Review

MOLECULAR METABOLISM

Islet cell plasticity and regeneration*

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ABSTRACT

Insulin-dependent diabetes is a complex multifactorial disorder characterized by loss or dysfunction of β-cells resulting in failure of metabolic control. Even though type 1 and 2 diabetes differ in their pathogenesis, restoring β-cell function is the overarching goal for improved therapy of both diseases. This could be achieved either by cell-replacement therapy or by triggering intrinsic regenerative mechanisms of the pancreas. For type 1 diabetes, a combination of β-cell replacement and immunosuppressive therapy could be a curative treatment, whereas for type 2 diabetes enhancing endogenous mechanisms of β-cell regeneration might optimize blood glucose control. This review will briefly summarize recent efforts to allow β-cell regeneration where the most promising approaches are currently (1) increasing β-cell self-replication or neogenesis from ductal progenitors and (2) conversion of α-cells into β-cells.

Keywords Diabetes; Islet architecture; β-cell regeneration; β-cell proliferation; β-cell neogenesis; Pancreas plasticity

1. β-CELL DEGENERATION AND REGENERATION

Diabetes mellitus is a metabolic disorder characterized by progressive loss or dysfunction of pancreatic insulin-producing β-cells. This results in hyperglycemia causing multiple complications and organ damage. Although diabetes is manageable, β-cell failure is progressive and no definitive curative treatment has yet been found for both major forms of diabetes. In type 1 diabetes mellitus (T1DM), deficit of insulin is caused by autoimmune destruction of β-cells. The only available curative therapy for T1DM is the replacement of the lost β-cell mass by islet transplantation from cadaveric donors [1]. Due to the shortage of transplantable material, *in vitro* generation of β-cells from an unlimited source of self-renewing stem cells, such as embryonic stem cells, might be an alternative approach [2]. However, until now no functional mature β-cells can be efficiently generated from stem cells *in vitro*. Thus, a major effort is on the way to improve differentiation protocols in order to increase transplantable material to allow successful cell-replacement therapies in the future.

Type 2 diabetes mellitus (T2DM), generally results from high insulin demand due to the insulin resistance of the peripheral tissues triggering β-cell mass expansion and hyperinsulinemia. This in turn leads to gradual β-cell exhaustion and dysfunction (insulin secretion defects), and eventually instigates loss of β-cell mass by apoptosis [3–7]. Recently, de-differentiation of mature insulin-producing β-cells to a "naïve" status has been reported as a novel mechanism of β-cell failure in T2DM [8]. Thus, the only way for a better treatment of insulin-dependent T2DM patients is to replace or regenerate the lost or dysfunctional β-cell mass. This could be achieved by triggering β-cell proliferation or neogenesis, reversing β-cell de-differentiation or blocking β-cell apoptosis [9–12]. However, this requires a thorough understanding of the natural and diseased islet cell niche and the signals and factors that influence β-cell degeneration and regeneration.

2. ISLET ARCHITECTURE AND NICHE

Adult murine pancreatic islets are constituted of 70–90% insulin-producing β-cells, surrounded by α-, δ-, ε-, and PP-cells secreting glucagon, somatostatin, ghrelin and pancreatic polypeptide, respectively [13,14]. These different endocrine cell types are the main regulators of nutrient metabolism and glucose homeostasis. Of note, islet architecture is highly variable from species to species [15]. In rodents for example, β-cells are located in the core of the islet, whereas in humans they are intermixed with other endocrine cell types [16,17]. In mice, β-cells are organized in polarized rosette-like structures around the islet capillaries, which provide oxygen and nutrient and collect hormones secreted by the islet endocrine cells into the blood stream [18–20]. Interaction between blood vessels and pancreatic progenitor cells takes place early during development where neighboring tissue interactions are essential for organ differentiation [21]. During adulthood, islet endothelial cells secrete several growth factors, such as hepatocyte growth factor (HGF) and connective tissue growth factor (CTGF), that together with a specialized extracellular matrix (ECM) control and support β-cell function and proliferation [22]. Interconnection between ECM, cell–cell adhesion and gap junctions maintains β-cells in higher three dimensional (3D)

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order which is crucial to coordinate their function [14]. For example, loss of the gap junction protein Connexin-36 is linked to impaired glucose sensitivity in mice and to increased the susceptibility for T2DM in humans [23,24]. Thus, 3D islet cell architecture with its intricate neighboring cell types and ECM interaction makes it likely to believe that the islet resembles an important niche for β-cell function, growth and homeostasis.

