

Mechanisms of plasticity of pancreatic β -cell mass

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ABSTRACT

Pancreatic β -cell mass exhibits plasticity under conditions of physiological metabolic challenge such as pregnancy, and in the presence of hyperglycemia and obesity. A failure of compensation can contribute to the onset of diabetes. Mechanistic models of β -cell plasticity can teach us much about the potential for targeted beta cell regeneration, and how this may alter with age. A key question is the identification of the cellular source(s) of β -cell expansion and, secondly, the nature of the stimuli that activate either a normally quiescent beta cell population or the differentiation of β -cell progenitors resident within the pancreas. Adult beta cells have a very low rate of proliferation, and the ability to generate new cells by neogenesis from pancreatic ducts, or by trans-differentiation from other islet endocrine cells, ceases shortly after birth. During metabolic stress, growth-arrested beta cells can re-enter the cell cycle due to a reduced expression of cell cycle inhibitory proteins, particularly in an immature population of β -cells that are normally resident in the small, but highly abundant, extra-islet endocrine clusters. These may contribute little to insulin release but represent a reserve of

proliferative plastic cells with multiple lineage potential from which to increase functional β -cell mass. The triggers to mobilization of β -cell progenitors and reactivation of β -cell proliferation include metabolic, endocrine and paracrine stimuli. Modulation of these pathways could represent exploitable strategies for the regeneration of β -cell mass to prevent or reverse diabetes.

KEYWORDS: pancreas, β -cell mass, plasticity, islet, neogenesis, growth factors, stem cells, diabetes, pregnancy

INTRODUCTION

Requirements for carbohydrate metabolism change with normal growth and development from birth to adulthood, as well as with physiological challenges such as pregnancy, feast and famine [1, 2]. Plasticity in β -cell mass is a physiological response to these physiological stressors, but also comes into play during pathological metabolic stress such as obesity [3, 4] and type 2 diabetes (T2D). A delicate balance of proliferation and apoptotic loss maintains β -cell mass during normal metabolic homeostasis. However, in humans there is histological evidence of β -cell turnover involving mitogenesis and apoptosis throughout life, in both children and adults with T1 or T2D [5-9]. The proliferation of remaining β -cells was identified in deceased patients with new onset T1D, but not in those with long-standing disease or T2D [10]. However, the regenerative capacity of human β -cells appears to be age dependent, since new cells were not generated in patients aged over 50 following

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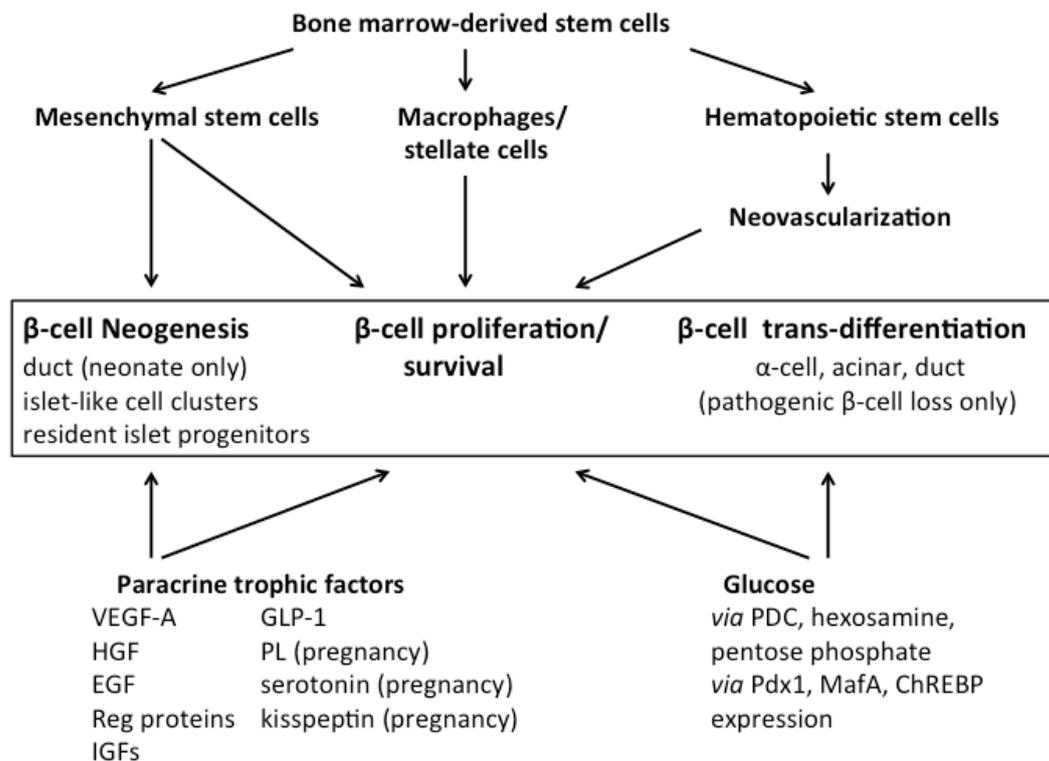


Figure 1. Summary of the mechanisms reported to contribute to β -cell plasticity in postnatal life resulting in an increased β -cell mass and/or function.

partial pancreatectomy [11]. Understanding the cellular and trophic mechanisms regulating the adaptability of β -cell mass is important for the potential design of preventative therapies for T1 or T2D, and potentially for regenerative tissue replacement. This review will focus on recent findings surrounding the potential cellular sources for the adaptability of β -cell mass, and on the likely physiological triggers for β -cell expansion (Figure 1).

A variety of animal models have been used to model β -cell plasticity postnatally. Partial pancreatectomy induced the expansion of both endocrine and exocrine pancreatic tissue [12, 13], while injection of streptozotocin (STZ) to selectively destroy β -cells in young rodents was shown to induce islet neogenesis as well as the expansion of remaining β -cells [14]. Pancreatic ductal ligation was followed by a doubling of β -cell mass in adult rats [15] resulting from both islet neogenesis and hypertrophy of existing β -cells [16]. However, if β -cells were first obliterated with STZ, then pancreatic duct ligation

failed to result in β -cell regeneration, suggesting that neogenesis from a non- β -cell source is not a major mechanism of renewal [17]. Surviving β -cells have been shown to spontaneously proliferate after cessation of cell-specific doxycycline-induced apoptosis by diphtheria toxin [18], supporting the concept that during regeneration β -cells are released from a tight suppression of cell replication. The increased β -cell mass that results from feeding a high fat diet to adult mice was associated with the expression of the regeneration gene, T cell factor 7-like 2 (TCF7L2) in small islet-like clusters (ICCs) [19]. Polymorphisms in TCF7L2 are associated with human T2D, suggesting a role for this gene in the homeostasis of β -cell mass [20]. When taken together, these models show that β -cell plasticity can occur in response to many physiological or pathological perturbations, but that there is no commonality in the cellular origins by which new β -cells might arise. This suggests that several separate mechanisms may occur, dependent on species and/or the severity of the metabolic insult.

Cellular origins of β -cell plasticity

Ontogeny of pancreatic β -cells

Islet endocrine cells and acinar tissue first develop from pancreatic epithelium, as demonstrated in both the rodent and human embryos [21-24]. The initial development of both lineages depends on the expression of key transcription factors such as pancreatic and duodenal homeobox 1 (Pdx1) and pancreas transcription factor 1 α (Ptf1 α) within the ductal cells [25]. Pdx1 is also required in the mature β -cell where it trans-activates the insulin and Glut2 gene promoters, and has recently been shown to actively repress genes characteristic of α -cells [26]. Other transcription factors including neurogenin-3 (Ngn3), Beta2/NeuroD, Pax-4 and Pax-6, and homeobox proteins Nkx2.2 and Nkx6.1 are necessary to complete the differentiation of individual endocrine cell lineages [27, 28]. Rodent islet β -cells are functionally immature at birth with poor glucose-sensitive insulin release [29], as are human fetal islets until close to parturition [30]. Functional maturation is accompanied by a down-regulation of the transcription factor, MafB, an up-regulation of MafA, and the expression of genes such as urocortin 3 [31, 32].

It has been proposed, and widely assumed, that a recapitulation of this ontogeny exists in postnatal life also to account for the development of new β -cells, starting with neogenesis from ductal progenitor endocrine cells, which give rise to small islet-like clusters (ICCs) that expand into mature islets [33]. However, this is countered by the findings that large islets form by fission from cord-like structures in the young mouse, with a core of β -cells and a mantle of α -cells, in addition to their expansion from ICCs [34]. The proliferation rate of insulin immunoreactive cells in mouse increases dramatically both in ICCs and islets up to 10 weeks of age, at which time 90% of the total postnatal insulin-expressing β -cells have already been generated [35]. Of these, over 90% are located in the ICCs, not within the mature islets. Following birth, most ICCs do not develop into larger islets; only ~250 ICCs, of the many thousands present in a single pancreas are able to grow into islets >200 μ m diameter [36] accounting for some 50% of the immediate postnatal gain in islet mass,

the remainder coming from intra-islet β -cell proliferation. The numbers of ICCs decline with age but remain at approximately 10/mm² after 1 year in both mouse and rat [35, 37]. Similarly, the abundance of ICCs in human pancreas is high, and slowly decreases with age [38]. In patients with T2D, a preferential loss of β -cell mass occurs from islets, predominantly located in the head of the pancreas, rather than from the ICCs [39]. Although the presence of ICCs is a common feature of both rodent and human pancreas, the architecture of the islets differs considerably between species. Unlike rodent islets where β -cells are located in the core, human islets have a more dispersed distribution of β - and α -cells throughout the islets and a different architecture of microvasculature [24].

Adaptive changes in β -cell mass postnatally can potentially arise from one or more cellular sources, including the replication of existing β -cells, neogenesis from pancreatic ducts, differentiation from resident pancreatic progenitor cells, or trans-differentiation from other functional pancreatic cell types.

β -cell proliferation

During postnatal growth ongoing β -cell proliferation in rodent and human islets requires the activation of the cell cycle genes, cyclin D1 and 2, and cyclin-dependent kinase 4 (Cdk4) [40, 41]. However, once adulthood is reached β -cell proliferation is rare, both in the normal and diabetic states [42-44]. Analysis of human islets from cadavers failed to demonstrate ongoing β -cell proliferation [45]. However, a low level of proliferation was found following the transplant of human islets into immune-deficient mice, which was enhanced by the glucagon-like peptide (GLP)-1 analog, exendin-4, although this declined with advancing age of the donor [46]. The resistance of functional β -cells to proliferate was thought to result from intrinsic cell cycle growth arrest, which progressed with age. This correlated with an age-associated increase in expression of the inhibitor of cell cycle progression, p16^{ink4a} [47]. The ability to expand beta-cell mass in mouse was associated with an expression of the polycomb protein Bmi1, which regulates p16^{ink4a}. Bmi1 knockout mice exhibit an increased expression of

p16^{ink4a} and do not undertake β -cell proliferation in response to exendin 4 [48]. A direct experimental reduction of p16^{ink4a} in β -cells maintained their proliferation, while over-expression of p16^{ink4a} caused a premature failure of β -cell proliferation [49, 50]. Endogenous p16^{ink4a} levels are inversely controlled by platelet-derived growth factor (PDGF) whose expression also declines with age in islets [51], whilst phosphatase and tensin homolog (PTEN) increases p16^{ink4a} activity and blocks β -cell proliferation [52]. Since the induction of cyclin D1 down-regulates PTEN *via* the activation of E2F transcription factors, a dynamic balance of β -cell proliferative capacity appears to exist with a resetting of the balance towards cell cycle arrest with advancing age.

The age at which β -cell proliferation normally ceases to be a significant mechanism for expanding β -cell mass is approximately 9-12 months in mouse and the early 20s in humans [53-55]. However, it was recently shown that adult, growth-arrested mouse β -cells grafted into young donors, but not older ones, were able to renew proliferation due to the presence of a circulating β -cell trophic factor in early life, and that this was independent of p16^{ink4a} expression [56]. This may be analogous to the circulating hormone, betatrophin that was recently described [57]. Betatrophin expression from the liver is a potent stimulus for β -cell proliferation in adult mouse, but was recently shown to have no effect on the proliferation of human β -cells within grafted islets [58]. Dor *et al.* [59, 60] showed that following partial pancreatectomy, re-population of β -cells within mouse islets occurred almost entirely by replication of existing β -cells. However, this study did not rule out the possible existence of resident β -cell progenitors within the islets. Thus, while differentiated β -cells do not normally continue to proliferate into adulthood, β -cell mass may be increased from within islets or ICCs during diabetes, and could be related to the presence of trophic factors.

Ductal neogenesis

The pancreatic ductal epithelium is a major source of new endocrine cells during intrauterine development, and it was shown by lineage tracking of carbonic anhydrase II-expressing ductal cells

that this continues for at least a month following birth in mice [61]. Conversely, cell lineage tracking of pancreatic duct cells marked by hepatocyte nuclear factor 1 homeobox β (HNF1 β) expression showed that no further endocrine cells were generated from ducts following birth, during normal development, or during subsequent induced β -cell regeneration [62]. Similarly, Kopp *et al.* [63] utilized an inducible SRY (Sex Determining Region Y)-Box 9 (Sox9) reporter construct in mice wherein 70% of pancreatic ductal cells were lineage labeled. They concluded that the ability of duct cells to give rise to either acinar or endocrine cells appears to decline rapidly postnatally, both in normal development and during β -cell regeneration following pancreatic duct ligation. Similar conclusions were drawn recently as a result of detailed pancreatic morphometry following pancreatic duct ligation [64]. Human pancreatic duct cells can adopt a β -cell phenotype *in vitro* following transfection with transcription factors such as Ngn3 [65], and histological analysis of human pancreas has demonstrated a lifelong presence of individual insulin-expressing cells in ducts and in the acinar compartment following the onset of diabetes [66]. However, it is not known if these isolated cells have the capacity to develop into functional islets *in vivo*. It appears, on balance, that the ductal epithelium is not a major source of new β -cells following birth, both during normal development and following the onset of diabetes.

