

Genetic Control of β -Cell Mass Homeostasis

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Abstract: Control of β -cell function and mass is tightly linked to glucose homeostasis. Failing β -cells inevitably lead to diabetes. Recently, several contradictory studies have been published arguing against or in favor of various mechanisms controlling β -cell mass regulation. Here we review the literature on control of adult β -cell mass and aim to reconcile thereby the contradictions. We discuss the role of β -cell proliferation and neogenesis, both in mice and man. We also discuss the influence of genetic predisposition on β -cell mass control. We conclude that β -cell generation in the adult human and mouse likely depends on many paths to assure sufficient numbers of β -cells at any given time, thereby balancing mechanisms for negative regulation of β -cell numbers. A simple model with only one pathway does not fit the current literature.

Keywords: Islet of Langerhans, diabetes, beta-cell, proliferation, neogenesis, genetics, mouse, human.

1. INTRODUCTION

Control of β -cell function and mass is tightly linked to glucose homeostasis. Normally, metabolic stress induced by environmental factors such as obesity or pregnancy is compensated by increased β -cell function or mass. Remarkably, already 1932 Rosenloecher described that human islets of Langerhans increase in size during mid to late pregnancy and then return to normal size post-partum [1]. He correlated his finding with the increased pancreatic function during the same periods. More than 40 years later Van Assche verified these findings in a quantitative study [2]. On the other hand, when adequate compensation fails, it leads to diabetes. Histologically, β -cell destruction and insufficient β -cell mass are evident in humans with diabetes [3-8]. However, β -cell mass dynamics are difficult to study in humans, still leaving us with limited insight into β -cell lifespan and renewal mechanisms in man. Consequently, animal models with differential regulation of β -cell mass serve as the main tool for understanding mechanisms of β -cell mass regulation. Hummel *et al.* demonstrated in the 1970s that β -cell atrophy is prominent in certain obese inbred mice but not in others, pointing towards specific genetic differences regulating β -cell mass homeostasis [9]. Recently, human population genetic studies have given new insights into β -cell function and mass regulation in human diabetes (see Table 1). Interestingly, susceptibility to diabetes is correlated to ethnic background. Caucasian type 2 diabetes mellitus (T2DM) is typically associated with high

body mass index (BMI), whereas Indian and Japanese subjects present with diabetes at significantly lower BMI, suggesting that genetic differences in β -cell mass regulation in different ethnic groups may be one reason for the differential ability to compensate for increased insulin demand [10, 11]. Here we will review the role of β -cell mass compensation as a means to control glucose homeostasis. In particular, we will revisit studies on mouse and human β -cell mass homeostasis and discuss the influence of genetic factors that influence control of β -cell mass.

2. ADULT β -CELL MASS HOMEOSTASIS

2.1. Animal Studies

Cell mass homeostasis depends on the balance between loss of cells, formation of new cells and changes in cell size. In this review, we will mainly focus on the aspect of new cell formation. In the adult pancreas the source of new β -cells is debated. Whether formation of new β -cells primarily depends on self-replication or neogenesis from progenitors, has been controversial since the very first indications in the early 1960s that 1.) duct cells may be β -cell progenitors [12] and 2.) mitotic β -cells are present in adult rodent pancreata [13]. Nevertheless, these studies were the beginning of the understanding that β -cell mass is dynamic and that β -cells may not be post-mitotic. They also led to the formulation of the hypothesis that β -cell mass dynamics would be necessary to meet changes in demand for insulin, an idea put forward in the 1970's by Hellerström and others [14]. Although β -cells were once thought to be post-mitotic, there is hardly any controversy about the existence of β -cell proliferation today. We have seen an explosion in the interest for regulation of β -cell proliferation over the last 15-20 years. As a result, a large number of high-quality mechanistic

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Table 1. Genetic Mutations Associated with Diabetes in Humans that have a Possible Role in Regulation of β -Cell Mass or β -Cell Function. The List should not be Viewed as Exhaustive

Gene	Function	Effect on β -Cells	Genetic Link	References and Race (when Known)
ABCC8 (SUR1)	Potassium channel subunit	Insulin exocytosis [111]	Monogenic	[112]
AKT2 (PKBβ)	Serine/threonine protein kinase	β -cell function and survival [113, 114]	Monogenic	[115]
CDKAL1	Cell cycle	Indirect link to insulin secretion [88-90]	Genetic association	Icelandic and Chinese [116], British [117], Asian [118], Finnish [119], Finnish and Swedish [120] subjects
CDKN2A/B (P16-INK4/P15INK4B)	Cell cycle arrest/tumor suppressor	Age-dependent β -cell generation [96, 121], indirect link to insulin secretion [88-90]	Genetic association	British [117], Asian [118], Finnish [119], Finnish and Swedish [120] subjects
GCK	Glucose metabolism	Glucose sensing /insulin secretion [reviewed in [122]], maintenance of islet mass [123]	Monogenic [MODY2]	[124, 125]
HHEX	Transcription factor	Indirect link to insulin secretion [88-90], pancreas development [105]	Genetic association	French [126], British [117], Asian [118], Finnish [119], Icelandic [116], Finnish and Swedish [120] subjects
HNF1A (TCF1)	Transcription factor	Insulin secretion [127], β -cell proliferation [128]	Monogenic [MODY3]	[129]
HNF1B (TCF2)	Transcription factor	Pancreas development [130]	Monogenic [MODY5]	[131]
HNF4A	Transcription factor	Glucose metabolism [132], insulin transcription [133]	Monogenic [MODY1]	[134]
IGF2BP2	Insulin like growth factor signaling	Indirect link to insulin secretion [88, 89]	Genetic association	French Caucasian [135], British [117], Asian [118], Finnish [119], Finnish and Swedish [120] subjects
IRS1	Insulin signaling	Insulin secretion [136]	Genetic association	French [137], Japanese [138], Mexican American [139], Chinese [140] subjects
KCNJ11 (Kir6.2)	Potassium channel subunit	Insulin exocytosis [141]	Monogenic, Genetic association	British [117, 142] Asian [118] Finnish [119] Finnish and Swedish [120] subjects; Monogenic [143]
NEUROD1	Transcription factor	Insulin transcription [144], β -cell development [145]	Monogenic [MODY6]	[146]
PDX1	Transcription factor	Insulin transcription [147], pancreas development [148]	Monogenic [MODY4]	[149]
PPARγ	Nuclear hormone receptor super-family/Transcription factor	β -cell proliferation [103]	Monogenic, Genetic association	British [117, 150], Finnish [119], Finnish and Swedish [120], Japanese [151], Scottish [152], young Italian obese [153], Asian [154] subjects; Monogenic [155]
SLC30A8	Zinc transporter	Insulin storage, maturation [156, 157], indirect link to insulin secretion [88, 89]	Genetic association	French [126], British [117], Asian [118], Finnish [119], Icelandic [116], Finnish and Swedish [120], Chinese [158] subjects
TCF7L2	Transcription factor [Wnt signaling]	β -cell apoptosis and proliferation [97], Insulin secretion [159]	Genetic association	French [126], British [117], Finnish [119], Icelandic and Chinese [116, 160], Finnish and Swedish [120] subjects
TRIB3	Transcription repressor/protein kinase inhibitor	β -cell apoptosis and ER-stress [110]	Genetic association	Italian [107], Chinese [106] subjects

papers have been published using mice as the model system. From such studies it has become clear that growth factors, metabolites, neural input, as well as cell-cell interaction between the β -cell and surrounding tissues are partaking in the regulation of β -cell proliferation (e.g. [15-19]). It is

beyond the scope of this article to review current knowledge about β -cell proliferation in experimental animals. Several other excellent reviews have recently covered this topic (e.g. [15-17]). Instead, in this section about animal studies we will focus on the controversy over β -cell neogenesis. We will,

however, discuss β -cell proliferation in detail in the context of human β -cells below (2.2.1.).

2.1.1. β -Cell Neogenesis

Over the years, the idea that exocrine and duct cells may serve as endocrine progenitors has become more and more supported by experimental data. For more than 50 years, partial pancreatectomy and duct ligation have been known to induce pancreatic islet regeneration [20, 21]. Later, several labs observed that β -cells in ducts are present in ample numbers after partial pancreatectomy and duct ligation [22-24]. Likewise, the Sarvetnick lab found evidence for islets budding out of ducts, as well as amylase/insulin double-positive cells in a transgenic model of β -cell regeneration [25, 26]. Such double-positive cells are also sometimes found in normal mouse pancreas (Fig. 1a). The Bouwens lab demonstrated that exocrine cells are plastic and can be induced to become β -cells *in vitro* [27, 28]. Together, these and other studies were compelling evidence for β -cell generation from duct and exocrine cells, at least under certain conditions.

The hypothesis that β -cells can arise from exocrine or duct cells was contradicted a few years ago by a landmark paper from the Melton lab [29]. They concluded that only pre-existing β -cells give rise to new β -cells with no contribution from progenitor cells in mice. Similarly, the Kushner lab concluded that β -cells form solely from self-replication in label retention tracing after CldU and IdU labeling of β -cells [30]. Lately, several other lineage-tracing studies, including our human tissue study (see section 2.2.2.), challenged these results and indeed demonstrate that β -cells in adult pancreatic tissue can arise from progenitors with ductal or exocrine cell characteristics.

The most unambiguous method for determining cell fate is clearly by genetic lineage tracing. By genetically labeling a population of cells with a marker like lacZ or GFP, the cell fate can be followed over time. Typically, the technique depends on expression of the marker gene controlled by the ubiquitous ROSA26 promoter. Under normal conditions, marker gene expression is blocked by insertion of a STOP codon flanked by loxP sites. Only in cells where Cre recombinase is expressed under the control of a cell-specific promoter, is the STOP codon excised leading to permanent marker gene expression even if the cell fate changes and the

cell-specific promoter is no longer active. Depending on the specificity of the Cre-driving promoter, the trace becomes very specific to the fate of one particular cell type. Consequently, lineage tracing duct- and exocrine cells seemed like an attractive approach to find evidence for β -cell neogenesis. The results from such studies have, however, been somewhat confusing: A study from the Seino lab demonstrated that mouse amylase- and elastase-positive cells could trans-differentiate to β -cells *in vitro* [32]. The authors observed less efficient differentiation from elastase-positive than amylase-positive cells, although both promoters would be expected to mark the same group of exocrine cells. In contrast, an attempt to demonstrate exocrine-to- β -cell differentiation *in vivo* in response to several modes of injury-induced regeneration failed, in an elastase-promoter driven lineage trace [33]. More recently, the Bonner-Weir lab published a carbonic anhydrase promoter-driven lineage trace and they were indeed able to demonstrate that new β -cell arise from cells with a ductal cell characteristic following duct ligation [34]. The Melton lab recently demonstrated that by expressing a subset of (pre) β -cell transcription factors in exocrine cells *in vivo*, a large number of cells took on a β -cell fate [35]. Those results indicate that a large portion of the cells in the pancreas have the capacity to become endocrine cells and are indeed promising for β -cell replacement efforts. Still, the question remains if this approach resembles a physiological phenomenon. As is now clear, somatic cells can be induced to become embryonic stem cell-like with overexpression of the right transcription factor cocktail (IPS cells [36]), indicating that a similar reprogramming might be induced in the Melton lab study rather than resembling normal physiology.