Apical-basal (AB) and planar cell polarity (PCP) of the different islet cell types is required as a base for this higher order 3D organization and function in the islets. One such protein, which regulates AB polarity and energy metabolism in different organs, is Liver Kinase B1 (Lkb1). Lkb1 is a serine threonine kinase that is highly conserved among different species [25,26]. Conditional deletion of Lkb1 in pancreatic tissue, display histological alteration in rosette-like structures of β-cells around the islet capillaries, suggesting that Lkb1 and its targets orchestrate β-cell polarity [27]. Moreover, it has been observed that the loss of Lkb1 leads to increased β-cell volume, improved glucose tolerance in the high-fat diet mouse model and increased proliferation of insulin-producing cells [28]. Thus, for an ordered arrangement of cells in a 3D space both AB and PCP are absolutely required. This is well supported with the presence of PCP proteins already at embryonic day (E) 11.5 in pancreatic epithelial progenitors. Celsr2 and Celsr3 are important PCP core components and their ablation lead to impaired β-cell differentiation from endocrine progenitors during fetal life [29]. Further evidence demonstrating the relevance of PCP in mature islets comes from a study focusing on the role of the Activating Transcription Factor 2 (ATF2). Han and colleagues demonstrated that ATF2, which is involved in Wnt/PCP signaling during morphogenesis [30], plays an important role in the regulation of insulin gene expression in mature islets. ATF2 interacts with key β-cell transcription factors such as MAFA, PDX1 and BETA2 [31]. Altogether, besides classical signaling pathways and growth factors such as Wnt, Hedgehog, Notch etc. known to control β-cell homeostasis, a new light has been shed on islet architecture and its components as factors regulating β-cell function. Thus, it is very likely that 3D architecture of the islet niche actively contributes to preserve β-cell function and if disturbed, might trigger compensatory regenerative mechanisms.

3. CELLULAR PLASTICITY IN THE PANCREAS: CAN WE HARNESS MECHANISMS OF CELLULAR PLASTICITY FOR REGENERATION?

Genetic analysis of endocrine and exocrine cells within the pancreas reveals a certain degree of cellular plasticity under pathological or experimental conditions, which is summarized in Figure 1 and will be discussed in the following sections. Cellular plasticity is defined by the capacity of a specialized cell type to convert into another cell type to compensate for the loss of cellular or systemic function. Thus, the inter-conversion of pancreatic cells into β-cells might be harnessed for novel regenerative therapies.

Lately, several studies have focused their efforts on the generation of β-cells from other pancreatic cells by expressing key transcription factors regulating β-cell development [32–35]. In 2008, Zhou et al. reported the ability of exocrine cells to be reprogrammed directly into insulin-producing β-cells *in vivo*, without cell replication or reversion into a progenitor-like stage [36]. By inducing ectopic expression of a subset of

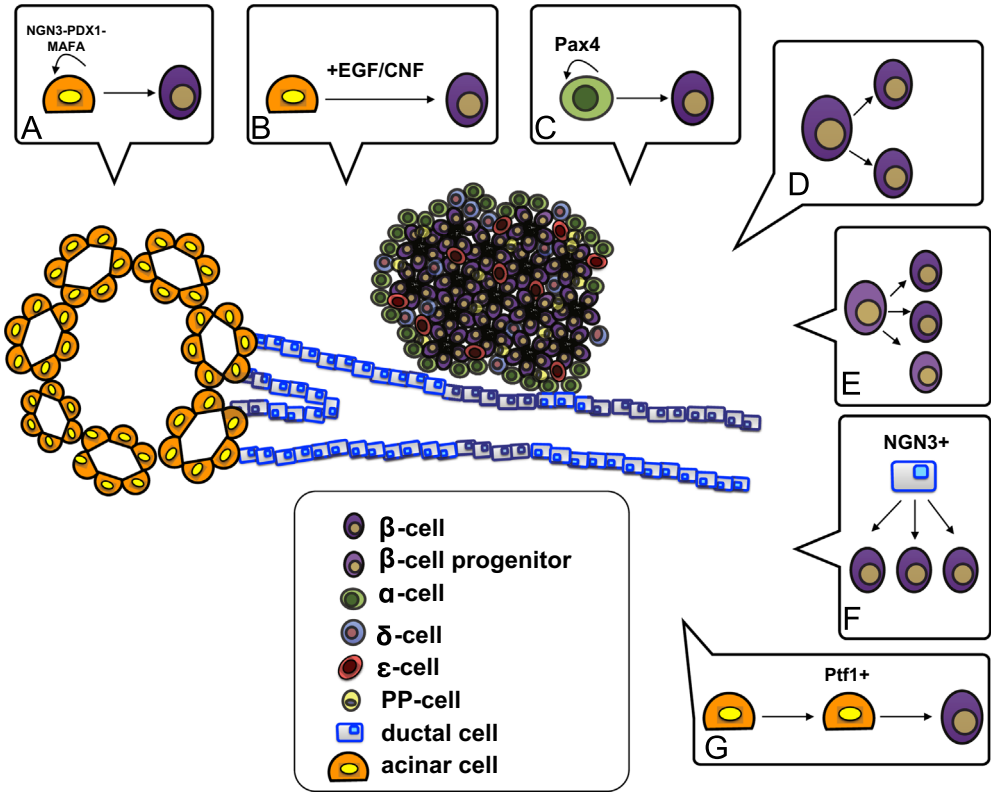

Figure 1: Potential ways of β-cell regeneration. (A) Ectopic expression of NGN3, PDX1 and MAFA in acinar cells triggers the formation of new β-like cells. (B) EGF and CNF treatment triggers the conversion of acinar cells into β-like cells. (C) Ectopic expression of Pax4 in α-cells drives their conversion into β-cells. (D) Proliferation of pre-existing mature β-cells. (E) β-cell regeneration from intra-islet multipotent pancreatic progenitors. (F) Neogenesis of β-cells from NGN3+ ductal progenitors. (G) β-cell regeneration from Ptf1+ acinar endocrine progenitors.