Resident β -cell progenitors

Liu *et al.* [67] used a mouse model where β -cells were lineage-tagged with human placental alkaline phosphatase (HPAP) to show that β -cell progenitors did exist within the islets, but with little or no insulin expression, and that these proliferated following β -cell depletion with STZ treatment. Similarly, Szabat *et al.* [68] identified cells in mouse islets that were Pdx1-expressing but insulin-negative, and which co-expressed MafB, a marker of immature β -cells. These matured into β -cells *in vitro* expressing insulin, MafA and Glut2, or could remain as progenitors. Seaberg *et al.* [69] showed that multi-potential pancreatic progenitors did exist within adult mouse pancreas, but were rare. Subsequently, Smukler *et al.* [70]

reported that both the adult human and mouse pancreas contain multi-potent progenitor cells that expressed insulin. These generated progeny in both the pancreatic and neural lineages, and were distinct from mature, differentiated β -cells in that they exhibited a lower expression of insulin, higher levels of pancreatic progenitor markers (Ngn3, Pdx1 and Nkx6.1), and importantly they lacked glucose transporter 2 (Glut2) expression. Cells expressing Thy1.1 and CD133, but not endocrine hormones have also been identified in adult rat pancreatic ducts, and subsequently expressed Pdx1 and both insulin and glucagon following differentiation [71]. Similar dual insulin and glucagon-expressing cells have been identified in neonatal rat islets during β -cell regeneration following STZ treatment [72], and could represent resident endocrine cell progenitors. Recently it has been postulated that such progenitors are pancreatic stellate cells that can be distinguished by expression of the STP-binding cassette G2 (ABCG2) transporter, and are retained into adulthood [73]. Taken together, these reports support the existence of perhaps multiple β -cell progenitor types with lineage plasticity within both adult mouse and humans that can contribute to the generation of new, functional β -cells, but they do not identify a common anatomical location aside from being present in pancreatic islets.

Cellular trans-differentiation

Yet another potential source of new β -cells was described by Thorel *et al.* [74], who showed that after near-total β -cell loss, new cells could be generated by trans-differentiation from α -cells. However, this mechanism only appears to be utilized following extreme β -cell loss. Conversely, β -cells may be able to trans-differentiate directly to α -cells, a process that has been proposed to contribute to the reduction of β -cell mass in T2D [75]. Acinar cell trans-differentiation has been demonstrated by genetic cell lineage tracing in explants of mouse pancreas [76], and in mice treated with transforming growth factor- α [77]. Acinar cells were able to form ductal cells, but this did not progress further to β -cell differentiation. Similarly, no endocrine cells were shown to arise normally by trans-differentiation of acinar cells postnatally [62]. Human islets may form tubular

structures during T2D [78], which could be an intermediary step towards an attempted re-derivation of β -cells. However, this does not appear to represent a robust mechanism of β -cell regeneration.

In summary, adaptive changes in β -cell mass as a physiological response most likely involves a re-activation of normally quiescent, existing β -cells or differentiation from resident pancreatic β -cell progenitor cells either within the islets or located within the ICCs. Only after pathological β -cell loss would pancreatic cell trans-differentiation be utilized in adulthood.

The regulation of β -cell regeneration

Glucose metabolism

Since glucose-stimulated insulin secretion (GSIS) is essential for glucose homeostasis it is logical that persistent glycemic challenge would be mechanistically linked to β -cell mass. Induced hyperglycemia for 24 h resulted in an elevated insulin release from rat pancreas, but without any evidence of β -cell mitogenesis or a changed β -cell mass [79]. However, a longer exposure of 3-4 days, or recurrent hyperglycemia, resulted in an adaptive increase in β -cell mass [80]. Following the uptake of glucose by β -cells, facilitated by Glut2, and the generation of glucose 6-phosphate, catalyzed by glucokinase, glucose can enter three separate metabolic pathways. The major glycolysis pathway generates pyruvate, which is converted to acetyl-CoA driven by the mitochondrial multi-enzyme pyruvate dehydrogenase (PDH) complex (PDC) [81]. This results in a generation of ATP, an influx of Ca^{2+} by the opening of L-type Ca^{2+} channels and insulin secretion [82, 83]. A second pathway results in the hexosamine biosynthetic pathway [84], and a third, the pentose phosphate pathway. The latter generates NADPH, which can then act as a cofactor to facilitate GSIS [85].

In addition to GSIS, glucose induces gene expression in β -cells [86, 87] and glucose metabolism increases the binding of transcription factors important for β -cell replication and differentiation such as Pdx1, MafA, B2/E47 and carbohydrate response element binding protein (ChREBP) [88-90]. Whilst Pdx1 is essential for β -cell neogenesis prior to birth, it is not essential

for the generation of new β -cells postnatally, although it is important for their functional maturation and for maintaining a β -cell fate, which will determine the percent of β -cells within an islet [91]. Using an *in vitro* model of rat embryonic pancreas culture [92], glucose was shown to be crucial for both α - and β -cell development in islets by regulating the transition between Ngn3 and NeuroD transcription factor expression. Such actions may be mediated by PDC since we found that β -PDH knockout (KO) mice not only demonstrated impaired GSIS but a deficiency of β -cells postnatally, and a decreased expression of the genes Pdx1 and Ngn3 in islets [93]. In addition to a reduction in β -cell mitogenic activity, PDHKO mice showed a decreased abundance of insulin-positive extra-islet endocrine clusters in comparison to wild type animals [93], which represent a possible source of β -cell progenitors postnatally [94].

The importance of PDC action for islet and β -cell growth and maturation is consistent with reports linking glucose metabolic pathways to β -cell plasticity. A targeted deletion of the glucokinase gene in mouse β -cells not only abolished GSIS but also prevented β -cell proliferation, and was associated with a reduced β -cell survival [95]. Similarly, hormonal inhibition of the pentose phosphate pathway in rat islets with dehydroepiandrosterone reduced insulin secretion [96]. The pentose phosphate pathway generates NADPH, and an intermediate pentose sugar, xylulose 5-phosphate that subsequently leads to the activation of the transcription factor ChREBP [97]. ChREBP is expressed within pancreatic endocrine cells, and inhibition of ChREBP decreased β -cell differentiation [98]. The hexosamine biosynthetic pathway of glucose metabolism mediates changes in β -cell proliferation and differentiation status [99]. Thus, it appears that disruption of glucose metabolism within each of the major biosynthetic pathways within β -cells results in compromised β -cell mass as well as function, reinforcing the hypothesis advanced by Porat *et al.* [95] that glucose is a direct regulator of β -cell mass and plasticity.

Glucose may regulate β -cell mass through several cellular targets, including existing β -cells. Using a zebrafish model, acute increases in nutrient

availability resulted in an immediate entry of otherwise quiescent β -cells into the cell replication cycle as well as the differentiation of β -cell progenitors associated with the pancreatic ducts [100]. Regulation of cell cycle control genes by glucose within β -cells, such as Cdk2, was lost in a mouse model as the animals aged, perhaps contributing to the loss of β -cell plasticity that accompanies the aging process [101, 102]. In agreement with these findings, analysis of cell cycle-associated genes in mouse islets showed that glucose increased the expression of proliferation-associated transcription factors throughout life, but that the induction of cell cycle-associated genes was lost with age, even after the removal of cell cycle inhibitor proteins such as p16^{ink4a} [103]. Possible indirect effects of glucose include a direct control of vascular endothelial growth factor A (VEGF-A) secretion by β -cells [104], which can regulate the proliferation and survival of adjacent endothelial cells within the islets, which are obligatory for β -cell plasticity. However, prolonged hyperglycemia can result in islet inflammation and a decrease in VEGF-A expression [105]. Since endothelial cells arise from stem cell lineages within the bone marrow, it is not surprising that a presence of endothelial progenitor cells within the pancreas accompanies β -cell regeneration following pathologic loss.

Bone marrow-derived stem cells

Transplantation of bone marrow cells, or subfractions of marrow progenitor cells, has been shown by us and others to facilitate the reversal of diabetes in experimental animals, and newly diagnosed individuals with T1D [106-109]. In some studies a direct trans-differentiation of bone marrow stem cells into insulin-expressing cells was demonstrated, either *in vivo* or following *in vitro* lineage manipulation [110-112], but the ability of hematopoietic or mesenchymal stem cells from bone marrow to trans-differentiate into new β -cells is generally low, and is inconsistent with the resulting increase in insulin secretion and normalization of blood glucose [106, 113-115]. One mechanism of β -cell regeneration would appear to result from the differentiation of bone marrow-derived vascular progenitors into endothelial progenitor cells, present either as isolated cells infiltrating the islets and pancreatic ducts, or

becoming incorporated as endothelial cells into the micro-vasculature during neovascularization [106]. Neovascularization was accompanied by an increase in endogenous β -cells by replication, or the neogenesis of new islets from the pancreatic ducts [106, 113].

It is not clear which bone marrow-derived cells 'induce' β -cell regeneration. Yoder [116] postulated that bone marrow contained both pro-angiogenic hematopoietic progenitors of myeloid/monocyte lineage, and true endothelial progenitor cells that were of mesenchymal lineage. The former were hypothesized to function as paracrine supportive cells that induce vasculogenesis and tissue regeneration, but the majority would not form functional endothelial cells. In the context of β -cell regeneration these cells could contribute to the trophic interactions known to occur between vascular endothelium and β -cells. The mesenchymal stem cell component of bone marrow has been demonstrated to reverse hyperglycemia in experimental T1D through an induction of endogenous β -cell regeneration [117], but these cells may additionally trans-differentiate into insulin-expressing cells within the islets and ducts, the latter possibly representing islet neogenesis [118, 119]. Such resident cells within the islets express nestin and vimentin in addition to mesenchymal stem cell markers [120-122]. Bone marrow-derived mesenchymal stem cells possess a number of chemokine receptors that enable them to hone to the diabetic pancreas in response to chemokines present in islet cell extracts [123], to reduce inflammation during pancreatitis [124], to improve islet vascularization by endothelial cells [125], and to maintain islet morphology *in vivo* [126].

Most studies on the contribution of bone marrow-derived stem cells to β -cell survival or regeneration have transplanted cells bearing a genetic tag, such as green fluorescent protein (GFP), into irradiated recipient animals made diabetic with STZ, or into diabetes-prone animals such as the NOD mouse [127, 128]. As little as 1% allogeneic chimerism of repopulated marrow was able to reverse diabetes in the latter. However, in human pancreata from individuals who had previously received hematopoietic stem cell transplants from the opposite gender, there was no evidence of

colonization of transplanted cells within the islets [129]. The mobilization of bone marrow stem cells to colonize the pancreas and contribute to vasculogenesis appears to be dependent on pancreatic tissue damage in either the endocrine or exocrine compartments [130, 131]. The entire environment of the pancreas following β -cell loss, including bone marrow-derived cells, the remodeled extra-cellular matrix, and the cytokine/growth factor milieu, is likely to represent the combined elements necessary for effective β -cell regeneration.

In most papers hematopoietic or mesenchymal stem cells derived from bone marrow are not separated before utilization. We utilized transgenic mice expressing a yellow fluorescent protein (YFP) reporter under the control of the Vav gene promoter to label the hematopoietic cell lineage. The Vav gene is ubiquitously expressed by all hematopoietic lineage cells where it functions as a signal transduction molecule, remaining active on differentiated cell progeny including T cells, B cells and macrophages [132]. YFP-tagged cells were located within the pancreas at all ages, lining the pancreatic ductal epithelium as well as around and within the islets [133]. Small islets originating from the ductal epithelium were found to be completely lined by hematopoietic lineage cells, while larger YFP-tagged cells were found within the islets. Following STZ treatment, the abundance of hematopoietic lineage cells within islets and around ducts significantly increased, temporally corresponding with a recovery of β -cell mass, but they did not express any endocrine hormones. Approximately 30% of YFP-expressing cells co-stained with the endothelial cell marker CD31, and this significantly increased after STZ treatment, strongly suggesting that endogenous bone marrow-derived endothelial progenitor cells are involved in the recovery of β -cell mass. In contrast, mesenchymal stem cells, identified by CD44 localization, were more abundant in pancreas after β -cell damage with STZ treatment, but were found dispersed within the exocrine tissue and not within the islets (D. Hill, unpublished observations).

An alternative mechanism whereby bone marrow-derived stem cells could contribute to β -cell regeneration is through the generation of pancreatic macrophages. In the macrophage-deficient colony

stimulating factor 1 (CSF-1) knockout mouse (*op/op*), animals develop osteopetrosis as adults, but young animals demonstrated abnormal islet morphogenesis with a much-reduced β -cell mass [134]. Islet neogenesis at the pancreatic ducts was enhanced, suggesting that islets could be formed but the β -cell population cannot expand. Macrophages are functionally involved in the formation of β -cells in the embryonic pancreas, and this can be manipulated by exposure to CSF-1 *in vitro* resulting in a replication of macrophages and increase in β -cell number [135, 136]. They can also enhance islet angiogenesis following transplantation [137]. Tissue-resident macrophages have a distinct, non-inflammatory M2 profile of cytokine release, involving the expression of interleukin (IL)-4 and IL-13, and enhance tissue repair following damage [138]. Such cells associated with β -cell generation express the distinct surface markers ER-MP23 and MOMA-1 that are absent from pro-inflammatory macrophages [136], but may not express the classical macrophage marker F4/80.

Evidence supports a role of bone marrow-derived stem cells in the induction of β -cell regeneration through several possible mechanisms, including vasculogenesis. However, the islet microvasculature *in situ* also has a direct role in β -cell adaptation to metabolic or pathological stress.

Islet microvasculature and β -cell plasticity

Whilst bone marrow-derived stem cells can contribute to β -cell plasticity in part through the generation and actions of endothelial progenitor cells, the existing islet microvasculature is also crucial. The microvasculature of the islets is five times more dense than that of the pancreatic exocrine tissue [139], and vascular endothelial cells and β -cells are intimately juxtaposed such that one aspect of every β -cell is adjacent to an arterial capillary and another is adjacent to a venous vessel [140]. This applies to both rodent and human islets although the microvascular arrangement and drainage of the islets differ [141]. The islet capillaries are uniquely fenestrated allowing for rapid exchange of nutrient and hormonal signals from the circulation, and an ability to distribute secreted insulin rapidly [142]. A localized intercellular matrix exists between

β - and endothelial cells that is rich in type IV collagen and laminin, and allows for paracrine and integrin signaling between the cell types. An intact intercellular basement membrane is required for optimal β -cell proliferation and differentiation, while also providing key signals for β -cell growth and function [143, 144].

Paracrine actions within the islet include a synthesis of VEGF from the β -cells that contributes to endothelial cell homeostasis, while a reciprocal production of hepatocyte growth factor (HGF) by the endothelial cells promotes β -cell proliferation [145]. The release, but not the expression, of VEGF-A by β -cells is under the direct control of glucose [146]. However, the relationships are likely to be complex since a transient over-expression of VEGF-A within β -cells with euglycemia caused an increase in endothelial cell number, but a net loss of β -cells [147]. Once VEGF expression was returned to normal a compensatory proliferation of existing β -cells occurred, which was also dependent on the recruitment of marrow-derived macrophages to the islets. The effects of VEGF are also likely to be age-dependent, since a deletion of β -cell-derived VEGF-A severely impaired the gain of β -cell mass during development, whilst a targeted deletion in adult mice did not greatly alter β -cell number, but did severely reduce islet cell vascularity [148]. However, the scenario may differ when β -cell damage first occurs. We demonstrated that following treatment of young rats with STZ there was a decrease in islet capillary density in parallel with the loss of β -cell mass [149]. A regeneration of capillary density through angiogenesis preceded the regeneration of β -cells. In a model of remission of T1D in mice, treatment with bone marrow-derived endothelial progenitor cells first resulted in islet angiogenesis, followed by β -cell regeneration [150]. Similarly, in T2D early microvascular changes within the islets precede the onset of hyperglycemia [151]. A reciprocal signaling of endothelial cell-derived HGF upon the β -cell appears to be essential for their plasticity, at least in mouse. Adult mice bearing a targeted deletion of the HGF receptor, c-Met, were unable to regenerate β -cells following either STZ treatment or partial pancreatectomy, whilst in wild type animals where regeneration

occurred the endogenous levels of c-Met on β -cells were elevated following these insults [152].

Other paracrine mechanisms

A number of other paracrine pathways have been linked to adaptation of β -cell mass that are not directly linked to islet-capillary interactions. The Reg genes encode proteins related to the C-type lectin family originally identified in a cDNA library from rat regenerating islets [153]. There are seven separate Reg proteins in mouse, and five in humans and rats [154]. Reg2 peptides are found in mice and hamsters only, but are structurally related to the Reg1. In human embryos Reg1 expression coincides with early islet formation [155], and in mouse Reg1 and 2 genes become active coincident with the expression of insulin I and II [156]. Reg genes are up-regulated in pancreas following partial pancreatectomy, and after STZ treatment Reg1 expression is induced in β -cells and ducts [157-160]. Both Reg1 and 2 expression are increased in islets from female non-obese diabetic (NOD) mice, which develop T1D early in life [161, 162]. Reg2 expression is high in islets both during diabetes development, after adjuvant treatment in diabetic NOD mice, which causes β -cell regeneration, and in C57BL/6 mice made diabetic by STZ treatment [163]. Reg gene expression would therefore appear to be an important paracrine islet regenerative pathway, with Reg2 being selectively expressed in β -cells, Reg1 and Reg3 in both β - and α -cells, and Reg3 δ in the α -cells and pancreatic ducts [154, 164]. Reg1 activates cyclin D1 expression and β -cell proliferation through a phosphatidylinositol 3-kinase (PI3K)-mediated phosphorylation of the transcription factor ATF-2 [165]. Treatment of normal adult mice with mouse or human Reg3 peptides caused an increase in the mean size of ICCs due to cell proliferation and the appearance of increased numbers of insulin-expressing cells [166]. Whilst exogenous Reg proteins can cause an increase in β -cell number the extent to which the endogenous peptides contribute to β -cell plasticity is not clear.

The epidermal growth factor (EGF) receptor is essential for normal development of the endocrine pancreas, as are receptor ligands such as betacellulin [167, 168]. Mice expressing a mutant EGF receptor

failed to demonstrate an increased β -cell mass following feeding of a high fat diet or during pregnancy, demonstrating that EGF signaling is also required for β -cell plasticity in adult animals [169]. Another EGF receptor ligand, heparin-binding EGF, is also up regulated in β -cells during their proliferation following a prolonged high lipid-hyperglycemic challenge [170]. Whether this is entirely due to the proliferation of existing β -cells is unclear, since administration of EGF together with ciliary neurotrophic factor to STZ- or alloxan-diabetic mice also resulted in an acinar to β -cell trans-differentiation, associated with an expression of Ngn3 [171]. Transforming growth factor β (TGF β) also regulates β -cell cycle progression by regulating the intracellular distribution of the CDK inhibitor, p27 [172], and inhibition of signaling resulted in an increased β -cell proliferation. The TGF β receptor 2 is particularly important for TGF β action in islets acting *via* the Smad7 signaling pathway [173].

The insulin-like growth factors (IGFs) are expressed within the pancreas rudiments and within pancreatic β -cells during both rodent and human intrauterine development, and we and others have shown that changes in the relative expression of IGF-II *vs.* IGF-I within the pancreas during neonatal life control β -cell survival and functional maturation in rat [174-176]. Over-expression of IGF-II within islets grafted into diabetic rats resulted in an increased β -cell proliferation and a return to euglycemia, although insulin release per β -cell was unaltered [177]. A contribution of IGF-II to the development of T2D is supported by the findings that its expression, and that of the signaling receptor, IGF-IR, were reduced in pancreas of the Goto-Kakizaki diabetic rat model prior to an observed reduction in β -cell number, and that treatment with IGF-II was protective against the β -cell loss [178]. The above findings could potentially be explained by the potent anti-apoptotic actions of IGF-II, but a recent report suggests that IGF-II may also be involved in compensatory β -cell proliferation in the adult mouse. Using a model of targeted, inducible c-Myc expression in β -cells approximately 90% of these cells were destroyed, but were regenerated following cessation of c-Myc expression [179]. Regeneration was accompanied by a re-expression of IGF-II within β -cells, and was

prevented in animals where the IGF-II gene was inactive. It remains possible that the predominant actions of IGF-II in compensatory β -cell growth are to ensure cell survival, and that other paracrine mechanisms overlay this effect to induce the mitogenic response.

Changes in the local expression of IGF-II within mouse islets may also account for the potent anti-apoptotic actions of GLP-1 and its more long-lasting analog, exendin 4. GLP-1 applied to isolated mouse islets increased the expression of the IGF-IR, and the expression of IGF-II by β -cells, whilst blocking the expression of either receptor or ligand abolished the anti-apoptotic effects of GLP-1 [180]. Endogenous GLP-1 was demonstrated to contribute to the regeneration of β -cell mass following partial pancreatectomy in mice [181]; the action being dependent on the release of cells from cycle arrest as well as stimulatory pathways activated downstream of the G-protein-coupled GLP-1 receptor [182]. Although GLP-1 synthesis and release is normally restricted to the intestinal L-cells and the central nervous system, a local production from the proglucagon gene expressed within α -cells has been reported following STZ-induced diabetes in mouse [183], and following islet isolation [184]. This suggests that an activation of prohormone convertase 1/3 can occur in α -cells following β -cell damage, or following prolonged hyperglycemic challenge, to alter the post-translational processing of pre-proglucagon away from glucagon and towards GLP-1 synthesis, thereby contributing to β -cell regeneration, at least in mouse models.

In summary, islets operate a complex homeostatic expression of paracrine-acting growth factors and trophic molecules whereby changes in the relative balance of expression can result in profound changes in β -cell survival and/or proliferation. This balance can be modulated by prolonged glycemic challenge, or by physical injury and induced β -cell death within the islets. Whilst paracrine factors can be manipulated to reactivate entry of quiescent β -cells back into cell proliferation in rodent models, this may be more difficult to achieve in human islets.

Adaptation of β -cell mass during pregnancy

Pregnancy represents an excellent model of adaptive expansion of β -cell mass, and subsequent

regression post-partum, within a physiological context that is well defined and reproducible. Many of the mechanisms described above in a developmental, pathological or experimental context can be observed to contribute to an increase in maternal β -cell mass during the course of pregnancy. Whilst this has been well validated in animal models, an expansion in β -cell number also occurs during human pregnancy. Van Assche *et al.* [185] examined the pancreata of women who had died during third trimester or at parturition compared with those of age-matched, non-pregnant trauma victims. A doubling of fractional area of β -cells was observed during pregnancy. Butler *et al.* [186] examined the fractional area of β -cells in pancreata from women who had died during pregnancy *vs.* the non-pregnant state and found a 1.4-fold increase during pregnancy. The reduced adaptive expansion during pregnancy reported by Butler *et al.* [186] compared to van Assche *et al.* [185] may be explained by the pooling of data, with samples obtained at first trimester as well as later pregnancy. If the adaptive changes to the pancreas during pregnancy are sub-optimal then gestational diabetes can result [187].

Adaptation of β -cell mass during pregnancy is necessary because of the increasing maternal peripheral insulin resistance that occurs throughout gestation, driven by the rise in placentally-derived variant growth hormone (GH-V) [188]. GH-V also suppresses maternal pituitary growth hormone and becomes the major regulator of an elevated circulating IGF-I. Placental lactogen (PL) release from the placental syncytiotrophoblasts into maternal blood also promotes insulin resistance, and the combination of GH-V and PL causes hepatic gluconeogenesis and lipolysis to maximize nutrient availability for the growing fetus. This tendency towards maternal hyperglycemia is compensated by the ability of PL to expand β -cell mass, predominantly through a re-activation of mitogenesis, at least in rodents. Interestingly, the adaptive β -cell changes occur independently of the presence of fetuses in a mouse model, demonstrating that the process is driven entirely under maternal control [189]. The actions of PL, and other hormones, such as estrogen, on maternal β -cells have been reviewed in detail by Rieck and Kaestner [190].

Mechanisms of action of placental lactogen

The increase in maternal β -cell mass through pregnancy in rodents correlates with the appearance and rise of PL [191], and targeted over-expression of PL in β -cells resulted in an increased proliferation [192]. PL interacts with the prolactin receptor on β -cells, and receptor deletion results in a failure of compensatory β -cell proliferation, impaired insulin release and glucose intolerance [193, 194]. Conversely, prolactin receptor over-expression caused overgrowth of β -cells [194]. Prolactin receptor signaling occurs through a number of second messenger pathways, including an association with Jak2 resulting in the activation of Stat5 [195], mitogen-activated protein kinases (MAPK), and PI3K leading to the activation of Akt [196]. In the mouse this leads to an increased rate of β -cell mitogenesis resulting in a 2-3-fold increase in β -cell mass, peaking at around gestational days 13-15 (term 19 days) [197]. Expression of the prolactin receptor and the cell cycle regulator Cdk4 were also elevated [198], as was islet vascularization [199], reinforcing the close relationship between micro-vasculature and β -cells during islet growth. An inhibition of β -cell apoptosis and potentiation of mitogenesis is also likely to be aided by the elevated levels of IGF-I found during pregnancy. Beta cell adaptation in the mouse may not depend solely on the proliferation of existing β -cells, since lineage marking of such cells showed a dilution of label within new β -cells that indicates some generation from progenitor cells [200]. Involution of β -cell mass back to pre-pregnancy levels occurs in rodents within 10 days after parturition, largely by apoptosis [201], and correlates with a reduction of circulating IGF-I post-partum.

The activation of Stat5 by placental lactogens and/or prolactin within β -cells of pregnant rodents induces B-cell lymphoma 6 (Bcl6) gene expression, which is a transcriptional repressor of the tumour suppressor gene, menin 1. Islets bearing a deletion of the prolactin receptor demonstrated an increased expression of menin 1 during pregnancy [202]. A specific down-regulation of menin 1 within islets during pregnancy resulted in an inhibition of p18 and p27, and released β -cells from cell cycle quiescence [203]. The down-regulation of menin 1 is rate-limiting to compensatory islet growth

since a targeted over-expression within mouse maternal β -cells prevented their proliferation and resulted in glucose intolerance [203]. It is unclear if Stat5 activation is the sole mechanism responsible for a down-regulation of menin 1 in β -cells since glucose alone can suppress menin 1 *in vitro* [204]. The actions of glucose were dependent on signaling *via* the PI3K/Akt pathway.

The actions of at least two transcription factors are necessary for compensatory β -cell growth to occur: hepatocyte nuclear factor-4 α (HNF-4 α), which activates the Ras-Erk1/2 kinase mitogenic pathway [205], and forkhead box protein M1 (FoxM1), which is activated downstream of the PI3K signaling pathway, suppresses menin 1 levels, and activates apoptosis-inhibiting factors such as baculoviral IAP repeat-containing 5 (Birc5) [206, 207]. FoxM1 is highly expressed within islets during fetal and neonatal endocrine pancreas development, and β -cell mass is reduced if FoxM1 is silenced [208].

Changes to the islet paracrine environment during pregnancy

Changes to the paracrine milieu of the islets have also been reported during pregnancy. A genomic study of islet adaptation to pregnancy in rats identified a 2.5-fold increase in the expression of Reg3a and a 3-fold increase in EGF expression [209]. Hepatocyte growth factor is obligatory for β -cell expansion in the pregnant mouse since targeted pancreatic deletion of the HGF receptor, c-Met resulted in a failure of adaptive β -cell proliferation and an increased rate of apoptosis [210]. This was associated with a reduction in the levels of prolactin receptor mRNA in islets, a reduced activation of Stat5, a lower expression of FoxM1, and a failure to suppress p27, all of which suggest that HGF is necessary for a PL-initiated mitogenic response by β -cells. The dams lacking c-Met expression became hyperglycemic with impaired glucose tolerance.

Placental lactogen actions *via* the prolactin receptor also result in an increase in islet levels of serotonin, which has been linked to an increase in GSIS in addition to increasing β -cell mass. This is mediated, at least in part, through an increased expression of the serotonin synthesizing enzymes, tryptophan hydroxylases 1 and 2 during pregnancy,

resulting in an increase in the islet content of serotonin [211]. Serotonin regulates glucose-stimulated insulin release through the activation of the 5-hydroxytryptamine (5-HT) 3a and b receptors, whilst the ability of serotonin to increase β -cell mass is mediated by HTr2b in mid-gestation in mouse and HTr1d in late gestation [212]. Consequently, animals null for HTr3a demonstrated glucose intolerance during pregnancy despite undergoing an adaptation of β -cell mass [213].

Kisspeptin

Whilst placental lactogen and changes to paracrine growth factor expression within the islets of Langerhans could account for the adaptation in β -cell mass that occurs during pregnancy, they do not appear to directly enhance GSIS. However, this role may be achieved by a novel group of peptides, the kisspeptins. Kisspeptins represent a family of post-translationally modified proteins expressed from the *kiss1* gene of which the amidated kisspeptin-54 form, also known as metastin, is the most abundant [214]. Although originally described as a tumour suppressor, kisspeptin has been shown to play a central permissive role in regulating the release of gonadotropin-releasing hormone (GnRH) from the hypothalamic neurons implicating a function in the initiation of puberty [215]. The receptor for kisspeptins has recently been identified as the 7-pass G-protein-coupled receptor (GPCR), GPR54, whose sequence shares homology (35-45%) to the galanin receptor family [216], a known neuropeptide receptor that exerts inhibitory actions on insulin release [217]. The mechanism of action of GPR54 involves coupling to the $G_{\alpha q}$ subunit, leading to an activation of PLC- β and a subsequent increase in intracellular Ca^{2+} and the activation of protein kinase C (PKC) [218, 219]. Chinese hamster ovary cells transfected with human or rat GPR54 also showed a strong activation of the MAP kinases, ERK1 and 2, suggesting the activation of mitogenic pathways [214], and consistent with a role for kisspeptin in placental growth.

Both kisspeptin and GPR54 are expressed by the placental trophoblasts, and kisspeptin is released into the maternal circulation [214, 220]. The molecular form of kisspeptin synthesized within

the placenta is kisspeptin-10, which represents the common C-terminal peptide sequence shared by all kisspeptin 1 (KISS1) derivatives, and is the shortest sequence capable of activating the GPR54 receptor [221]. Peak mRNA levels for both KISS1 and GPR54 occur in the first trimester coincident with the peak of trophoblast invasiveness [220], and expression levels are reduced in pregnancies associated with small of gestational age infants, pre-eclampsia or T1D [222, 223]. Circulating levels of kisspeptin are normally low in humans but increase substantially during pregnancy and a peak concentration of 20 nM or more was observed in the third trimester [222, 224]. The increase in kisspeptin levels with the progression of pregnancy suggests that the peptide might also have effects on maternal metabolism in addition to paracrine actions within the placenta.

In addition to the placenta, both kisspeptin and the GPR54 receptor have also been reported to be expressed within pancreatic islets of Langerhans of mouse and humans, as well as within mouse MIN6 β -cell and the α TC1 α -cell lines [225]. Furthermore, immunopositive staining has identified both ligand and receptor to be co-localized with insulin and glucagon, confirming expression by both the α - and β -cells, but not the exocrine tissue [225]. The intravenous administration of kisspeptin has recently been shown in rodents to cause a prompt, four-fold increase in plasma insulin, which persisted for 90 min [226], with similar findings in rhesus monkey [227]. Similar effects have been shown *in vitro*, whereby direct administration of exogenous kisspeptin was found to potentiate GSIS by approximately 3-fold from isolated mouse islets during perfusion, acting on both first and second phase insulin release [226]. Similar findings were observed in response to both kisspeptin-10 and -13 following the perfusion of mouse, rat, piglet and human islets [228], but not the MIN6 β -cell line [225]. The active levels of kisspeptin for promoting GSIS *in vitro* were found to be 100 pM-1 μ M [226], only marginally less than the circulating maternal levels in the third trimester, suggesting that placental production may augment the effects of islet-derived kisspeptin on GSIS. These findings, however, have been difficult to replicate using different methodologies, as others have reported that kisspeptin-10, -13 or -54 inhibited GSIS following

whole pancreas perfusion or static culture of isolated islets [229-231]. These inhibitory effects were consistently seen at lower concentrations of kisspeptin, and may indicate that a sustained presence of kisspeptin is detrimental to insulin release, whilst a transient presence is stimulatory. Kisspeptin had no effect on glucagon release from isolated mouse islets [225], although glucagon negatively regulated kisspeptin expression in liver [231].

The ability of kisspeptin to promote GSIS from isolated rodent islets was inhibited by specific inhibitors for PLC and p42/44 MAPK, whilst inhibitors against p38 MAPK, and diacylglycerol-dependent PKC isoforms had no effect [228]. This would indicate that kisspeptin is unlikely to promote β -cell proliferation *via* p38 MAPK and that the activation of GSIS is likely to involve PLC and a mobilization of intracellular Ca^{2+} . Indeed, kisspeptin had a rapid stimulatory action on intracellular Ca^{2+} flux within mouse islets [228]. In summary, a paracrine effect of kisspeptin within the pancreas may contribute to the increased demand for GSIS during pregnancy, and could complement the actions of hormones such as placental lactogen in increasing β -cell mass. Hypothalamic studies suggest that the kisspeptin/GPR54 axis responds to reduced nutrition by lowering kiss1 expression and delaying the onset of puberty and reproductive function [232]. As such, it is possible that kisspeptin may play a similar metabolic modulatory role in pregnancy whereby ligand derived from the placenta could enhance maternal GSIS *via* GPR54.

Beta-cell adaptation during human pregnancy

The extent to which the mechanisms underlying an adaptive change in β -cell mass during human pregnancy are similar to those revealed from rodent models needs to be examined critically. Changes in the presence of growth factors known to stimulate β -cell proliferation and suppress apoptosis do occur in the human maternal circulation, such as HGF [233] and IGF-I [234]. However, Butler *et al.* [186] using *post mortem* samples found no change in β -cell mitotic index or the relative area of β -cells per islet between pregnant and non-pregnant states, but did observe an increase in the numbers of isolated insulin-expressing cells scattered throughout the acinus

and associated with the pancreatic ducts. This would argue in favour of neogenesis of β -cells from progenitors as a preferred mechanism rather than the re-entry into the cell cycle of existing quiescent β -cells. This conclusion is supported by the reappearance of C-peptide during pregnancy in a cohort of 90 pregnant women with pre-existing T1D with mean duration of disease of 17 years [235]. Since little residual β -cell mass would be expected to exist in these women the appearance of endogenous insulin production suggests that a degree of regeneration occurs, and that perhaps this is derived from progenitors as well as the replication of residual β -cells. Differences exist in the regulatory control of the β -cell replicative cycle between rodents and humans since in mouse Cdk4 is a key regulator whose expression is increased in the presence of PL, and disruption of Cdk4 results in cell cycle arrest. However, in human islets Cdk4 and 6 are equally expressed [236] and it is possible that cell cycle kinetics in β -cells during pregnancy is less responsive to changes in pregnancy-associated hormones than in rodents.

Beta-cell regeneration is intimately associated with the microvasculature, at least in rodents, and can be induced by grafting of endothelial progenitor cells. Endothelial progenitor cell presence in the human maternal circulation increases throughout the course of pregnancy, and is correlated with increasing levels of serum estradiol [237, 238]. In mouse the proliferation rate of bone marrow hematopoietic stem cells, of which endothelial precursors are a sub-set, is enhanced by estrogen [239]. Women with gestational diabetes or impaired glucose tolerance during pregnancy demonstrated a reduced number of circulating endothelial progenitor cells [240]. Whether endothelial progenitor cell presence is a stimulus to adaptive changes to human β -cell mass during pregnancy remains to be demonstrated. In summary, whilst adaptive changes in β -cell mass clearly occur during human pregnancy, the balance of mechanisms responsible may differ from that in rodents.

CONCLUSION

Information gained from rodent models on the adaptation of β -cells to metabolic stress show that a predominant mechanism for the generation of

new β -cells is by relaxation of the normally quiescent state of pre-existing β -cells, allowing increased mitotic activity. However, the generation of new β -cells from resident progenitors located within the ICCs and islets can also occur. A major stimulus to such adaptations is persistent glycemic challenge and direct actions of glucose, although an activation of trophic paracrine pathways by hyperglycemia can also occur. Only following a pathological loss of β -cells could other mechanisms such as ductal neogenesis or pancreatic cell trans-differentiation be theoretically engaged for β -cell renewal. An expansion of endogenous β -cell mass can be induced experimentally by the introduction of bone marrow-derived stem cells of either mesenchymal or hematopoietic origin, with a mechanism of action that involves vasculogenesis and modulation of the important and mutually supportive signaling environment between endothelial cells and β -cells. Several of these mechanisms may contribute to the adaptive increase of β -cell mass during pregnancy where a major initiating signal is the presence of PL.

In humans, relative differences in cell cycle gene abundance compared to rodents may make a reactivation of β -cell mitogenesis less effective than in rodents, such that an increase in β -cell mass may depend more on differentiation from resident progenitor cells. However, the 2-fold increase in β -cell mass observed in late human pregnancy demonstrates that adaptability exists in adulthood, and could potentially be therapeutically manipulated to improve metabolic control.

OUTSTANDING QUESTIONS

- What is the predominant cellular source of new β -cells in humans responsive to 'physiological' metabolic challenges such as obesity and pregnancy?
- How might the cellular source of new β -cells differ in humans under pathological metabolic stress such as type 2 diabetes?
- Of the numerous paracrine regulators of β -cell proliferation and survival described in adult rodent models of β -cell regeneration, which are relevant to humans with the potential to be regenerative agents?

- What are the mechanisms by which bone-marrow-derived stem cells of mesenchymal or hematopoietic lineage induce β -cell regeneration in diabetic conditions?
- Can modulation of intracellular suppressors of β -cell cycle progression be used to expand human β -cell number?

GLOSSARY

ATP binding cassette (ABC) transporter G2 (ABCG2): a 72-kDa transporter protein first identified as a breast cancer resistance protein. ABCG2 represents half of a transporter unit consisting of six transmembrane domains and must form homodimers to be functional.

Carbohydrate response element binding protein (ChREBP): a glucose-dependent protein that interacts with the carbohydrate response element sequences of DNA. De-phosphorylation causes the trans-location of ChREBP from the cytoplasm to the nucleus where it is able to activate expression of enzymes involved with both carbohydrate and lipid metabolism.

Cellular trans-differentiation: the process whereby one differentiated cell type can convert into another, usually within the same ontological lineage. This may require an intermediary less differentiated phenotype.

Deoxycycline: a variant of the antibiotic tetracycline that is frequently used to activate recombinant gene expression in transgenic mouse models.

Glucagon-like polypeptide-1 (GLP-1): an 'incretin' hormone generated by differential post-translational processing of the proglucagon gene product. It is synthesized predominantly in the intestinal L-cells and is released into the circulation in response to a rise in intestinal carbohydrate and lipid content. GLP-1 enhances glucose-stimulated insulin release from the pancreatic β -cells, and in rodents also has mitogenic and anti-apoptotic effects. The biologically active forms are GLP-1-(7-37) and GLP-1-(7-36)NH₂.

Neogenesis: the process whereby new, differentiated cell types are derived from un-differentiated resident stem cells or progenitors.

Neovascularization: the formation of a new microvascular bed capable of the perfusion of

blood without the need to be generated from existing capillaries.

p16^{INK4a}: a tumour suppressor protein encoded by the human CDKN2A gene. p16 is involved with cell cycle regulation by preventing the progression of cells from G1 to S phase of the cell cycle. It is an inhibitor of cyclin-dependent kinases such as Cdk4 and Cdk6. Mutations in the CDKN2A gene are associated with various tumours.

Pyruvate dehydrogenase complex (PDC): a mitochondrial enzyme complex able to metabolize pyruvate to form acetyl-CoA. Further metabolism to citrate generates ATP and is necessary for glucose-stimulated insulin secretion in pancreatic β -cells.

Streptozotocin (STZ): a glucosamine-nitrosourea molecule that damages DNA and is cytotoxic, and binds to the glucose transporter 2 (Glut2) with high affinity. Since Glut2 is abundant on rodent β -cells STZ can be used to selectively destroy β -cells as a model of type 1 diabetes. Titration of STZ can result in total or sub-total β -cell destruction.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest with regard to any of the statements and opinions included within this paper.

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