The studies discussed above have made the assumption that β -cell progenitors have similar characteristics to mature cells of exocrine or ductal phenotype, in that promoter activity (amylase, elastase or carbonic anhydrase) is equal in mature cells and progenitors. Most studies have also assumed that all or many cells of ductal and/or exocrine origin would have the same capacity to differentiate to β -cells under the right conditions. Another line of thought is that adult pancreatic progenitors may reside in the ductal and/or exocrine compartment of the pancreas, but that they are phenotypically distinct and may only represent a small subset of the cells in that compartment. A likely candidate

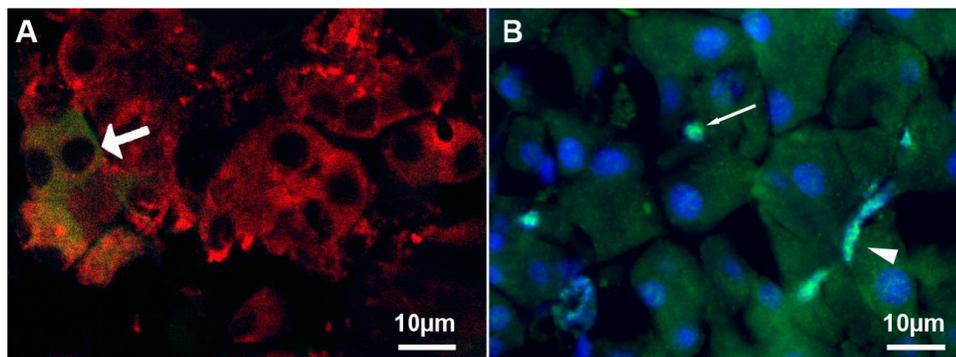


Fig. (1). Confocal micrographs of normal adult exocrine mouse pancreas. A. Insulin (green) and amylase (red) are co-expressed in some cells in a normal mouse pancreas. **B.** Sox9 (green) is expressed in cells that with morphological identification resemble centroacinar cells (arrow) and intercalated duct cells (arrowhead). Nuclei are stained with DAPI (blue). Note overlap of blue and green in Sox9 positive cells (arrow and arrowhead).

marker for such a cell would be a transcription factor driving embryonic β -cell differentiation, such as Ngn3 [37]. A recent study from the Heimberg lab demonstrates that Ngn3 expressing cells are activated during regeneration in the adult pancreas and that they act as endocrine progenitors similar to such cells in the embryonic pancreas [38]. Only a relatively small number of Ngn3-positive cells were found in or adjacent to small ducts, arguing that there may only exist a small number of true progenitors in the adult pancreas within the ductal compartment capable of activating an endocrine differentiation program upon stimulation. Appearance of Ngn3-positive cells has also been confirmed in other studies inducing regeneration in adult mouse pancreas [39].

Another interesting candidate marker for such progenitors is Sox9, which is required for embryonic differentiation of β -cell progenitors [40, 41]. Sox9 is expressed in an interesting pattern in the adult pancreas. We find that Sox9 preferentially is expressed in cells that morphologically resemble centroacinar and intercalated duct cells (Fig. 1b). Indeed, recently centroacinar cells were shown to be capable of spontaneous differentiation into exocrine and endocrine lineages *in vitro* [42]. As of yet, we have no evidence that Sox9 is necessary for adult β -cell neogenesis, but its embryonic function and adult expression pattern fits the expected profile of a progenitor cell that comprises a small subset of the exocrine and duct cell pool. Further indirect evidence for centroacinar and/or intercalated duct cells serving as adult pancreatic progenitor cells come from the cancer field. It has been suggested that malignant transformation in the pancreas originates from these cells and not from acinar or duct cells [43, 44], consequently supporting a hypothesis where they serve as stem cells as well as cancer stem cells. Another study argues a similar conclusion [45]. These authors attempted to take an approach to pancreatic stem cells similar to what is common practice in the study of neural stem cells: *in vitro* formation of neurospheres from single progenitor cells. By seeding single-cell whole pancreas digests into culture dishes they observed formation of a small number of spheres that seemed to contain self-renewable cells with progenitor cell characteristics that could be induced to differentiate to endocrine cells. Consequently, the conclusion would be that only a small subset of adult pancreatic cells has progenitor cell characteristics.

The contradictory results in animal studies and the failure so far to isolate a pure population of true progenitor cells from the normal adult pancreas has resulted in skepticism towards adult β -cell differentiation from progenitors as a valid route to generate new β -cells. However, when analyzing available data in more detail, there might very well be valid explanations for the discrepancies between studies. First, lineage traces have been performed in mice with different genetic background. We do not know much about how genetic background influences β -cell neogenesis, but knowing how important genetic background is for regulation of β -cell proliferation (see section 3), it is a fair assumption that regulation of neogenesis might also depend on genetic background. Second, as the Seino lab study demonstrated [32], the promoter choice might influence the results. It was expected that amylase and elastase promoter driven Cre expression would lead to similar results in the lineage traces, but it did not. Consequently, heterogeneity in the pancreatic

non-endocrine cell compartment might preclude unambiguous lineage traces until we have a better understanding of the gene expression profile of potential progenitor cells. As discussed above, centroacinar and/or intercalated duct cells are possible candidates for adult progenitor cells. Centroacinar and intercalated duct cells do not express exocrine enzymes [46], but they do on the other hand express carbonic anhydrase [47]. Consequently, if these cells function as progenitors, their expression pattern may explain the discrepancy between lineage tracing elastase and carbonic anhydrase *in vivo*. Third, since the precise cell type serving as adult endocrine progenitors is still somewhat elusive, we do not know how to explicitly induce differentiation. Different studies have used different techniques to induce β -cell neogenesis. The Bonner-Weir and Heimberg studies that succeeded in inducing β -cell neogenesis, induced differentiation with duct ligation [34, 38]. This may therefore be the best experimental approach to induce β -cell differentiation at this point. Duct-ligation was not performed in the Melton and Kushner lab studies that contradict the progenitor cell hypothesis [29, 30], leaving the possibility that their approaches may result in different results if ligation experiments were performed.

