeneration of the pancreatic beta cell. J Clin Invest 2005;115:5-12

140. Jopling C, Boue S, Izpisua Belmonte JC. Dedifferentiation, transdifferentiation and reprogramming: three routes to regeneration. Nat Rev Mol Cell Biol 2011;12:79-89

141. Zhou Q, Melton DA. Extreme makeover: converting one cell into another. Cell Stem Cell 2008;3:382-388

142. Jopling C, Sleep E, Raya M, Marti M, Raya A, Izpisua Belmonte JC. Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. Nature 2010;464:606-609

143. Chen ZL, Yu WM, Strickland S. Peripheral regeneration. Annu Rev Neurosci 2007;30:209-233

144. Kragl M, Knapp D, Nacu E, Khattak S, Maden M, Epperlein HH, Tanaka EM. Cells keep a memory of their tissue origin during axolotl limb regeneration. Nature 2009;460:60-65

145. Tsonis PA, Madhavan M, Tancous EE, Del Rio-Tsonis K. A newt's eye view of lens regeneration. Int J Dev Biol 2004;48:975-980

146. Vierbuchen T, Wernig M. Direct lineage conversions: unnatural but useful? Nat Biotechnol 2011;29:892-907

147. Ferber S, Halkin A, Cohen H, Ber I, Einav Y, Goldberg I, Barshack I, Seijffers R, Kopolovic J, Kaiser N, Karasik A. Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. Nat Med 2000;6:568-572

148. Sapir T, Shternhall K, Meivar-Levy I, Blumenfeld T, Cohen H, Skutelsky E, Eventov-Friedman S, Barshack I, Goldberg I, Pri-Chen S, Ben-Dor L, Polak-Charcon S, Karasik A, Shimon I, Mor E,

Ferber S. Cell-replacement therapy for diabetes: Generating functional insulin-producing tissue from adult human liver cells. Proc Natl Acad Sci U S A 2005;102:7964-7969

149. Ariyachet C, Tovaglieri A, Xiang G, Lu J, Shah MS, Richmond CA, Verbeke C, Melton DA, Stanger BZ, Mooney D, Shivdasani RA, Mahony S, Xia Q, Breault DT, Zhou Q. Reprogrammed Stomach Tissue as a Renewable Source of Functional beta Cells for Blood Glucose Regulation. Cell Stem Cell 2016;18:410-421

150. Houbracken I, de WE, Lardon J, Ling Z, Heimberg H, Rooman I, Bouwens L. Lineage tracing evidence for transdifferentiation of acinar to duct cells and plasticity of human pancreas. Gastroenterology 2011;141:731-41, 741

151. Minami K, Okuno M, Miyawaki K, Okumachi A, Ishizaki K, Oyama K, Kawaguchi M, Ishizuka N, Iwanaga T, Seino S. Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells. Proc Natl Acad Sci U S A 2005;102:15116-15121

152. Baeyens L, De BS, Lardon J, Mfopou JK, Rooman I, Bouwens L. In vitro generation of insulin-producing beta cells from adult exocrine pancreatic cells. Diabetologia 2005;48:49-57

153. Baeyens L, Lemper M, Leuckx G, De GS, Bonfanti P, Stange G, Shemer R, Nord C, Scheel DW, Pan FC, Ahlgren U, Gu G, Stoffers DA, Dor Y, Ferrer J, Gradwohl G, Wright CV, Van de Casteele M, German MS, Bouwens L, Heimberg H. Transient cytokine treatment induces acinar cell reprogramming and regenerates functional beta cell mass in diabetic mice. Nat Biotechnol 2014;32:76-83

154. 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 beta-like Cells Able to Counter Repeated Cycles of Toxin-Induced Diabetes. Dev Cell 2013;26:86-100

155. 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

156. Yang YP, Thorel F, Boyer DF, Herrera PL, Wright CV. Context-specific alpha- to-beta-cell reprogramming by forced Pdx1 expression. Genes Dev 2011;25:1680-1685

157. Thorel F, Nepote 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

158. 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 delta-cells into insulin producers. Nature 2014;514:503-507

159. 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 alpha to beta cell reprogramming. J Clin Invest 2013;123:1275-1284

160. 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 beta cell identity and function by repressing an alpha cell program. Cell Metab 2014;19:259-271

161. Gao N, Le LJ, Qin W, Doliba N, Schug J, Fox AJ, Smirnova O, Matschinsky FM, Kaestner KH. Foxa1 and Foxa2 maintain the metabolic and secretory features of the mature beta-cell. Mol Endocrinol 2010;24:1594-1604

162. Hennessy E, O'Driscoll L. Molecular medicine of microRNAs: structure, function and implications for diabetes. Expert Rev Mol Med 2008;10:e24

163. Tattikota SG, Poy MN. Re-dicing the pancreatic beta-cell: do microRNAs define cellular identity? EMBO J 2011;30:797-799

164. Baroukh N, Ravier MA, Loder MK, Hill EV, Bounacer A, Scharfmann R, Rutter GA, Van OE. MicroRNA-124a regulates Foxa2 expression and intracellular signaling in pancreatic beta-cell lines. J Biol Chem 2007;282:19575-19588

165. 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

166. Papizan JB, Singer RA, Tschen SI, Dhawan S, Friel JM, Hipkens SB, Magnuson MA, Bhushan A, Sussel L. Nkx2.2 repressor complex regulates islet beta-cell specification and prevents beta-to-alpha-cell reprogramming. Genes Dev 2011;25:2291-2305

167. Schaffer AE, Taylor BL, Benthuysen JR, Liu J, Thorel F, Yuan W, Jiao Y, Kaestner KH, Herrera PL, Magnuson MA, May CL, Sander M. Nkx6.1 controls a gene regulatory network required for establishing and maintaining pancreatic Beta cell identity. PLoS Genet 2013;9:e1003274

168. Russ HA, Ravassard P, Kerr-Conte J, Pattou F, Efrat S. Epithelial-mesenchymal transition in cells expanded in vitro from lineage-traced adult human pancreatic beta cells. PLoS One 2009;4:e6417

169. Nikolova G, Jabs N, Konstantinova I, Domogatskaya A, Tryggvason K, Sorokin L, Fassler R, Gu G, Gerber HP, Ferrara N, Melton DA, Lammert E. The vascular basement membrane: a niche for insulin gene expression and Beta cell proliferation. Dev Cell 2006;10:397-405

170. Parnaud G, Hammar E, Rouiller DG, Armanet M, Halban PA, Bosco D. Blockade of beta1 integrin-laminin-5 interaction affects spreading and insulin secretion of rat beta-cells attached on extracellular matrix. Diabetes 2006;55:1413-1420

171. Goulley J, Dahl U, Baeza N, Mishina Y, Edlund H. BMP4-BMPR1A signaling in beta cells is required for and augments glucose-stimulated insulin secretion. Cell Metab 2007;5:207-219

172. Jonas JC, Sharma A, Hasenkamp W, Ilkova H, Patane G, Laybutt R, Bonner-Weir S, Weir GC. Chronic hyperglycemia triggers loss of pancreatic beta cell differentiation in an animal model of diabetes. J Biol Chem 1999;274:14112-14121

173. Guo S, Dai C, Guo M, Taylor B, Harmon JS, Sander M, Robertson RP, Powers AC, Stein R. Inactivation of specific beta cell transcription factors in type 2 diabetes. J Clin Invest 2013;

174. Talchai C, Xuan S, Lin HV, Sussel L, Accili D. Pancreatic beta cell dedifferentiation as a mechanism of diabetic beta cell failure. Cell 2012;150:1223-1234

175. Blau HM. Differentiation requires continuous active control. Annu Rev Biochem 1992;61:1213-1230