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Weighing up beta-cell mass in mice and humans: self renewal, progenitors or stem cells?

Neil A. Hanley^{1,2,*}, Karen Piper Hanley^{1,2}, Päivi J. Miettinen³, Timo Otonkoski^{3,*}

¹Centre for Human Development, Stem Cells & Regeneration; ²Human Genetics Division, University of Southampton, Southampton, UK.

³Biomedicum Helsinki and Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland.

***Corresponding authors:**

Neil A Hanley

Human Genetics Division, Duthie Building, Mailpoint 808,

Southampton General Hospital, Tremona Road, Southampton, SO16 6YD, UK

Email: N.A.Hanley@soton.ac.uk

Timo Otonkoski

Biomedicum Helsinki

Room C507b, P.O. Box 63, FIN-00014

University of Helsinki

Helsinki, Finland

Email: timo.otonkoski@helsinki.fi

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Abstract

Understanding how beta-cells maintain themselves in the adult pancreas is important for prioritizing strategies aimed at ameliorating or ideally curing different forms of diabetes. There has been much debate over whether beta-cell proliferation, as a means of self-renewal, predominates over the existence and differentiation of a pancreatic stem cell or progenitor cell population. This article describes the two opposing positions based largely on research in laboratory rodents and its extrapolation to humans.

Key words: human, mouse, pancreas, stem cell, hormone, beta-cell, diabetes

Abbreviations: PDX1, pancreas duodenal homeobox factor 1; NGN3, neurogenin 3; PAX4, paired box factor 4; NKX2.2, NK homeobox factor 2; IA1, insulinoma associated 1; EGF, epidermal growth factor 1; PMP, pancreatic multipotent precursor; LIF, leukaemia inhibitory factor; GLP-1, glucagon-like peptide 1, CDK, cyclin dependent kinase; FOX, forkhead homeobox transcription factor; PITX2, paired-like homeodomain factor 2; WNT, wiggless-type MMTV integration site family; MAPK, mitogen-associated protein kinase; HNF, hepatocyte nuclear factor; BrdU, bromodeoxyuridine; PTEN, phosphatase and tensin homologue; MEN, multiple endocrine neoplasia; MET, mesenchymal-to-epithelial transition; DPP-IV, dipeptidyl peptidase IV

1. Introduction

In recent years few debates have divided the fields of pancreas development and diabetes research like the one on how the body maintains beta-cells during post-natal life. The beta-cell is responsible for insulin secretion, which regulates blood glucose levels. It is the cell-type that is functionally lacking in diabetes, a disorder defined by elevated blood glucose and commonly diagnosed by its measurement after fasting or after the administration of an oral glucose ‘challenge’. The total population of beta-cells in the pancreas has been termed ‘beta-cell mass’ (Bouwens and Rooman, 2005). The shortfall in beta-cell mass is absolute in type 1 diabetes, where beta-cells have been destroyed by an autoimmune process. In type 2 diabetes the deficiency is relative, although functionally inadequate to maintain blood glucose levels within the normal range. Accordingly, understanding how beta-cell mass is maintained during life is important, not just as a biological conundrum, but as a forerunner for minimizing beta-cell loss and devising the best strategy for beta-cell regeneration as potentially curative treatment of diabetes. Nevertheless, precise regulation is critical: too much could be as dangerous as too little. Excessive amplification of beta-cells could potentially be associated with disproportionate insulin release and cause life-threatening hypoglycaemia.

Compared to organs such as the intestine and skin, covered elsewhere in this issue of *Molecular & Cellular Endocrinology*, the potential existence of a stem cell to maintain adult pancreatic cell lineages has proven highly contentious. In this article, we discuss two broadly opposing viewpoints: the differentiation of new beta-cells from stem cells or progenitors versus maintenance of beta-cell mass by proliferation of pre-existing mature cells. The discussion is timely as recent years have seen a shift in stance for many researchers. During the later twentieth century, a significant dataset gathered, supporting a progenitor cell population able to give rise to new beta-cells, a process often termed ‘neogenesis’. However, a special underlying stem cell has never been identified and skepticism persists over potential progenitor sources. By the turn of the millennium, the mouse genetics ‘revolution’ had

created an ever greater technical ability to mark cells indelibly and trace their lineage *in vivo* under very specific circumstances or according to developmental origin. For many, these latter experiments provided incontrovertible proof for the second viewpoint; namely, that the adult beta-cell population perdures close to entirely, if not entirely, by self-renewal. However, the debate persists with very recent data in mice reinvigorating arguments in favour of neogenesis; and of course, there still remains the unsubstantiated extrapolation from proof in laboratory animals to regenerative therapy in diabetic patients.

In attempting to reconcile these opinions, the debate can be distilled, somewhat provocatively, into two components: there might be misinterpretations or over-interpretations by one side or the other (or both) as to the predominant mechanism in rodents; superimposed on which, there may also be a species difference between mouse and human as to the ‘preferred’ regenerative mechanism. To resolve the former, time and ongoing research should provide the answers; for the latter, it is worth considering why and how rodent models might cease to mimic human physiology and pathophysiology. The regulation of mammalian beta-cell mass has been the subject of some excellent, highly comprehensive reviews elsewhere (Bouwens and Rooman, 2005; Heit et al., 2006). The following sections focus on the central reasoning behind the differing viewpoints and introduce an additional concept that may underlie an inter-species difference.