three endocrine transcription factors (NGN3, PDX1 and MAFA) the authors found formation of new β-cells from acinar cells. These β-like cells expressed β-cell-specific genes, showed morphological characteristics of β-cells and ameliorated blood glucose levels in diabetic mice. However, these acinar-derived β-cells did not cluster to resemble islets leaving some uncertainty about their maturation and functional state [37]. Recently, another study reported *in vivo* conversion of pancreatic acinar cells into β-like cells by triggering similar mechanisms [38]. This has been achieved by transient treatment with epidermal growth factor (EGF) and ciliary neurotrophic factor (CNF) in hyperglycemic adult mice. Taken together, acinar to β-cell conversion through intrinsic or extrinsic signaling factors might open new therapeutic treatment options in the future.

The exocrine–endocrine lineage decision occurs early during development. As the endocrine lineages are closely related, it seems likely that these cells resemble a better source for generating new β-cells. In this regard, it is interesting to note that chromatin immunoprecipitation followed by next generation sequencing and mRNA profiling of human α- and β-cells revealed new details regarding the close epigenomic relationship between these cells [32]. Accordingly, several studies have used single gene manipulations to induce inter-conversion of islet cells towards the β-cell fate [39,40]. For example, Collombat et al. reported that ectopic expression of Pax4 in α-cells drives their conversion to the β-cell fate, leading to progressive amelioration of systemic glycemia in a β-cell depletion model [41]. Al-Hasani et al. also recently linked Pax4-mediated α- to β-cell conversion to enhanced β-cell regeneration by pancreatic duct-lining precursor cells [42]. *Vice versa*, expression of the α-cell-specific transcription factor Arx in β-cells lead to transdifferentation into the α- or PP-cell lineage, highlighting the possibility that induced ablation of this factor in α- or PP-cells could induce α-to-β-cell conversion [43]. Lately, work from Schaffer et al. reported the critical role of the transcription factor Nkx6.1 as β-cell specific reprogramming factor that was able to define α- versus β-cell fate [33].

Beside all the efforts to understand how to reprogram endocrine cells towards the β-cell fate, accumulating evidence support the hypothesis that α-cells can be considered to be a natural source for generating β-cells. Using a genetic lineage tracing system to follow α-cells, it was demonstrated that after almost complete ablation of β-cells, α-cells convert into β-cells *in vivo* [44]. Therefore, a better understanding of the heterogeneity and epigenetic status of endocrine cells might open up a complete new field of research focusing on genetic and epigenetic manipulation to achieve reprogramming towards the β-cell fate. Additional studies will be required to comprehend the usefulness and potential threats of inter- and intra-endocrine conversion towards the β-cell fate for therapeutical purpose.

4. ISLET CELL REGENERATION

In 2004 Dor et al. reported that proliferation of pre-existing β-cells is the major mechanism regulating β-cell expansion in adulthood [45]. The β-cell self-replication model originally observed by the Melton laboratory has been confirmed by several other groups [46,47]. For example, Nir and colleagues used a transgenic model for β-cell depletion combined with a double *in vivo* thymidine analogue-labeling strategy to show that even upon β-cell depletion, increased proliferation of remaining β-cells is the major process contributing to β-cell regeneration. This was confirmed recently by following the fate of insulin-producing cells in several injury models, which also argued against β-cell neogenesis from other cell types than insulin-producing cells [48]. A major concern about genetic lineage tracing systems is their poor labeling efficiency and the limited time window provided for investigation [49,50]. Furthermore, all these genetic labeling systems were based on the assumption that a putative β-cell progenitor should be characterized by *de novo* expression of insulin. This does not take into account that progenitors might already express insulin. Evidence for this scenario was provided recently by the identification of a rare pancreatic multipotent precursor (PMP) cell population expressing insulin and low levels of the glucose transporter Glut2 in mouse and in human islets. PMPs are able to generate pancreatic and neuronal progeny *in vitro*, whereby it is unclear how endoderm-derived PMPs can cross the lineage barrier to form neurons. In any case, the PMPs are capable of generating mature and glucose responsive β-cells *in vitro* and *in vivo* [51,52]. Intriguingly, latest data from human suggest that β-cell neogenesis from progenitor cells might preferentially occur rather than β-cell proliferation, as a compensatory mechanism to counteract decreased glucose tolerance [53]. This might reflect that different species have evolved different regenerative mechanisms or that depending on the injury different regenerative programs are triggered. It is also likely that neogenesis and proliferation of pre-existing β-cells co-exist to regulate β-cell mass [50].

During embryonic development, the pancreatic epithelium contains multipotent progenitors that will give rise to all pancreatic cell types: the ductal, endocrine and exocrine lineages [54,55]. While the tip cells become committed to an acinar fate, the ductal epithelium provides a source for endocrine progenitors that delaminate and aggregate to form the islets of Langerhans. Based on this knowledge, research today has also focused on the adult ductal epithelium, as potential facultative progenitors. These cells are thought to be able to reactivate an embryonic program and differentiate into the endocrine lineage. The concept is based on the hypothesis that upon injury or prolonged metabolic stress, a fully differentiated pancreatic cell might de-differentiate and gain stem cell characteristic for endocrine regeneration [56]. Evidence for this scenario was provided by Xu et al. who observed the induction of Neurogenin3+ endocrine cells in the ductal region that were able to give rise to all islet cell types in the duct ligation injury model [57]. Additionally, facultative CAII+ ductal progenitor cells were reported to differentiate into all endocrine lineages and contributed to compensate for tissue loss [58]. In contrast, several other studies exist that contradict the ductal progenitor hypothesis. For example, Solar et al. showed that Hnf1b+ pancreatic progenitors from the embryonic duct do not contribute to postnatal endocrine growth or to β-cell neogenesis after pancreatic duct ligation or chemical-induced β-cell ablation [59]. Notably, Hnf1b-CreER mice showed low labeling efficiency during embryonic development and in adulthood, leaving most of the duct cells unlabeled [60]. Moreover, a recent study from Rankin and colleague reported that the classical pancreatic duct ligation model leads to a massive pancreatic injury and alters pancreatic composition rather than influencing β-cell mass and consequently activates conversion of ductal-endocrine progenitors into the β-cell lineage [61]. Indeed, in a recent study from Pan et al., new evidence about the identity and existence of a pancreatic multipotent progenitor has emerged. By using an elegant lineage tracing approach, the authors described spatiotemporal patterns of multipotentiality in Ptf1 expressing cells during pancreas development. Interestingly, the authors provide evidence that acinar cells can trans-differentiate into ductal and β-cells without exogenous factors upon injury [62]. And as mentioned above, Al-Hasani et al. revealed that after Pax4-mediated conversion of α- into β-cells, ductal progenitors differentiate into endocrine cells. This is thought to happen through reawakening of the embryonic epithelial–mesenchymal transition (EMT) process and provides convincing

evidence that under certain circumstances ductal cells can serve as facultative endocrine progenitors [42]. Supporting evidence comes from human studies where 15% of all β-cells are located in clusters along the duct. *In vitro*, human ductal cells seem to retain the capacity to differentiate into endocrine, exocrine and hepatic cells [63,64]. Altogether, a thorough understanding of pancreatic cell plasticity, β-cell proliferation and neogenesis from progenitors is urgently needed. Identification of specific progenitors will allow to trigger receptors and molecular pathways responsible for β-cell regeneration.

5. HUMORAL RESPONSE REGULATING -CELL REGENERATION

Currently, self-replication of β-cells seems to be one of the major mechanisms regulating β-cell expansion. Hence, a major goal is the identification of factors that induce β-cell proliferation. It is long known that the proliferative capacity of β-cells is age dependent, differs from species to species and declines over time [65]. For example, in rodents the β-cell replication rate is reported to be 2.5%, whereas in human it is only around 0.2% [65,66]. Several studies have suggested that β-cells regenerate upon injury-driven tissue loss, but how and to what extent secreted molecules regulate this process is currently unknown [2,66]. Betacellulin (BTC) was one of the first identified secreted proteins regulating β-cell proliferation. BTC is a member of the epidermal growth factor family and is expressed in a broad range of cells, including β-cells where it was identified in 1993. *In vitro*, BTC together with Activin-A have been shown to induce acinar to β-cell conversion [67]. Interestingly, ectopic expression of BTC has been reported to ameliorate hyperglycemia in a diabetic murine model by promoting β-cell re-entry into the cell cycle [68,69]. Organ cross talk does not control only systemic metabolism, but also regulates tissue homeostasis and growth. This is mostly modulated by secreted factors, which influence cell behavior and turnover. In this respect, gut-derived hormones profoundly regulate β-cell mass and function. For example, upon food intake, duodenal L- and K-cells secrete glucagon like peptide 1 (Glp-1) and gastric inhibitor polypeptide (GIP), respectively. These hormones have a pleiotropic effect on β-cells and both of them amplify glucose-stimulated insulin release of β-cells by stabilizing the postprandial hyperglycemic spike. They also trigger activation of pro-survival and anti-apoptotic pathways in pancreatic β-cells [70,71]. Moreover, Glp-1 was reported to increase the β-cell mass in rodents by activating the IRS2-Akt pathway leading to enhanced β-cell proliferation [72–74]. Additionally, endogenous expression of Glp-1 in murine models of streptozotocin (STZ)-induced diabetes has been shown to prevent hyperglycemia and to improve β-cell survival [75–77]. Clinical trials targeting the GLP-1 pathway by the administration of Lixisenatide have reported encouraging data in terms of novel therapeutic approaches for T2DM, however, if GLP-1 contributes to β-cell regeneration in humans remains to be clarified [78–80]. Years of research indicate that β-cells retain the capacity to undergo dynamic changes to compensate for both physiological (pregnancy) and pathological (e.g. insulin resistance) metabolic alterations [81,82]. During pregnancy, β-cell expansion is regulated by hormones, such as prolactin (Prl), placental lactogens, and serotonin [83,84]. Prolactin and placental lactogens seem to act via Prl-receptor (Prl-r) and the Jak2/Stat5 pathway, thus activating mTOR signaling leading to enhancement of β-cell replication [85]. Moreover, studies conducted in Prl-r knock-out animals revealed an active involvement of Prl signaling pathway during embryogenesis and early postnatal period. These animals displayed 30% reduction of β-cell mass and impaired proliferation of β-cell progenitors at E18.5 [86], but the exact β-cell specific mechanism of action of the lactogenic hormones is not fully understood. A new player, serotonin has recently been reported to increase β-cell proliferation. Serotonin works downstream of the pathway activated by lactogens and thus leads to β-cell proliferation [83]. Besides β-cell mass regulation occurring under physiological condition, other secreted molecules orchestrate β-cell mass growth in response to increased insulin demand, i.e. during systemic insulin resistance. Interesting data reported by El Quaamari et al., highlight the contribution of a liver-derived growth factor modulating β-cell mass expansion upon insulin resistance. Using an *in vivo* parabiosis model of LIRKO (liver-specific insulin receptor knock-out) and control mice, combined by *in vitro* experiments with human islets, the authors demonstrated that a humoral liver-derived response plays a crucial role in regulating β-cell proliferation upon insulin resistance [87].

Accordingly, Yi et al. identified such a systemic acting factor that shows increased expression in liver and fat in mouse models that expand the β-cell mass upon insulin resistance, which they named Betatrophin. Ectopic expression of this hormone from the liver induces a rapid, robust, and specific increase of β-cell proliferation and improves glucose tolerance in young adult mice [12]. However, phenotypic analysis of Betatrophin knock-out mice has not shown abnormal glucose regulation, but reduced levels of triglyceride were observed after re-feeding [88]. It is noteworthy that, elevated plasmatic concentration of Betatrophins was found in patients with long standing T1DM, suggesting that Betatrophin treatment alone might not be beneficial for patients with T1DM [89]. Additionally, human β-cells showed limited proliferative capacity in response to increased Betatrophin expression in transplant settings [90]. In the future it will be important to identify the receptor and signaling pathways that are triggered by Betatrophin to understand how this hormone induces such a potent β-cell proliferation response in the mouse model [9–11]. The extensive search for a secreted factor regulating β-cell expansion has not been limited to hepatocyte-derived factors, but has been extended to several factors secreted from diverse tissues. Thus, macrophage-derived cytokines, muscle-derived myokines, and adipocyte-derived adipokines have all been shown to regulate β-cell mass [91–96]. Altogether, former and recent work point into the direction that regulation of β-cell mass is orchestrated by a systemic cross talk between organs as well as autocrine and paracrine interactions between cells in the pancreas. Thus, several ways might exist to trigger endogenous mechanisms of β-cell regeneration.

6. CONCLUSION

Considerable challenges remain before regeneration of functional β-cells can become reality. β-cell replacement therapy combined with novel immunosuppressive treatments is an encouraging perspective to restore β-cell mass in T1DM. Furthermore, neogenesis of β-cells from intra-islet (α- or β-cells) or extra-islet (acinar or duct) progenitors might uncover novel strategies for regeneration. In contrast, key components in the pathophysiology of T2DM are the progressive β-cell exhaustion, due to unrestrained metabolic alteration, and consequently loss of β-cell function caused by de-differentiation. Thus therapies aiming to reduce metabolic stress and to trigger maturation of de-differentiated β-cells might preserve β-cell mass to regain blood glucose control. This requires novel markers that identify mature β-cells to develop new drugs targeting specific β-cell subpopulations and to trigger endogenous regenerative mechanism, e.g. β-cell self-replication or neogenesis. Currently it is not clear which approach might be the most suitable

treatment for both types of diabetes, however, the recent worldwide efforts in β-cell regeneration might allow therapy in the near future.

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CONFLICT OF INTEREST

None declared.

REFERENCES

[1] Shapiro, A.M., et al., 2000. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. New England Journal of Medicine 343 (4):230–238.
[2] Pagliuca, F.W., Melton, D.A., 2013. How to make a functional beta-cell. Development 140 (12):2472–2483.
[3] Rahier, J., et al., 2008. Pancreatic beta-cell mass in European subjects with type 2 diabetes. Diabetes, Obesity and Metabolism 10 (4):32–42.
[4] Butler, A.E., et al., 2003. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 52 (1):102–110.
[5] Butler, A.E., et al., 2003. Increased beta-cell apoptosis prevents adaptive increase in beta-cell mass in mouse model of type 2 diabetes: evidence for role of islet amyloid formation rather than direct action of amyloid. Diabetes 52 (9):2304–2314.
[6] Rhodes, C.J., 2005. Type 2 diabetes—a matter of beta-cell life and death? Science 307 (5708):380–384.
[7] Araujo, T.G., Oliveira, A.G., Saad, M.J., 2013. Insulin-resistance-associated compensatory mechanisms of pancreatic beta cells: a current opinion. Frontiers in Endocrinology (Lausanne) 4. (p. 146).
[8] Talchai, C., et al., 2012. Pancreatic beta cell dedifferentiation as a mechanism of diabetic beta cell failure. Cell 150 (6):1223–1234.
[9] Lickert, H., 2013. Betatrophin fuels beta cell proliferation: first step toward regenerative therapy? Cell Metabolism 18 (1):5–6.
[10] Crunkhorn, S., 2013. Metabolic disorders: Betatrophin boosts beta-cells. Nature Reviews Drug Discovery 12 (7). (p. 504).
[11] Kugelberg, E., 2013. Diabetes: Betatrophin-inducing beta-cell expansion to treat diabetes mellitus? Nature Reviews Endocrinology 9 (7). (p. 379).
[12] Yi, P., Park, J.S., Melton, D.A., 2013. Betatrophin: a hormone that controls pancreatic beta cell proliferation. Cell 153 (4):747–758.
[13] Collombat, P., et al., 2006. Specifying pancreatic endocrine cell fates. Mechanisms of Development 123 (7):501–512.
[14] Jain, R., Lammert, E., 2009. Cell–cell interactions in the endocrine pancreas. Diabetes, Obesity and Metabolism 11 (4):159–167.
[15] Steiner, D.J., et al., 2010. Pancreatic islet plasticity: interspecies comparison of islet architecture and composition. Islets 2 (3):135–145.
[16] Brissova, M., et al., 2005. Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. Journal of Histochemistry and Cytochemistry 53 (9):1087–1097.
[17] Kim, A., et al., 2009. Islet architecture: a comparative study. Islets 1 (2):129–136.
[18] Bonner-Weir, S., 1988. Morphological evidence for pancreatic polarity of beta-cell within islets of Langerhans. Diabetes 37 (5):616–621.
[19] Nikolova, G., et al., 2006. The vascular basement membrane: a niche for insulin gene expression and Beta cell proliferation. Developmental Cell 10 (3):397–405.
[20] Zaret, K.S., 2006. Pancreatic beta cells: responding to the matrix. Cell Metabolism 3 (3):148–150.
[21] Lammert, E., Cleaver, O., Melton, D., 2001. Induction of pancreatic differentiation by signals from blood vessels. Science 294 (5542):564–567.
[22] Eberhard, D., Kragl, M., Lammert, E., 2010. Giving and taking: endothelial and beta-cells in the islets of Langerhans. Trends in Endocrinology and Metabolism 21 (8):457–463.
[23] Speier, S., et al., 2007. Cx36-mediated coupling reduces beta-cell heterogeneity, confines the stimulating glucose concentration range, and affects insulin release kinetics. Diabetes 56 (4):1078–1086.
[24] Mori, Y., et al., 2002. Genome-wide search for type 2 diabetes in Japanese affected sib-pairs confirms susceptibility genes on 3q, 15q, and 20q and identifies two new candidate loci on 7p and 11p. Diabetes 51 (4):1247–1255.
[25] Hezel, A.F., et al., 2008. Pancreatic LKB1 deletion leads to acinar polarity defects and cystic neoplasms. Molecular and Cellular Biology 28 (7):2414–2425.
[26] Woods, A., et al., 2003. LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. Current Biology 13 (22):2004–2008.
[27] Granot, Z., et al., 2009. LKB1 regulates pancreatic beta cell size, polarity, and function. Cell Metabolism 10 (4):296–308.
[28] Fu, A., et al., 2009. Loss of Lkb1 in adult beta cells increases beta cell mass and enhances glucose tolerance in mice. Cell Metabolism 10 (4):285–295.
[29] Cortijo, C., et al., 2012. Planar cell polarity controls pancreatic beta cell differentiation and glucose homeostasis. Cell Reports 2 (6):1593–1606.
[30] Schambony, A., Wedlich, D., 2007. Wnt-5A/Ror2 regulate expression of XPAPC through an alternative noncanonical signaling pathway. Developmental Cell 12 (5):779–792.
[31] Han, S.I., Yasuda, K., Kataoka, K., 2011. ATF2 interacts with beta-cell-enriched transcription factors, MafA, Pdx1, and beta2, and activates insulin gene transcription. Journal of Biological Chemistry 286 (12):10449–10456.
[32] Bramswig, N.C., et al., 2013. Epigenomic plasticity enables human pancreatic alpha to beta cell reprogramming. Journal of Clinical Investigation 123 (3):1275–1284.
[33] Schaffer, A.E., et al., 2013. Nkx6.1 controls a gene regulatory network required for establishing and maintaining pancreatic Beta cell identity. PLoS Genetics 9 (1). (p. e1003274).
[34] St-Onge, L., et al., 1997. Pax6 is required for differentiation of glucagon-producing alpha-cells in mouse pancreas. Nature 387 (6631):406–409.
[35] Sosa-Pineda, B., et al., 1997. The Pax4 gene is essential for differentiation of insulin-producing beta cells in the mammalian pancreas. Nature 386 (6623):399–402.
[36] Zhou, Q., et al., 2008. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature 455 (7213):627–632.
[37] Puri, S., Hebrok, M., 2010. Cellular plasticity within the pancreas – lessons learned from development. Developmental Cell 18 (3):342–356.
[38] Baeyens, L., et al., 2014. Transient cytokine treatment induces acinar cell reprogramming and regenerates functional beta cell mass in diabetic mice. Nature Biotechnology 32:76–83.
[39] Collombat, P., et al., 2003. Opposing actions of Arx and Pax4 in endocrine pancreas development. Genes and Development 17 (20):2591–2603.
[40] Biressi, S., et al., 2008. The homeobox gene Arx is a novel positive regulator of embryonic myogenesis. Cell Death and Differentiation 15 (1):94–104.
[41] Collombat, P., et al., 2009. The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into alpha and subsequently beta cells. Cell 138 (3):449–462.

[42] Al-Hasani, K., et al., 2013. Adult duct-lining cells can reprogram into beta-like cells able to counter repeated cycles of toxin-induced diabetes. Developmental Cell 26 (1):86–100.
[43] Collombat, P., et al., 2007. Embryonic endocrine pancreas and mature beta cells acquire alpha and PP cell phenotypes upon Arx misexpression. Journal of Clinical Investigation 117 (4):961–970.
[44] Thorel, F., et al., 2010. Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. Nature 464 (7292):1149–1154.
[45] Dor, Y., et al., 2004. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature 429 (6987):41–46.
[46] Nir, T., Melton, D.A., Dor, Y., 2007. Recovery from diabetes in mice by beta cell regeneration. Journal of Clinical Investigation 117 (9):2553–2561.
[47] Teta, M., et al., 2007. Growth and regeneration of adult beta cells does not involve specialized progenitors. Developmental Cell 12 (5):817–826.
[48] Xiao, X., et al., 2013. No evidence for beta cell neogenesis in murine adult pancreas. Journal of Clinical Investigation 123 (5):2207–2217.
[49] Magnuson, M.A., Osipovich, A.B., 2013. Pancreas-specific cre driver lines and considerations for their prudent use. Cell Metabolism 18 (1):9–20.
[50] Bonner-Weir, S., et al., 2010. Beta-cell growth and regeneration: replication is only part of the story. Diabetes 59 (10):2340–2348.
[51] Seaberg, R.M., et al., 2004. Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. Nature Biotechnology 22 (9):1115–1124.
[52] Smukler, S.R., et al., 2011. The adult mouse and human pancreas contain rare multipotent stem cells that express insulin. Cell Stem Cell 8 (3):281–293.
[53] Yoneda, S., et al., 2013. Predominance of beta-cell neogenesis rather than replication in humans with an impaired glucose tolerance and newly diagnosed diabetes. Journal of Clinical Endocrinology and Metabolism 98 (5):2053–2061.
[54] Pan, F.C., Wright, C., 2011. Pancreas organogenesis: from bud to plexus to gland. Developmental Dynamics 240 (3):530–565.
[55] Raducanu, A., Lickert, H., 2012. Understanding pancreas development for beta-cell repair and replacement therapies. Current Diabetes Reports 12 (5):481–489.
[56] Ziv, O., Glaser, B., Dor, Y., 2013. The plastic pancreas. Developmental Cell 26 (1):3–7.
[57] Xu, X., et al., 2008. Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 132 (2):197–207.
[58] Inada, A., et al., 2008. Carbonic anhydrase II-positive pancreatic cells are progenitors for both endocrine and exocrine pancreas after birth. Proceedings of the National Academy of Sciences of the United States of America 105 (50):19915–19919.
[59] Solar, M., et al., 2009. Pancreatic exocrine duct cells give rise to insulin-producing beta cells during embryogenesis but not after birth. Developmental Cell 17 (6):849–860.
[60] Kushner, J.A., Weir, G.C., Bonner-Weir, S., 2010. Ductal origin hypothesis of pancreatic regeneration under attack. Cell Metabolism 11 (1):2–3.
[61] Rankin, M.M., et al., 2013. Beta-cells are not generated in pancreatic duct ligation-induced injury in adult mice. Diabetes 62 (5):1634–1645.
[62] Pan, F.C., et al., 2013. Spatiotemporal patterns of multipotentiality in Ptf1a-expressing cells during pancreas organogenesis and injury-induced facultative restoration. Development 140 (4):751–764.
[63] Bouwens, L., Pipeleers, D.G., 1998. Extra-insular beta cells associated with ductules are frequent in adult human pancreas. Diabetologia 41 (6):629–633.
[64] Zulewski, H., et al., 2001. Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. Diabetes 50 (3):521–533.
[65] Kushner, J.A., 2013. The role of aging upon beta cell turnover. Journal of Clinical Investigation 123 (3):990–995.
[66] Cano, D.A., et al., 2008. Regulated beta-cell regeneration in the adult mouse pancreas. Diabetes 57 (4):958–966.
[67] Nagaoka, T., et al., 2008. A betacellulin mutant promotes differentiation of pancreatic acinar AR42J cells into insulin-producing cells with low affinity of binding to ErbB1. Journal of Molecular Biology 380 (1):83–94.
[68] Oh, Y.S., et al., 2011. Betacellulin-induced beta cell proliferation and regeneration is mediated by activation of ErbB-1 and ErbB-2 receptors. PLoS One 6 (8):e23894.
[69] Shin, S., et al., 2008. Remission of diabetes by beta-cell regeneration in diabetic mice treated with a recombinant adenovirus expressing betacellulin. Molecular Therapy 16 (5):854–861.
[70] Yabe, D., Seino, Y., 2011. Two incretin hormones GLP-1 and GIP: comparison of their actions in insulin secretion and beta cell preservation. Progress in Biophysics and Molecular Biology 107 (2):248–256.
[71] Li, Y., et al., 2003. Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. Journal of Biological Chemistry 278 (1):471–478.
[72] Meloni, A.R., et al., 2013. GLP-1 receptor activated insulin secretion from pancreatic beta-cells: mechanism and glucose dependence. Diabetes, Obesity and Metabolism 15 (1):15–27.
[73] Wang, Q., Brubaker, P.L., 2002. Glucagon-like peptide-1 treatment delays the onset of diabetes in 8 week-old db/db mice. Diabetologia 45 (9): 1263–1273.
[74] Xu, G., et al., 1999. Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. Diabetes 48 (12):2270–2276.
[75] Gaddy, D.F., et al., 2010. In vivo expression of HGF/NK1 and GLP-1 From dsAAV vectors enhances pancreatic ss-cell proliferation and improves pathology in the db/db mouse model of diabetes. Diabetes 59 (12):3108–3116.
[76] Tonne, J.M., et al., 2013. Global gene expression profiling of pancreatic islets in mice during streptozotocin-induced beta-cell damage and pancreatic Glp-1 gene therapy. Disease Models and Mechanisms 6 (5):1236–1245.
[77] Farilla, L., et al., 2002. Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in Zucker diabetic rats. Endocrinology 143 (11): 4397–4408.
[78] Elkinson, S., Keating, G.M., 2013. Lixisenatide: first global approval. Drugs 73 (4):383–391.
[79] Garber, A.J., 2012. Novel GLP-1 receptor agonists for diabetes. Expert Opinion on Investigational Drugs 21 (1):45–57.
[80] Christensen, M., et al., 2009. Lixisenatide, a novel GLP-1 receptor agonist for the treatment of type 2 diabetes mellitus. IDrugs 12 (8):503–513.
[81] Maclean, N., Ogilvie, R.F., 1955. Quantitative estimation of the pancreatic islet tissue in diabetic subjects. Diabetes 4 (5):367–376.
[82] Sorenson, R.L., Brelje, T.C., 1997. Adaptation of islets of Langerhans to pregnancy: beta-cell growth, enhanced insulin secretion and the role of lactogenic hormones. Hormone and Metabolic Research 29 (6):301–307.
[83] Kim, H., et al., 2010. Serotonin regulates pancreatic beta cell mass during pregnancy. Nature Medicine 16 (7):804–808.
[84] Rieck, S., et al., 2009. The transcriptional response of the islet to pregnancy in mice. Molecular Endocrinology 23 (10):1702–1712.
[85] Rieck, S., Kaestner, K.H., 2010. Expansion of beta-cell mass in response to pregnancy. Trends in Endocrinology and Metabolism 21 (3):151–158.
[86] Auffret, J., et al., 2013. Defective prolactin signaling impairs pancreatic beta-cell development during the perinatal period. American Journal of Physiology – Endocrinology and Metabolism 305 (10):E1309–E1318.
[87] El Ouaamari, A., et al., 2013. Liver-derived systemic factors drive beta cell hyperplasia in insulin-resistant states. Cell Reports 3 (2):401–410.
[88] Wang, Y., et al., 2013. Mice lacking ANGPTL8 (Betatrophin) manifest disrupted triglyceride metabolism without impaired glucose homeostasis. Proceedings of the National Academy of Sciences of the United States of America 110 (40):16109–16114.
[89] Espes, D., Lau, J., Carlsson, P.O., 2013. Increased circulating levels of betatrophin in individuals with long-standing type 1 diabetes. Diabetologia.

[90] Jiao, Y., et al., 2013. Elevated mouse hepatic betatrophin expression does not increase human beta-cell replication in the transplant setting. Diabetes [Epub ahead of print].
[91] Vasavada, R.C., et al., 2006. Growth factors and beta cell replication. International Journal of Biochemistry and Cell Biology 38 (5–6):931–950.
[92] Charrier, A., Brigstock, D.R., 2013. Regulation of pancreatic function by connective tissue growth factor (CTGF, CCN2). Cytokine and Growth Factor Reviews 24 (1):59–68.
[93] Lee, Y.C., Nielsen, J.H., 2009. Regulation of beta cell replication. Molecular and Cellular Endocrinology 297 (1–2):18–27.
[94] Dunmore, S.J., Brown, J.E., 2013. The role of adipokines in beta-cell failure of type 2 diabetes. Journal of Endocrinology 216 (1):T37–T45.
[95] Plomgaard, P., Halban, P.A., Bouzakri, K., 2012. Bimodal impact of skeletal muscle on pancreatic beta-cell function in health and disease. Diabetes, Obesity and Metabolism 14 (3):78–84.
[96] Wang, C., Guan, Y., Yang, J., 2010. Cytokines in the progression of pancreatic beta-cell dysfunction. International Journal of Endocrinology 2010:515136.