2.2. Human Studies

2.2.1. β -Cell Proliferation

Until about 15 years ago, virtually nothing was known about β -cell generation in the human pancreas. β -cell mass dynamics as a mechanism to regulate glucose homeostasis in man had little supporting evidence. Two old studies in pregnant females had demonstrated a significant β -cell increase in women diseased during pregnancy compared to control subjects [1, 2]. Then, within a short time period, several labs, including ours, demonstrated that human β -cells indeed have a capacity to proliferate. Even though β -cell proliferation in experimental animals had been an accepted phenomenon for some time, many still believed that human β -cells were post-mitotic. Evidence that started to overturn this notion includes those emanating from the Sorenson lab, where it was demonstrated that treatment of isolated human islets with placental lactogens results in increased β -cell proliferation paralleled with increased insulin secretion [48]. The Hayek lab seeded dispersed human islets onto a growth matrix and found hepatocyte growth factor dependent proliferation in the β -cells [49], although this finding was later contradicted [50]. In our lab, we demonstrated that human β -cells have a capacity to proliferate both *in vitro* and *in vivo* after transplantation to immunocompromised mice [51]. We were also able to conclude that human β -cell proliferation is stimulated by growth factors as well as metabolic stimuli. When human islets were transplanted to obese mildly hyperglycemic immunocompromised mice we observed a 2-fold induction in proliferation. On the other hand, when human islets were transplanted to mice with alloxan-diabetes, we found no stimulation of proliferation, indicating that glucotoxicity may prevail under severely hyperglycemic conditions. When human islets were transplanted to lean mice that underwent unilateral nephrectomy, a process inducing growth factor dependent growth of the opposite (islet-graft-bearing) kidney [52], human β -cell proliferation increased 3-fold. Still, even in light of these data, the role of β -cell proliferation in

maintenance of human β -cell mass remained controversial. One reason is that the rate of proliferation is about 10-time slower in human β -cells than in some rodent models [53]. We favor a simple explanation for that discrepancy: the life span of the human β -cell is likely longer than in rodents and, therefore, β -cell mass in humans is maintained with a lower proliferative rate. The rate of proliferation that others and we have observed in human β -cells would double the β -cell mass in the human pancreas in about 6-8 month, unless β -cell loss occurs at the same time to maintain homeostasis [51]. A recent study indeed suggests that the life-span of adult human β -cells are very long [54]. They examined human pancreata from individuals aged 1-81 years for long-lived β -cells using Lipofuscin accumulation as a marker for long-lived cells. They found that β -cell numbers were established at 20 years and did not increase further with age. Similarly, we found that human β -cell proliferation declines from adolescence to age 70 [51]. β -cell proliferation in young adults (<30) was more than double than in older adults. The same trend was later confirmed in a larger data set [8]. In summary, it is hard to argue that human β -cell proliferation is negligible, particularly in younger individuals.

About 10 years after these initial studies on human β -cell proliferation were published several studies followed examining proliferation in pancreata from diseased humans. In particular, the Butler lab has contributed to the field's knowledge base with several quite extensive examinations of pathological samples with the help of proliferative markers such as Ki-67 [55, 56]. The proliferative indices that they found are, interestingly, quite comparable to our previous studies addressing this issue. β -cell proliferation was even reported in pancreata from type 1 diabetics [56]. However, the Butler lab has later questioned the validity of studies based on markers for proliferation, such as Ki-67 [57]. They show that the number of actual mitotic events is much lower than the corresponding number of Ki-67 positive cells. Consequently, the already low proliferative index in human β -cells may be even lower than expected.

To somewhat address this controversy; we have recently evaluated a limited number of the initial samples from organ-donor quality human pancreata collected by the JDRF-nPOD initiative (www.jdrfnpod.org). Interestingly, we found a remarkable variability among pancreata. In both type 1 diabetics (not shown) and normal controls we observed examples of ample as well as near absent β -cell proliferation (Fig. 2a vs b). In the specimen in Fig. (2a), the β -cell proliferation seems equal to, or even exceeding, comparable mouse data, where two to three Ki-67 positive cells per islet is not unusual. Given that β -cell proliferation clearly is important in mice and that proliferation in humans at least sometimes seems equal to mice, the likely conclusion is that β -cell proliferation is indeed important for maintenance of β -cell mass in humans. No doubt, additional studies are needed, particularly aiming to understand what regulates human β -cell proliferation. Beyond that, studies further assessing the influence of age, as well as temporal and/or induced conditions that might influence β -cell proliferation (e.g., pregnancy, pancreatitis, etc.) would be beneficial to the understanding of β -cell proliferation in the human. One goal clearly is to understand mechanisms underlying the large variability in proliferation among subjects (see Fig. 2), and

to utilize that knowledge for interventional therapies to induce proliferation when needed. The question remains if the lower rate of proliferation in the aging population still is necessary to maintain β -cell mass or if it is negligible in most individuals.

2.2.2. β -Cell Neogenesis

Equal to experimental animals, duct cells may serve as β -cell progenitors in the human. Similar to the histological picture in rodents after partial pancreatectomy or duct ligation [22-24], single β -cells and β -cells in ducts are often found in samples from human pancreata (Fig. 2b and [58]), indirectly indicating β -cell neogenesis. An increase in duct cells expressing β -cell transcription factors and insulin has also been observed in various experimental conditions and interpreted as an indication of differentiation. For example, we observed Nkx6.1 expression in human duct cells after transplantation to obese mice [52]. The Rabinovitch lab reported an increased number of duct cells expressing Pdx-1 after *in vitro* culture with EGF and gastrin [59]. Pipeleers lab transplanted human duct cells to mice and observed frequent insulin-CK19 (duct cell marker) double positive cells [60].

The Bonner-Weir lab developed the first *in vitro* protocol to induce differentiation of duct cells to insulin-producing cells about 10 years ago [61]. Another protocol inducing duct to β -cell differentiation was developed later in the Heimberg lab, which demonstrated differentiation in human duct cells when over-expressing Ngn3. Interestingly, these data suggest that in parallel to their study in mice, in which endogenous expression of Ngn3 was observed in adult mouse pancreas during regeneration [38], if endogenous expression of Ngn3 is activated, adult human β -cell differentiation may be induced. In all these studies, the evidence for duct to β -cell differentiation is indirect and the findings are, therefore, not indisputable. The culture protocol in some cases employed high concentrations of insulin, that later was shown to result in unspecific uptake of insulin into non-endocrine cells giving rise to artifactual conclusions about β -cell differentiation from stem cells [62]. β -cell dedifferentiation, leading to later redifferentiation is another concern [63].