2. Potential sources for *in vivo* beta-cell regeneration

Within the postnatal pancreas, various cell-types have been investigated, mainly in rodents, as the potential source for beta-cell renewal *in vivo* (Fig. 1).

2.1. Differentiation of islet stem cells or progenitors residing within the ductal epithelium

For many researchers, cells within the pancreatic ducts possess a capacity for regenerating beta-cells both *in vitro* and *in vivo*. At its most simplistic level, theorizing on the potential of ductal progenitors has been encouraged for two reasons.

Within the adult human pancreas, islets may lie close to the ducts and can even appear connected to them, and single insulin-positive cells can be observed within an intra-ductal location (Bouwens and Pipeleers, 1998). This creates the impression of an islet birth or budding process emanating from the ducts. Morphologically, the budding of islets from ducts mirrors normal development. It seems reasonable in pre-supposing that regeneration might recapitulate embryonic and fetal development. *In utero* a series of transcription factors enacts differentiation of multipotent pancreatic progenitors, capable of exocrine, ductal or endocrine differentiation, to terminally differentiated islet cell lineages, including beta-cells (Murtaugh, 2007). This process sees the movement of cells out of the duct-like structures into the pancreatic mesenchymal stroma. Unlike conjecture over beta-cell neogenesis in the adult pancreas, firm evidence for the developmental pathway comes from a series of mouse knockout and transgenic analyses. The embryonic pancreas is composed of branching ductal-like structures made up of epithelial cells that express the transcription factor, Pancreas-duodenal homeobox 1 (Pdx1) (Murtaugh, 2007). A subset of these cells within the centre of the embryonic pancreas commit to an endocrine cell fate by expressing Neurogenin 3 (Ngn3), a basic helix-loop-helix transcription factor (Schwitzgebel et al., 2000). Lack of Ngn3 leads to an absence of islets (Gradwohl et al., 2000); its ectopic expression over-commits cells prematurely to an endocrine cell fate (Apelqvist et al., 1999). A series of further transcription factors lie downstream of Ngn3 to differentiate endocrine precursors to beta-cells. Characterization of promoter and enhancer regions has demonstrated direct regulation by Ngn3 of target genes, such as *NeuroD1*, *Paired domain homeobox gene 4 (Pax4)*, *NK family member Nkx2.2* and *Insulinoma associated 1 (Ia1)*, all of which when inactivated impair differentiation,

downstream of Ngn3, to a beta-cell fate (Huang et al., 2000; Mellitzer et al., 2006; Smith et al., 2004; Sosa-Pineda et al., 1997; Sussel et al., 1998). Ectopic expression of NGN3 in adult ductal cells, either carcinoma cell lines or preparations of fresh adult pancreatic cells, has also been shown to recapitulate something similar to this normal beta-cell differentiation programme enacted during fetal development (Gasa et al., 2004; Heremans et al., 2002).

If, under normal circumstances, there is a balance between regulating beta-cell mass in order to maintain euglycaemia, artificially reducing beta-cell mass should provoke mechanisms that try to restore the equilibrium. Several models have tested this hypothesis and determined that ductal cells can act as islet precursors (Bouwens and Rooman, 2005). The most straightforward method for reducing beta-cell mass is surgical: partial pancreatectomy in rats stimulates limited re-growth of the remnant organ. The process is less robust than for the liver and appears proportionate to the scale of the excision. For a marked growth response, near total (>90%) removal is necessary, a measure which also induces hyperglycaemia (Bouwens and Rooman, 2005). Alternatively, when given to animals, streptozotocin is selectively taken up by beta-cells and alkylates target cell DNA leading to cell death. Combining these two models provides a surgical stimulus to re-growth with chemical ablation of remaining beta-cells. When this is performed, beta-cells reappear, a finding which has been interpreted as evidence of differentiation from precursors (Finegood et al., 1999). Furthermore, their appearance adjacent to ducts is persuasive that cells from the latter location serve as the source of precursor cells. Alloxan is another beta-cell toxic agent, which causes a rapid destruction of beta-cells by generating reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration. The administration of streptozotocin or alloxan without surgical resection is also instructive. If a dose is injected that is insufficient to eliminate all beta-cells, analysis can be made of those that remain. Calculations have indicated that proliferation from these 'escapee' beta-cells appears insufficient to account for the observed regeneration implying neogenesis from precursors (Bonner-Weir et al., 1981).

Another intervention is to ligate the pancreatic duct, which results in local inflammation and stimulates ductal cell proliferation (Bouwens and Rومان, 2005). Alongside ductal cell proliferation there is an increase in insulin-positive cell number that is unaccounted for by proliferation of existing beta-cells (Wang et al., 1995). Similar results have been obtained using adenoviral mediated ductal overexpression of epidermal growth factor (EGF) receptor (EGFR) ligands betacellulin and heparin-binding EGF (HB-EGF). In these models both beta-cell proliferation and neogenesis of insulin producing cells from ducts has been reported together with amelioration of alloxan-induced diabetes (Kozawa et al., 2005; Tokui et al., 2006). Interestingly, using the above mentioned models, data on *Ngn3* are conflicting. Some have reported the transcription factor not to be involved (Lee et al., 2006), whereas others have detected its expression during regeneration after streptozotocin (Kodama et al., 2005). This latter presence is very persuasive that pancreatic regeneration from progenitor to beta-cell is real and mimics the same pathway followed during normal development. Recently, the case for *Ngn3* in facilitating beta-cell regeneration from ductal cells within the adult mouse pancreas has gained further backing (Xu et al., 2008). