Open Access

Genetic Control of β-Cell Mass Homeostasis

Mangala M. Soundarapandian[1], Maria L. Nieves[1], Roxane Pasquier[1], Ulrika Bergström[1], Mark A. Atkinson[2] and Björn Tyrberg[*,1]

[1]*Metabolic Signaling and Disease, Diabetes and Obesity Research Center, Sanford-Burnham Medical Research Institute, Orlando, FL, USA*

[2]*Department of Pathology, Immunology and Laboratory Medicine, University of Florida College of Medicine, Gainesville, FL, USA*

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

*Address correspondence to this author at the Metabolic Signaling and Disease Program, Diabetes and Obesity Research Center, Sanford-Burnham Medical Research Institute, 6400 Sanger Road, Orlando, FL 32827, USA; Tel: +1-407-745-2063; Fax: +1-407-745-2013; E-mail: btyrberg@burnham.org

1874-2165/10 2010 Bentham Open

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 that 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

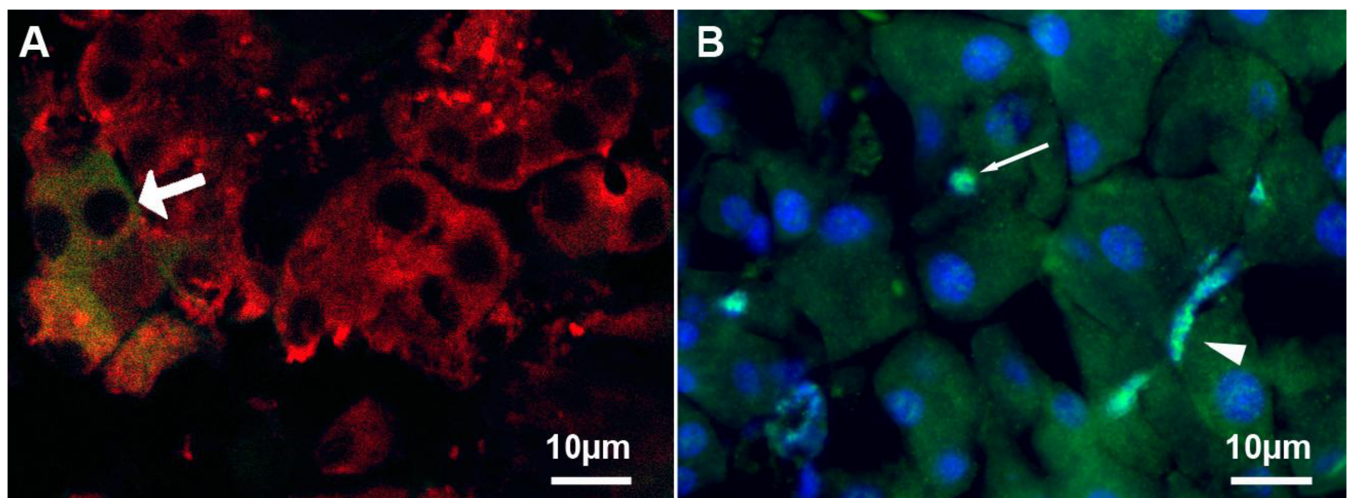

Fig. (1). Confocal micrographs of normal adult exocrine mouse pancreas. A. Insulin (green) and amylase (red) are co-expressed in some cells in a normal mouse pancreas. **B**. Sox9 (green) is expressed in cells that with morphological identification resemble centroacinar cells (arrow) and intercalated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

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 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 functions 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 Lipofuschin 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-