In an effort to unambiguously demonstrate that duct and/or exocrine cells indeed have the capacity to differentiate to β -cells in the human, we designed a genetic lineage trace experiment [31]. By selectively eliminating all endocrine and mesenchymal cells from digested human pancreata we were able to study the fate of adult pancreatic non-endocrine epithelial cells (NEPECs) of duct and exocrine origin without interference from preexisting β -cells or mesenchymal cells. We genetically labeled NEPECs with GFP and transplanted them to immunocompromised mice. Interestingly, we found that if adult NEPECs were co-transplanted with fetal pancreatic cells, β -cell differentiation was induced in the genetically labeled adult cells. Presumably, signals from fetal cells interacted with adult progenitors and induced adult β -cell differentiation. The Bonner-Weir lab later showed that the presence of fetal tissue is not absolutely necessary for NEPEC differentiation [64]. Instead, *in vitro* induction of a partial epithelial to mesenchymal transition (EMT) seems sufficient in combination with co-transplantation of mesenchymal support cells from either fetal or adult human pancreas. In

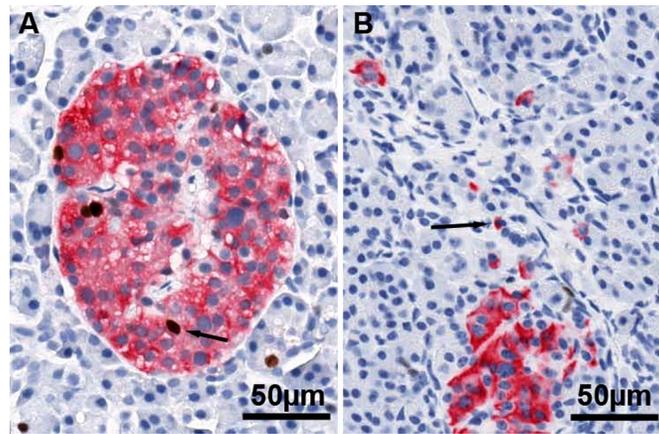


Fig. (2). Human pancreata stained for insulin (red) and Ki-67 (brown). A. Normal human pancreas with four proliferating β -cells in one islet (arrow points to Ki-67 positive β -cell). B. Normal human pancreas with very few Ki-67 positive β -cells. Instead, a large number of single β -cells that are not associated with islets is observed, and some are residing in ducts (arrow, morphological identification). Images are representative of respective pancreas.

both their and our study, NEPECs were subjected to a culture period *in vitro* before transplantation, which induced expression of mesenchymal markers such as vimentin while retaining epithelial marker expression such as E-cadherin, *i.e.* the cells underwent partial EMT. Similarly, in experimental animals it has been suggested that a partial EMT is necessary for β -cell formation in embryonic development [65].

Taken together these data support the idea that β -cell progenitors reside in the duct and/or exocrine compartment of the adult human pancreas. It still remains unclear under which physiological conditions the β -cell differentiation machinery is induced *in vivo*. Obviously, that question will be extremely difficult to address in human beings, unless methods to trace cell fate *in vivo* in humans are developed.

As discussed above in section 2.1.1., it is unclear if β -cell progenitors are rare specialized cells in the exocrine or duct cell compartment, or if all or most cells in these compartments can be induced to differentiate to β -cells with the right stimulus. Although the NEPECs had uniform characteristics based on select marker genes, we observed heterogeneity in affinity for some epithelial-specific antibodies (34bE12, Ber-Ep4), supporting the idea that β -cell differentiation might only be induced in a subset of NEPECs with a certain unknown gene expression pattern [31]. The actual efficiency of differentiation in this study also supports that assumption (about 10% of the epithelial cell population were induced to differentiate). Consequently, at this point we lack data supporting either hypotheses that progenitors in the human pancreas are specialized or not.

3. GENETIC CONTROL OF β -CELL MASS HOMEOSTASIS

When studying regulation of β -cell mass maintenance in both human and mouse tissue it is clear that variability in the response to stimuli is quite substantial. This is particularly well established when studying β -cell proliferation. Within a group of human pancreas donors, proliferation varies significantly, as discussed above (Fig. 2a, b). β -cell proliferation also varies between different strains of mice. Some strains, like FVB, seems to have higher β -cell proliferation indices, whereas others, like B6 and BKS have more moderate basal β -cell proliferation rates. Consequently,

genetic factors seem to influence regulation of β -cell mass maintenance.

3.1. Animal Studies

3.1.1. C57Bl/Ks Mice

The first evidence of genetic regulation of β -cell mass homeostasis was published in 1972-73 when Hummel and Coleman *et al.* described the phenotype of db/db and ob/ob mice with different genetic background [9, 66]. Both mutations, interestingly, precipitate severe diabetes and lack of increased islet size in mice on the C57Bl/Ks (BKS) background, but not in mice on the C57Bl/6 (B6) background. This is despite the fact that these mice develop equally severe obesity and grossly look identical. The db (diabetes) and ob (obesity) loci, were much later identified as the Leptin receptor gene [67] and Leptin gene [68], respectively. The db mice on B6 and BKS backgrounds present with very different islet morphology. B6 islets are enlarged and well granulated, whereas BKS islets are small and degranulated [9]. Consequently, it could be established that regulation of β -cell mass homeostasis is linked to genetic heritage. At this time, β -cell proliferation was not widely studied and it was not until 10 years later that it became clear that the capacity for β -cells to respond to a growth stimulus (glucose) was impaired in islets from BKS vs B6 mice [69]. Consequently, β -cell proliferation may be important for the phenotypic difference in response to obesity in B6 and BKS mice; and genetic background seems to influence the capacity for β -cells to proliferate. Indeed, the increase in β -cell mass in B6-ob mice was later linked to increased β -cell proliferation [70]. Several attempts have been made to determine which genes are responsible for differential diabetes susceptibility in these strains. The first genetic mapping study, performed by Coleman, revealed that low-activity of a malic enzyme regulatory locus was linked to diabetes in BKS mice [71]. It is unclear how that would relate to regulation of β -cell mass. It is possible that dysregulation of malic enzyme activity leads to disturbances in nutrient-induced β -cell proliferation, but also possible that malic enzyme activity is unrelated to β -cell mass regulation and instead is involved in some other pathway necessary for glucose homeostasis. A study by the Permut lab indicated that BKS diabetes susceptibility indeed is tightly linked to pancreatic function by showing a strong correlation

between pancreatic proinsulin mRNA and insulin content to glycemic control in a BKS x 129/J backcross study [72]. A recent intercross study with BKS and C3H mice rendered six candidate genes linked to diabetes susceptibility [73]. One of these genes reminds us about the other side of the coin, β -cell loss as a cause of type 2 diabetes: *Txndc11*, a gene likely to play a role in redox homeostasis. β -cell apoptosis is prominent in BKS-db mice [74, 75] and overexpression of glutathione peroxidase or thioredoxin, which both increase oxidative stress protection, rescues the phenotype in BKS-db mice [76, 77]. Lately, the phenotype in BKS-db mice has been rescued in several other genetic experiments. Deleting the cell cycle inhibitory kinase *p27^{Kip1}* rescued diabetes and loss of β -cell mass in BKS-db mice [78]. Similarly, overexpression of a constitutively active form of cyclin dependent kinase 4 resulted in rescue of the diabetic phenotype in BKS-db mice [79]. Again, diabetes susceptibility seems tightly linked to control of β -cell mass homeostasis in BKS-db mice.

To be able to further study the mechanisms underlying genetic control of β -cells mass, we recently started to revisit B6 and BKS mice as models for genetic control of β -cell homeostasis. We performed direct comparisons of metabolic phenotype and β -cell proliferative response in B6 and BKS mice carrying the db mutation. Clearly, the BKS-db mice quickly diverge from the B6-db mice and develop severe hyperglycemia and abnormal glucose homeostasis between 6 and 8 weeks of age (Fig. 3a). In parallel, the β -cell proliferative response is prominent in both strains postnatally, but starts failing in BKS mice between 4 and 6 weeks of age (unpublished data and Fig. 3b). BKS mice consequently do not increase β -cell mass in response to the increased insulin demand leading to subsequent islet atrophic lesions (Fig. 3c), likely due to the lack of compensation and consequent severe hyperglycemia. BKS and B6 mice increase body weight similarly up to about 13 weeks of age when BKS mice start losing weight and die from severe hyperglycemia between 16 and 23 weeks of age (Fig. 3d). Possibly some of the β -cell proliferative increase in B6-db mice could be attributed to lack of leptin signaling, as pancreatic leptinR mutant mice exhibit increased β -cell mass without an increase in body weight [80]. We think this is an unlikely explanation, as both B6-db and BKS-db lack leptin signaling but presents with differential regulation of β -cell proliferation. To determine if the difference in β -cell proliferation is unique to mice with disrupted leptin signaling, we also studied β -cell proliferation in pregnant B6 and BKS mice. Interestingly, we found that BKS β -cells proliferate at about half the rate of B6 β -cells at day 14.5 of pregnancy (unpublished data). Consequently, it is likely that β -cell proliferation deficiency in BKS mice depends on a genetic defect that is independent of the stimulus for proliferation. We are currently analyzing global gene expression in islets from genetically obese or pregnant B6 and BKS mice with the goal of finding genes that are differentially regulated between the two strains but equally regulated in obesity and pregnancy. Ultimately, we hope that this will lead to novel mechanistic insights related to regulation of β -cell proliferation and, consequently, β -cell mass.

3.1.2. Other Strains

Metabolic differences in response to obesity have been observed in several other inbred mouse strains, with more or less well-characterized phenotypic differences (for review see [81]). Of particular interest are studies of B6 vs BTBR mice

carrying the ob mutation. BTBR mice, like BKS, develop severe diabetes when carrying the ob mutation. The Attie lab has devoted gigantic efforts to genotyping intercrossed B6 x BTBR mice [82], which eventually lead to the cloning of *SorCS1* as the major affected gene in the T2dm2 quantitative trait locus [82]. It is unclear if *SorCS1* play a role in regulation of β -cell mass. *SorCS1* is, however, known to bind platelet derived growth factor and may, therefore, play a role in islet vascular development and remodeling, a process known to be important for β -cell proliferation [83, 84]. The Attie lab later followed up with gene expression analyses and built networks of genes linked to diabetes susceptibility in B6 vs BTBR mice [85]. Interestingly, among several expression networks, the cell cycle machinery in β -cells was one of the strongest linked networks to diabetes-susceptibility. A correlation was found between increased expression of cell cycle genes in B6-ob mice with increasing age from 4 to 10 weeks, but this correlation was lacking in BTBR mice. In particular, cyclins of the A, B, and E type, as well as the A and E cyclin partner *cdk2* in islets correlated with diabetes-susceptibility. Again, strongly supporting the idea that regulation of β -cell proliferation is tightly linked to T2DM susceptibility.

3.2. Human Studies

Given the central role of β -cell mass and function in glucose homeostasis it is conceivable that human diabetes-predisposing genetic variations are affecting maintenance of β -cell mass and functional homeostasis. Indeed, recent genome wide association and candidate gene studies have identified β -cell relevant genetic variations that predispose to various types of diabetes ranging from the juvenile monogenic to polygenic forms exhibited in the adult and influenced by the environment. A summary of such genes relevant to β -cell physiology is found in Table 1. It is striking that the vast majority of genes identified in human genetic studies have a known or likely role in β -cell function. In this section, we will discuss some of the genes that are potentially involved in maintaining β -cell mass. We will focus on the genes involved in polygenic diabetes, as monogenic diabetes have been comprehensively reviewed elsewhere [86].

3.2.1. *CDKAL1* and *CDKNA2A/B*

The ability of a cell to replicate is dependent on the cell cycle machinery. Cyclins, cyclin dependent kinases, and cyclin dependent kinase inhibitors are central to this process. Their role in regulating human β -cell replication has been suggested by viral over-expression studies. For instance, lenti-viral expression of a hyperactive form of *cdk4* in human islets increases β -cell proliferation [87]. The mechanism is probably due to the ability of the *cdk4/cyclinD1* complex to increase phosphorylation of the G1/S cell cycle checkpoint retinoblastoma protein. In mice, cell cycle proteins are equally important to maintenance of β -cell mass (reviewed in [15]). The identification of a genetic association of *CDK5* regulatory subunit associated protein 1-like-1 (*CDKAL1*) and Cyclin-Dependent Kinase Inhibitor 2A/B (*CDKN2A/B*, p16-Ink4/p15-Ink4b) to T2DM [88-90] is, therefore, intriguing.

Although the function of *CDKAL1* is obscure at best, it supposedly associates with *CDK5*, an atypical cyclin dependent kinase (reviewed in [91]). *CDK5* is best known for its role in regulating migration and maturation of post-mitotic neurons. Recently, it became clear that *CDK5* also acts as a cell cycle

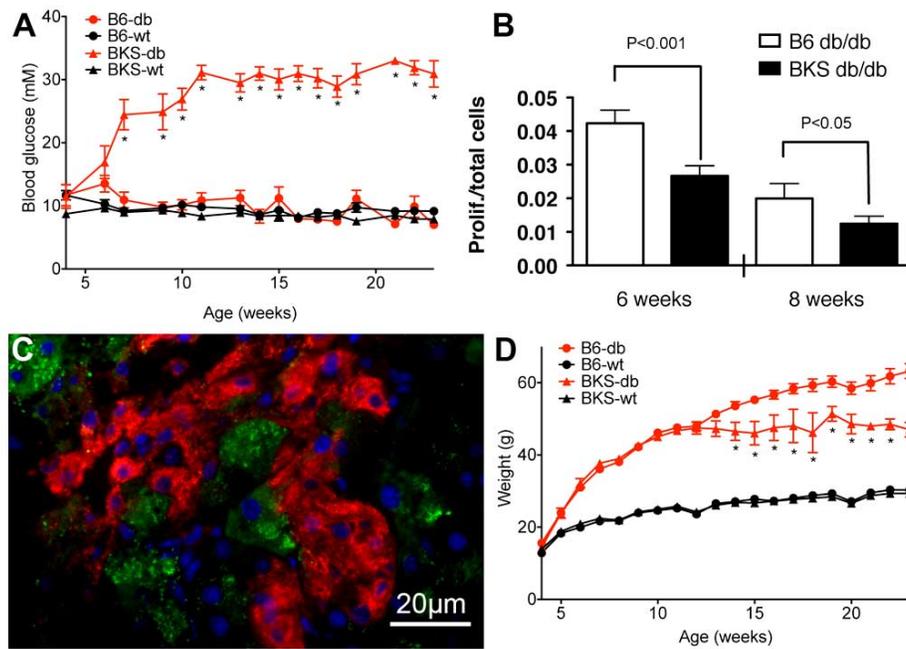


Fig. (3). Phenotypic characteristics of BKS and B6 mice. **A.** Non-fasted blood glucose in B6 and BKS mice over time (n=10-12). Only BKS-db/db mice develop hyperglycemia, which become apparent at 8 weeks of age. **B.** β -cell proliferation in BKS-db/db and B6-db/db mice (n=6-8). BKS-db/db β -cells proliferate at half the rate compared to B6-db/db. **C.** Typical example of islet atrophy in BKS-db/db mice at 12 weeks of age. Islet architecture is lost and β -cells are replaced by exocrine cells. Insulin (red), amylase (green), and nuclei (blue). **D.** Weight gain in BKS and B6 mice over time (n=10-12). BKS-db/db and B6-db/db gain equal amount of weight until 13 weeks of age. Thereafter BKS-db/db mice stop gaining weight and finally succumb to diabetes at 16-23 weeks of age.

inhibitory signal. The favored model suggest that CDK5 enters the nucleus in G_0 and binds to the cyclin dependent kinase inhibitor p27^{Kip1} blocking its exist from the nucleus and thereby blocking cell cycle entry [91]. Interestingly, in β -cells, CDK5 also plays a role in the insulin exocytotic process. Its exact role is debated, both CDK5 over-expression and loss of function seems to result in reduced insulin exocytosis. One study suggests that CDK5 is necessary for Munc18-dependent vesicle priming in β -cells [92]. Another study suggests that CDK5 inhibits the function of voltage dependent calcium channels through phosphorylation [93]. Consequently, extrapolating data on CDK5 and CDKAL1 function in β -cells is difficult, but the data suggest that they may play a role both in cell cycle control and in regulation of insulin secretion.

CDKN2A (p16-Ink4a), CDKN2B (p15-Ink4b) as well as p14(19)Arf are cyclin dependent kinase inhibitors and part of

the same locus, which is often deleted in tumors, resulting in uncontrolled cell growth [94]. The functions of these genes have only recently been studied separately, as tumor cell lines typically lack all three genes together. The role of p15-Ink4b in β -cell function is largely unknown. Loss-of-function studies indicate that p15-Ink4b is not necessary for maintenance of cell cycle control in the β -cell [95]. On the other hand, p16-Ink4a is known to accumulate in aging β -cells leading to decreased β -cell proliferation, whereas p16-Ink4a knock out restores β -cell proliferation in aging mice [96].

3.2.2. TCF7L2

TCF7L2 single nucleotide polymorphisms are strongly associated with T2DM. Depleting TCF7L2 in human islets lead to a decrease in β -cell proliferation and increased β -cell apoptosis [97]. The possible mechanism is that depletion of

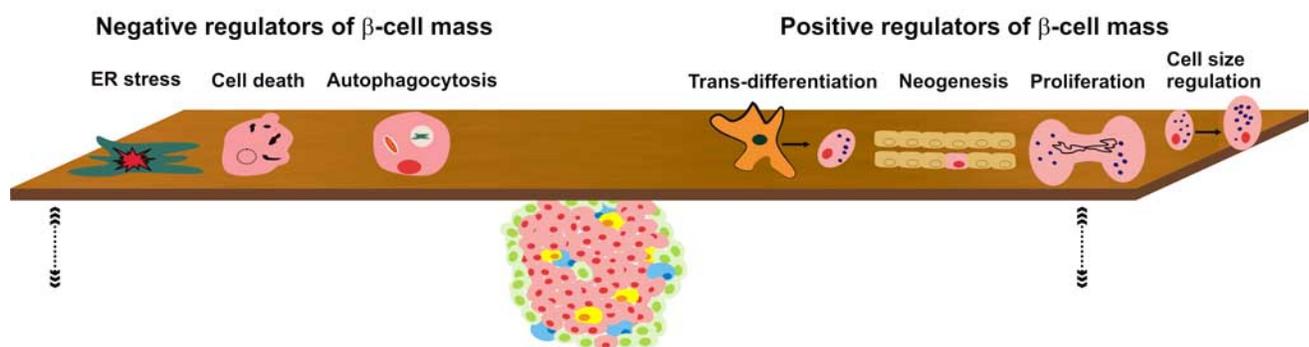


Fig. (4). β -cell mass homeostasis. Although some controversy remains, we believe that regulation of β -cell mass depends on several parallel pathways to maintain a balance with negative regulation of β -cell numbers. As long as β -cell mass homeostasis is in balance, glucose homeostasis is controlled, emphasizing the importance of β -cells in diabetes etiology.

TCF7L2 causes FOXO1 phosphorylation and nuclear seclusion [98]. TCF7L2 and FOXO1 compete with each other in the canonical Wnt signaling pathway to promote cell proliferation and survival. Hence, depletion of TCF7L2 might favor a FOXO1/ β -catenin complex that translocates to the nucleus and causes cell cycle arrest and apoptosis of β -cells (for review see [99]). Taking together, it is not unlikely that TCF7L2 plays a central role in maintaining β -cell mass homeostasis *in vivo*.

3.2.3. PPAR γ

Peroxisome Proliferator Activated receptor gamma (PPAR γ) belongs to a family of nuclear receptors that initiates transcription from PPAR response elements and is involved in regulation of lipid and glucose metabolism (for review see [100]). Although, best known for its function in peripheral tissues, in rodents, synthetic ligands of PPAR γ improve β -cell survival and proliferation [101, 102]. β -cell specific PPAR γ mutant mice exhibit increased β -cell proliferation and mass on normal chow but fail to increase mass further on high fat diet [103]. The latter could possibly be explained by lipid-induced nitric oxide production that is reduced in islets treated with rosiglitazone (PPAR γ agonist) [104]. Consequently, PPAR γ may play a role in protecting β -cells in a high fat diet induced hyperlipidemic environment. PPAR γ loss of function, thereby, may preclude such protection and prevent high fat diet induced β -cell mass compensation.

3.2.4. HHEX and TRIB3

HHEX was identified in recent genome-wide association studies [88-90]. HHEX plays a crucial role in early pancreas development by controlling ventral pancreas specification [105]. At this point, HHEX function in the adult pancreas is unknown, but transcription factors that are essential for pancreas development are often also important in adult β -cell function or growth control. A polymorphism in the TRIB3 gene linked to T2DM and the metabolic syndrome was identified this year in two independent studies [106, 107]. TRIB3 is a marker for ER-stress downstream of CHOP and TRIB3 loss of function decreases apoptosis in response to ER-stress [108]. Interestingly, TRIB3 is controlled by nutrient availability and might act as a nutrient sensor [109]. In β -cells, TRIB3 over-expression synergistically increases glucotoxic effects on cell growth and apoptosis, whereas TRIB3 knock down has the opposite effect [110]. Consequently, TRIB3 may be an important regulator of β -cell survival and mass in hyperglycemia.

4. CONCLUSIONS

Based on overwhelming evidence, it is clear that maintenance of β -cell mass is essential for control of glucose homeostasis. At this time, the source of new β -cells in adulthood remains controversial with evidence both for and against self-proliferation and neogenesis from various possible progenitors. Our conclusion is that β -cell generation in the adult depends on many paths to assure sufficient numbers of β -cells at any given time, balancing mechanisms for negative regulation of β -cell numbers (Fig. 4). A simple model with only one pathway does not fit the current literature. However, even with such a variety of mechanisms contributing to β -cell generation in the adult, as we know, β -cell function fails in diabetes. Recent human genetic studies have made an important contribution to our understanding of which genes regulate β -cell mass and functional homeostasis. The large number of genes identified, as well as morphological

heterogeneity in human pancreatic tissue samples, indicate that type 2 diabetes may etiologically very well be several, if not many, different diseases with a similar outcome with respect to glucose homeostasis.

In our opinion, it is certainly worthwhile to target central components of the proliferative or cell fate machinery to expand β -cell mass *in vivo*. One consideration to keep in mind is that intervening with such pathways might increase the risks for tumors, not only in β -cells. Consequently, we still need to find genes that uniquely regulate cell cycle processes in β -cells so that future therapies can be tightly controlled. It is also important to remember that many of the genes important in mice have yet to be confirmed in human β -cells. Similarly, many of the genes identified in human genetic studies have unclear function, which will be best elucidated in mice. Hence, studies in mice are important for delineating regulation of β -cell mass. Most studies in animal models will likely correlate well with mechanisms in humans, as long as we realize that kinetics of β -cell generation is different in humans and mice. In summary, we should be optimistic that interfering with human β -cell mass homeostasis will be a successful strategy to combat diabetes in the future.

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