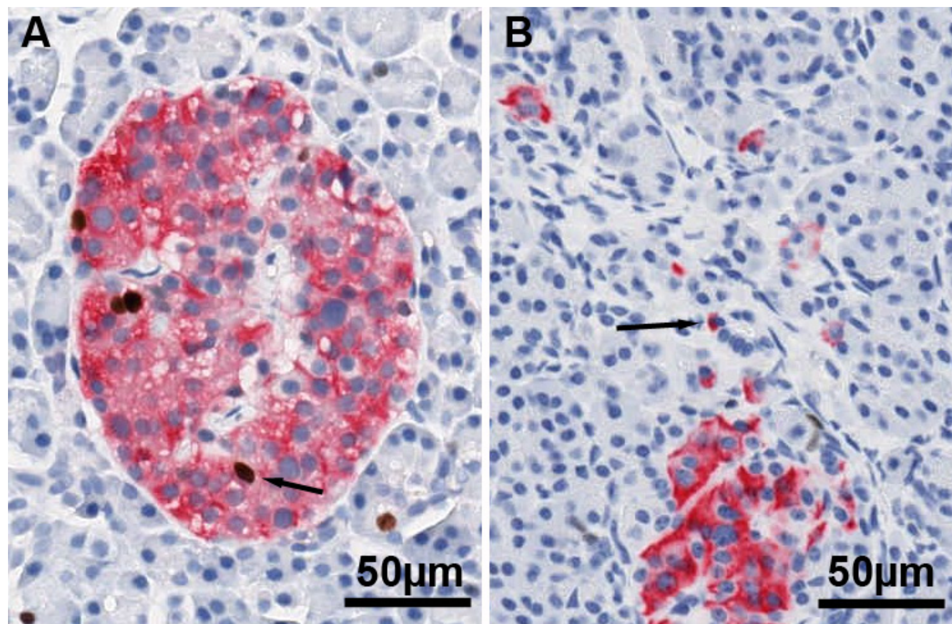

Fig. (2). Human pancreata stained for insulin (red) and Ki-67 (brown). A. Normal human pancreas with four proliferating β-cells in one islet (arrow points to Ki-67 positive β-cell). **B**. Normal human pancreas with very few Ki-67 positive β-cells. Instead, a large number of single β-cells that are not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

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 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 epithelia 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 fist 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 postnataly, 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 loosing 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

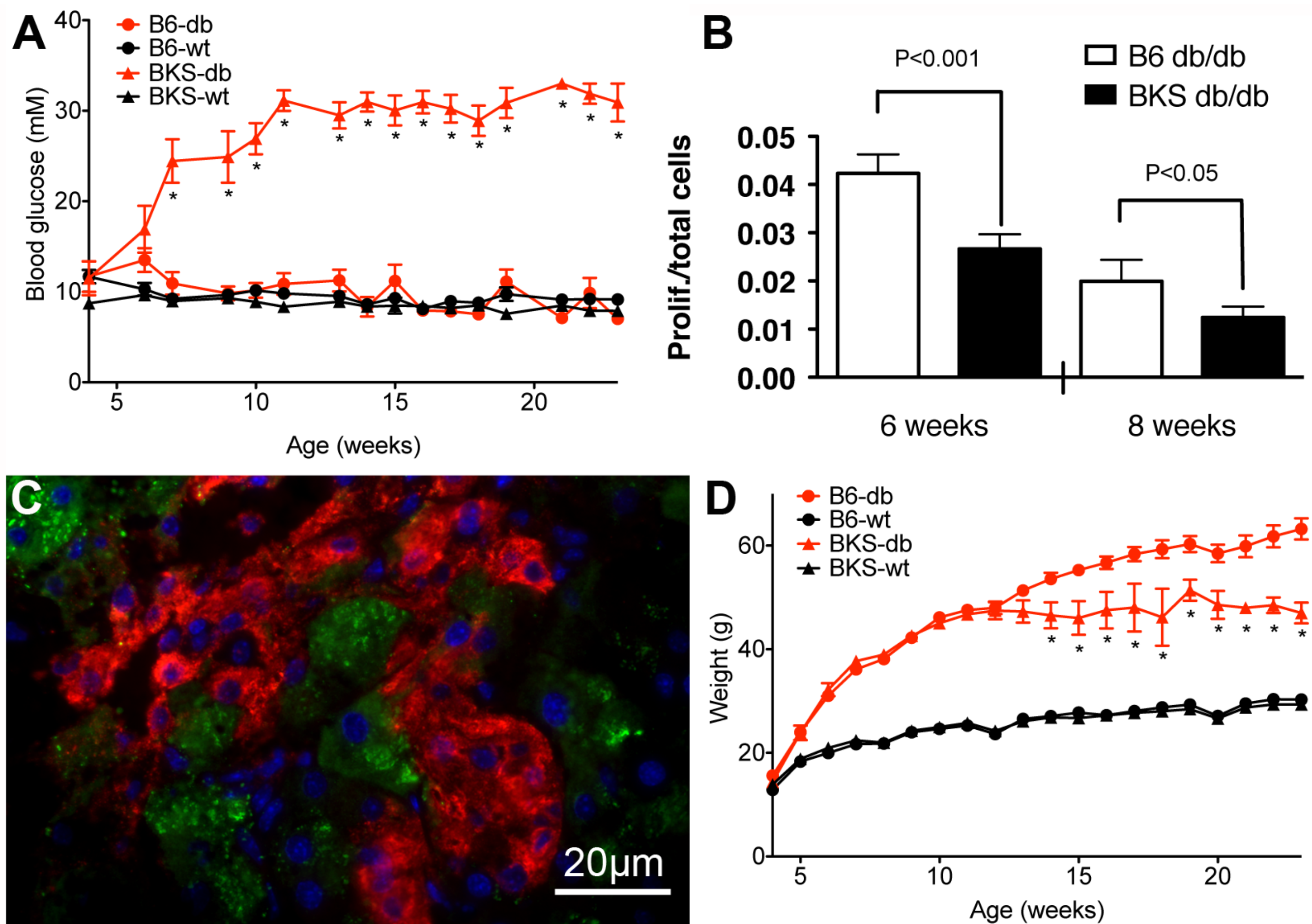

Fig. (3). Phenotypic characteristics of BKS and B6 mice. A. Non-fasted blood glucose in B6 and BKS mice over time (n=10-12). Only BKS-db/db mice develop hyperglycemia, which become apparent at 8 weeks of age. **B**. β-cell proliferation in BKS-db/db and B6-db/db mice (n=6-8). BKS-db/db β-cells proliferate 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.

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 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 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. PPARγ

Peroxisome Proliferator Activated receptor gamma (PPARγ) 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 know for its function in peripheral tissues, in rodents, synthetic ligands of PPARγ improve β-cell survival and proliferation [101, 102]. β-cell specific PPARγ 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 (PPARγ agonist) [104]. Consequently, PPARγ may play role in protecting β-cells in a high fat diet induced hyperlipidemic environment. PPARγ 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

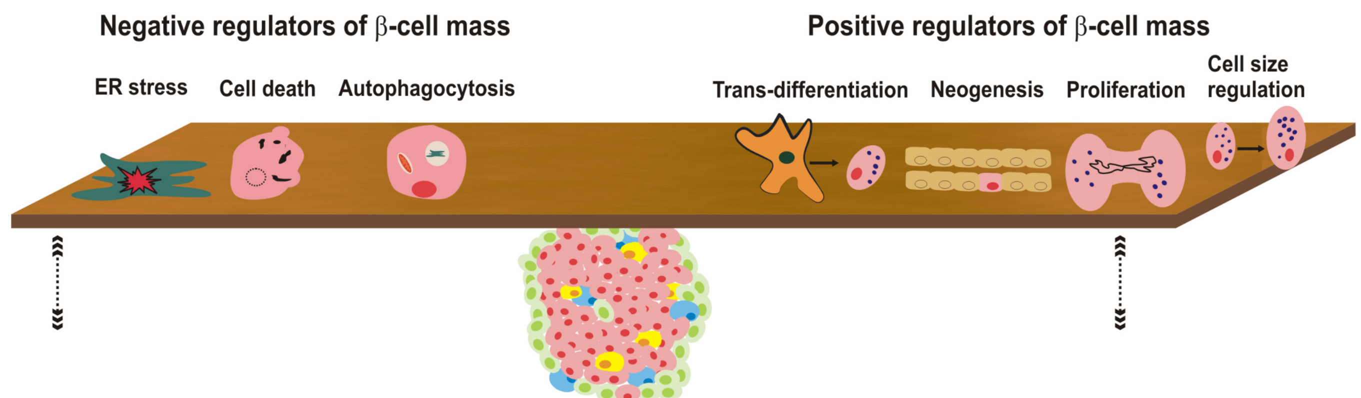

Fig. (4). β-cell mass homeostasis. Although some controversy remains, we believe that regulation of β-cell mass depends on several parallel pathways to maintain a balance with negative regulation of β-cell numbers. As long as β-cell mass homeostasis is in balance, glucose homeostasis is controlled, emphasizing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

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.

REFERENCES

[1] Rosenloecher K. Die Veränderungen des Pankreas in der Schwangenschaft bei Mensch und Tier. Arch Gynäkol 1932; 151: 567-75.

[2] Van Assche FA, Aerts L, De Prins F. A morphological study of the endocrine pancreas in human pregnancy. Br J Obstet Gynaecol 1978; 85: 818-20.

[3] Klöppel G, Lohr M, Habich K, Oberholzer M, Heitz PU. Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. Surv Synth Pathol Res 1985; 4: 110-25.

[4] Clark A, Wells CA, Buley ID, *et al.* Islet amyloid, increased A-cells, reduced B-cells and exocrine fibrosis: quantitative changes in the pancreas in type 2 diabetes. Diabetes Res 1988; 9: 151-9.

[5] Stefan Y, Orci L, Malaisse-Lagae F, Perrelet A, Patel Y, Unger RH. Quantitation of endocrine cell content in the pancreas of nondiabetic and diabetic humans. Diabetes 1982; 31: 694-700.

[6] Sakuraba H, Mizukami H, Yagihashi N, Wada R, Hanyu C, Yagihashi S. Reduced beta-cell mass and expression of oxidative stress-related DNA damage in the islet of Japanese Type II diabetic patients. Diabetologia 2002; 45: 85-96.

[7] Yoon KH, Ko SH, Cho JH, *et al.* Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea. J Clin Endocrinol Metab 2003; 88: 2300-8.

[8] 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: 102-10.

[9] Hummel KP, Coleman DL, Lane PW. The influence of genetic background on expression of mutations at the diabetes locus in the mouse. I. C57BL-KsJ and C57BL-6J strains. Biochem Genet 1972; 7: 1-13.

[10] Kuroe A, Fukushima M, Usami M, *et al.* Impaired beta-cell function and insulin sensitivity in Japanese subjects with normal glucose tolerance. Diabetes Res Clin Pract 2003; 59: 71-7.

[11] Nakagami T, Qiao Q, Carstensen B, *et al.* Age, body mass index and Type 2 diabetes-associations modified by ethnicity. Diabetologia 2003; 46: 1063-70.

[12] Lazarus SS, Volk BW. Pancreas in maturity-onset diabetes. Pathogenetic considerations. Arch Pathol 1961; 71: 44-59.

[13] Blum B, Heggestad C, Lazarow A. DNA synthesis in pancreatic islet tissue from fetal and young postnatal rats. Anat Rec 1963; 145: 309-10.

[14] Hellerström C, Andersson A, Gunnarsson R. Regeneration of islet cells. Acta Endocrinol Suppl (Copenh) 1976; 205: 145-60.

[15] Cozar-Castellano I, Fiaschi-Taesch N, Bigatel TA, *et al.* Molecular control of cell cycle progression in the pancreatic beta-cell. Endocr Rev 2006; 27: 356-70.

[16] Heit JJ, Karnik SK, Kim SK. Intrinsic regulators of pancreatic beta-cell proliferation. Annu Rev Cell Dev Biol 2006; 22: 311-38.

[17] Ackermann AM, Gannon M. Molecular regulation of pancreatic beta-cell mass development, maintenance, and expansion. J Mol Endocrinol 2007; 38: 193-206.

[18] Olerud J, Kanaykina N, Vasilovska S, *et al.* Neural crest stem cells increase beta cell proliferation and improve islet function in co-transplanted murine pancreatic islets. Diabetologia 2009; 52: 2594-601.

[19] Imai J, Katagiri H, Yamada T, *et al.* Regulation of pancreatic beta cell mass by neuronal signals from the liver. Science 2008; 322: 1250-4.

[20] Hieronymi G. Effect of alloxan in the white mouse after ligation of the pancreatic duct, with a contribution on the regeneration of the islet cells. Frankf Z Pathol 1951; 62: 430-41.

[21] Martin JM, Lacy PE. The prediabetic period in partially pancreatectomized rats. Diabetes 1963; 12: 238-42.

[22] Edström C, Falkmer S. Pancreatic morphology and blood glucose level in rats at various intervals after duct ligation. Virchows Arch A Pathol Pathol Anat 1968; 345: 139-53.

[23] Prado MLM, Cruz AR. Adaptation of the endocrine tissue of rat pancreas after partial pancreatectomy-a morphometric study. Acta Anat (Basel) 1983; 116: 346-52.

[24] Brockenbrough JS, Weir GC, Bonner-Weir S. Discordance of exocrine and endocrine growth after 90% pancreatectomy in rats. Diabetes 1988; 37: 232-6.

[25] Gu D, Sarvetnick N. Epithelial cell proliferation and islet neogenesis in IFN-γ transgenic mice. Development 1993; 118: 33-46.

[26] Gu D, Lee MS, Krahl T, Sarvetnick N. Transitional cells in the regenerating pancreas. Development 1994; 120: 1873-81.

[27] Baeyens L, De Breuck S, 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.

[28] Lardon J, Huyens N, Rooman I, Bouwens L. Exocrine cell transdifferentiation in dexamethasone-treated rat pancreas. Virchows Arch 2004; 444: 61-5.

[29] 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: 41-6.

[30] Teta M, Rankin MM, Long SY, Stein GM, Kushner JA. Growth and regeneration of adult Beta cells does not involve specialized progenitors. Dev Cell 2007; 12: 817-26.

[31] Minami K, Okuno M, Miyawaki K, *et al.* Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells. Proc Natl Acad Sci USA 2005; 102: 15116-21.

[32] Desai BM, Oliver-Krasinski J, De Leon DD, *et al.* Preexisting pancreatic acinar cells contribute to acinar cell, but not islet beta cell, regeneration. J Clin Invest 2007; 117: 971-7.

[33] Inada A, Nienaber C, Katsuta H, *et al.* Carbonic anhydrase II-positive pancreatic cells are progenitors for both endocrine and exocrine pancreas after birth. Proc Natl Acad Sci USA 2008; 105: 19915-9.

[34] Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. *In vivo* reprogramming of adult pancreatic exocrine cells to beta-cells. Nature 2008; 455: 627-32.

[35] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126: 663-76.

[36] Gradwohl G, Dierich A, LeMeur M, Guillemot F. Neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. Proc Natl Acad Sci USA 2000; 97: 1607-11.

[37] Xu X, D'Hoker J, Stange G, *et al.* Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 2008; 132: 197-207.

[38] Ackermann Misfeldt A, Costa RH, Gannon M. Beta-cell proliferation, but not neogenesis, following 60% partial pancreatectomy is impaired in the absence of FoxM1. Diabetes 2008; 57: 3069-77.

[39] Seymour PA, Freude KK, Dubois CL, Shih HP, Patel NA, Sander M. A dosage-dependent requirement for Sox9 in pancreatic endocrine cell formation. Dev Biol 2008; 323: 19-30.

[40] Seymour PA, Freude KK, Tran MN, *et al.* SOX9 is required for maintenance of the pancreatic progenitor cell pool. Proc Natl Acad Sci USA 2007; 104: 1865-70.

[41] Rovira M, Scott SG, Liss AS, Jensen J, Thayer SP, Leach SD. Isolation and characterization of centroacinar/terminal ductal progenitor cells in adult mouse pancreas. Proc Natl Acad Sci USA 2010; 107: 75-80.

[42] Stanger BZ, Stiles B, Lauwers GY, *et al.* Pten constrains centroacinar cell expansion and malignant transformation in the pancreas. Cancer Cell 2005; 8: 185-95.

[43] Tanaka H, Fukamachi K, Futakuchi M, *et al.* Mature acinar cells are refractory to carcinoma development by targeted activation of Ras oncogene in adult rats. Cancer Sci 2010; 101: 341-6.

[44] Seaberg RM, Smukler SR, Kieffer TJ, *et al.* Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. Nat Biotechnol 2004; 22: 1115-24.

[45] Ohta T, Terada T, Nagakawa T, Itoh H, Tajima H, Miyazaki I. Presence of pancreatic alpha-amylase, trypsinogen, and lipase immunoreactivity in normal human pancreatic ducts. Pancreas 1994; 9: 382-6.

[46] Boquist L, Hagstrom S. Carbonic anhydrase activity in mouse endocrine pancreas. Acta Pathol Microbiol Scand A 1979; 87A: 157-64.

[47] Brelje TC, Scharp DW, Lacy PE, *et al.* Effect of homologous placental lactogens, prolactins, and growth hormones on islet B-cell division and insulin secretion in rat, mouse, and human islets: implication for placental lactogen regulation of islet function during pregnancy. Endocrinology 1993; 132: 879-87.

[48] Hayek A, Beattie GM, Cirulli V, Lopez AD, Ricordi C, Rubin JS. Growth factor/matrix-induced proliferation of human adult beta-cells. Diabetes 1995; 44: 1458-60.

[49] Lefebvre VH, Otonkoski T, Ustinov J, Huotari A-M, Pipeleers DG, Bouwens L. Culture of adult human islet preparations with hepatocyte growth factor and 804G matrix is mitogenic for duct cells but not for beta-cells. Diabetes 1998; 47: 134-7.

[50] Tyrberg B, Eizirik DL, Hellerström C, Pipeleers DG, Andersson A. Human pancreatic beta-cell deoxyribonucleic acid-synthesis in islet grafts decreases with increasing organ donor age but increases in response to glucose stimulation *in vitro*. Endocrinology 1996; 137: 5694-9.

[51] Tyrberg B, Ustinov J, Otonkoski T, Andersson A. Stimulated endocrine cell proliferation and differentiation in transplanted human pancreatic islets: effects of the ob gene and compensatory growth of the implantation organ. Diabetes 2001; 50: 301-7.

[52] Tyrberg B, Andersson A, Borg LAH. Species differences in susceptibility of transplanted and cultured pancreatic islets to the beta-cell toxin alloxan. Gen Comp Endocrinol 2001; 122: 238-51.

[53] Cnop M, Hughes SJ, Igoillo-Esteve M, *et al.* The long lifespan and low turnover of human islet beta cells estimated by mathematical modelling of lipofuscin accumulation. Diabetologia 2010; 53: 321-30.

[54] Meier JJ, Butler AE, Saisho Y, *et al.* Beta-cell replication is the primary mechanism subserving the postnatal expansion of beta-cell mass in humans. Diabetes 2008; 57: 1584-94.

[55] Meier JJ, Lin JC, Butler AE, Galasso R, Martinez DS, Butler PC. Direct evidence of attempted beta cell regeneration in an 89-year-old patient with recent-onset type 1 diabetes. Diabetologia 2006; 49: 1838-44.

[56] Saisho Y, Manesso E, Gurlo T, *et al.* Development of factors to convert frequency to rate for beta-cell replication and apoptosis quantified by time-lapse video microscopy and immunohistochemistry. Am J Physiol Endocrinol Metab 2009; 296: E89-96.

[57] Martin-Pagola A, Sisino G, Allende G, *et al.* Insulin protein and proliferation in ductal cells in the transplanted pancreas of patients with type 1 diabetes and recurrence of autoimmunity. Diabetologia 2008; 51: 1803-13.

[58] Suarez-Pinzon WL, Lakey JR, Brand SJ, Rabinovitch A. Combination therapy with epidermal growth factor and gastrin induces neogenesis of human islet {beta}-cells from pancreatic duct cells and an increase in functional {beta}-cell mass. J Clin Endocrinol Metab 2005; 90: 3401-9.

[59] Bogdani M, Lefebvre V, Buelens N, *et al.* Formation of insulin-positive cells in implants of human pancreatic duct cell preparations from young donors. Diabetologia 2003; 46: 830-8.

[60] Bonner-Weir S, Taneja M, Weir GC, *et al. In vitro* cultivation of human islets from expanded ductal tissue. Proc Natl Acad Sci USA 2000; 97: 7999-8004.

[61] Rajagopal J, Anderson WJ, Kume S, Martinez OI, Melton DA. Insulin staining of ES cell progeny from insulin uptake. Science 2003; 299: 363.

[62] 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: 2296-304.

[63] Hao E, Tyrberg B, Itkin-Ansari P, *et al.* Beta-cell differentiation from nonendocrine epithelial cells of the human pancreas. EH, BT: Equal contribution. Nat Med 2006; 12: 310-6.

[64] Yatoh S, Dodge R, Akashi T, *et al.* Differentiation of affinity-purified human pancreatic duct cells to beta-cells. Diabetes 2007; 56: 1802-9.

[65] Cole L, Anderson M, Antin PB, Limesand SW. One process for pancreatic beta-cell coalescence into islets involves an epithelial-mesenchymal transition. J Endocrinol 2009; 203: 19-31.

[66] Coleman DL, Hummel KP. The influence of genetic background on the expression of the obese (Ob) gene in the mouse. Diabetologia 1973; 9: 287-93.

[67] Tartaglia LA, Dembski M, Weng X, *et al.* Identification and expression cloning of a leptin receptor, OB-R. Cell 1995; 83: 1263-71.

[68] Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372: 425-32.

[69] Swenne I, Andersson A. Effect of genetic background on the capacity for islet cell replication in mice. Diabetologia 1984; 27: 464-7.

[70] Andersson A, Korsgren O, Naeser P. DNA replication in transplanted and endogenous pancreatic islets of obese-hyperglycemic mice at different stages of the syndrome. Metabolism 1989; 38: 974-8.

[71] Coleman DL. The influence of genetic background on the expression of mutations at the diabetes (db) locus in the mouse. VI: Hepatic malic enzyme activity is associated with diabetes severity. Metabolism 1992; 41: 1134-6.

[72] Kaku K, Province M, Permutt MA. Genetic analysis of obesity-induced diabetes associated with a limited capacity to synthesize insulin in C57BL/KS mice: evidence for polygenic control. Diabetologia 1989; 32: 636-43.

[73] Moritani M, Togawa K, Yaguchi H, *et al.* Identification of diabetes susceptibility loci in db mice by combined quantitative trait loci analysis and haplotype mapping. Genomics 2006; 88: 719-30.

[74] Wang Q, Brubaker PL. Glucagon-like peptide-1 treatment delays the onset of diabetes in 8 week-old db/db mice. Diabetologia 2002; 45: 1263-73.

[75] Garris DR. Cytochemical analysis of pancreatic islet lipoapoptosis: hyperlipidemia-induced cytoinvolution following expression of the diabetes (db/db) mutation. Pathobiology 2005; 72: 124-32.

[76] Harmon JS, Bogdani M, Parazzoli SD, *et al.* beta-Cell-specific overexpression of glutathione peroxidase preserves intranuclear MafA and reverses diabetes in db/db mice. Endocrinology 2009; 150: 4855-62.

[77] Yamamoto M, Yamato E, Toyoda S, *et al.* Transgenic expression of antioxidant protein thioredoxin in pancreatic beta cells prevents progression of type 2 diabetes mellitus. Antioxid Redox Signal 2008; 10: 43-9.

[78] Uchida T, Nakamura T, Hashimoto N, *et al.* Deletion of Cdkn1b ameliorates hyperglycemia by maintaining compensatory hyperinsulinemia in diabetic mice. Nat Med 2005; 11: 175-82.

[79] Miyawaki K, Inoue H, Keshavarz P, *et al.* Transgenic expression of a mutated cyclin-dependent kinase 4 (CDK4/R24C) in pancreatic beta-cells prevents progression of diabetes in db/db mice. Diabetes Res Clin Pract 2008; 82: 33-41.

[80] Morioka T, Asilmaz E, Hu J, *et al.* Disruption of leptin receptor expression in the pancreas directly affects beta cell growth and function in mice. J Clin Invest 2007; 117: 2860-8.

[81] Clee SM, Attie AD. The genetic landscape of type 2 diabetes in mice. Endocr Rev 2007; 28: 48-83.

[82] Clee SM, Yandell BS, Schueler KM, *et al.* Positional cloning of Sorcs1, a type 2 diabetes quantitative trait locus. Nat Genet 2006; 38: 688-93.

[83] Nikolova G, Jabs N, Konstantinova I, *et al.* The vascular basement membrane: a niche for insulin gene expression and Beta cell proliferation. Dev Cell 2006; 10: 397-405.

[84] Johansson M, Mattsson G, Andersson A, Jansson L, Carlsson PO. Islet endothelial cells and pancreatic beta-cell proliferation: studies *in vitro* and during pregnancy in adult rats. Endocrinology 2006; 147: 2315-24.
[85] Keller MP, Choi Y, Wang P, *et al.* A gene expression network model of type 2 diabetes links cell cycle regulation in islets with diabetes susceptibility. Genome Res 2008; 18: 706-16.
[86] Vaxillaire M, Froguel P. Monogenic diabetes in the young, pharmacogenetics and relevance to multifactorial forms of type 2 diabetes. Endocr Rev 2008; 29: 254-64.
[87] Marzo N, Mora C, Fabregat ME, *et al.* Pancreatic islets from cyclin-dependent kinase 4/R24C (Cdk4) knockin mice have significantly increased beta cell mass and are physiologically functional, indicating that Cdk4 is a potential target for pancreatic beta cell mass regeneration in Type 1 diabetes. Diabetologia 2004; 47: 686-94.
[88] t Hart LM, Simonis-Bik AM, Nijpels G, *et al.* A Combined Risk Allele Score of Eight Type 2 Diabetes Genes Is Associated With Reduced First Phase Glucose Stimulated Insulin Secretion During Hyperglycemic Clamps. Diabetes 2010; 59: 287-92.
[89] Ruchat SM, Elks CE, Loos RJ, *et al.* Association between insulin secretion, insulin sensitivity and type 2 diabetes susceptibility variants identified in genome-wide association studies. Acta Diabetol 2009; 46: 217-26.
[90] Pascoe L, Tura A, Patel SK, *et al.* Common variants of the novel type 2 diabetes genes CDKAL1 and HHEX/IDE are associated with decreased pancreatic beta-cell function. Diabetes 2007; 56: 3101-4.
[91] Zhang J, Herrup K. Cdk5 and the non-catalytic arrest of the neuronal cell cycle. Cell Cycle 2008; 7: 3487-90.
[92] Lilja L, Johansson JU, Gromada J, *et al.* Cyclin-dependent kinase 5 associated with p39 promotes Munc18-1 phosphorylation and Ca(2+)-dependent exocytosis. J Biol Chem 2004; 279: 29534-41.
[93] Wei FY, Nagashima K, Ohshima T, *et al.* Cdk5-dependent regulation of glucose-stimulated insulin secretion. Nat Med 2005; 11: 1104-8.
[94] Krimpenfort P, Ijpenberg A, Song JY, *et al.* p15Ink4b is a critical tumour suppressor in the absence of p16Ink4a. Nature 2007; 448: 943-6.
[95] Latres E, Malumbres M, Sotillo R, *et al.* Limited overlapping roles of P15(INK4b) and P18(INK4c) cell cycle inhibitors in proliferation and tumorigenesis. EMBO J 2000; 19: 3496-506.
[96] Krishnamurthy J, Ramsey MR, Ligon KL, *et al.* p16INK4a induces an age-dependent decline in islet regenerative potential. Nature 2006; 443: 453-7.
[97] Shu L, Sauter NS, Schulthess FT, Matveyenko AV, Oberholzer J, Maedler K. Transcription factor 7-like 2 regulates beta-cell survival and function in human pancreatic islets. Diabetes 2008; 57: 645-53.
[98] Shu L, Matveyenko AV, Kerr-Conte J, Cho JH, McIntosh CH, Maedler K. Decreased TCF7L2 protein levels in type 2 diabetes mellitus correlate with downregulation of GIP- and GLP-1 receptors and impaired beta-cell function. Hum Mol Genet 2009; 18: 2388-99.
[99] Jin T. The WNT signalling pathway and diabetes mellitus. Diabetologia 2008; 51: 1771-80.
[100] Jeninga EH, Gurnell M, Kalkhoven E. Functional implications of genetic variation in human PPARgamma. Trends Endocrinol Metab 2009; 20: 380-7.
[101] Finegood DT, McArthur MD, Kojwang D, *et al.* Beta-cell mass dynamics in Zucker diabetic fatty rats. Rosiglitazone prevents the rise in net cell death. Diabetes 2001; 50: 1021-9.
[102] Higa M, Zhou YT, Ravazzola M, Baetens D, Orci L, Unger RH. Troglitazone prevents mitochondrial alterations, beta cell destruction, and diabetes in obese prediabetic rats. Proc Natl Acad Sci USA 1999; 96: 11513-8.
[103] Rosen ED, Kulkarni RN, Sarraf P, *et al.* Targeted elimination of peroxisome proliferator-activated receptor gamma in beta cells leads to abnormalities in islet mass without compromising glucose homeostasis. Mol Cell Biol 2003; 23: 7222-9.
[104] Vandewalle B, Moerman E, Lefebvre B, *et al.* PPARgamma-dependent and -independent effects of rosiglitazone on lipotoxic human pancreatic islets. Biochem Biophys Res Commun 2008; 366: 1096-101.
[105] Bort R, Martinez-Barbera JP, Beddington RS, Zaret KS. Hex homeobox gene-dependent tissue positioning is required for organogenesis of the ventral pancreas. Development 2004; 131: 797-806.
[106] Gong HP, Wang ZH, Jiang H, *et al.* TRIB3 functional Q84R polymorphism is a risk factor for metabolic syndrome and carotid atherosclerosis. Diabetes Care 2009; 32: 1311-3.
[107] Prudente S, Scarpelli D, Chandalia M, *et al.* The TRIB3 Q84R polymorphism and risk of early-onset type 2 diabetes. J Clin Endocrinol Metab 2009; 94: 190-6.
[108] Ohoka N, Yoshii S, Hattori T, Onozaki K, Hayashi H. TRB3, a novel ER stress-inducible gene, is induced via ATF4-CHOP pathway and is involved in cell death. EMBO J 2005; 24: 1243-55.
[109] Schwarzer R, Dames S, Tondera D, Klippel A, Kaufmann J. TRB3 is a PI 3-kinase dependent indicator for nutrient starvation. Cell Signal 2006; 18: 899-909.
[110] Qian B, Wang H, Men X, *et al.* TRI3 is implicated in glucotoxicity- and endoplasmic reticulum-stress-induced beta-cell apoptosis. J Endocrinol 2008; 199: 407-16.3.

Received: December 22, 2009 Revised: April 6, 2010 Accepted: April 13, 2010

© Soundarapandian *et al.*; Licensee *Bentham Open*.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.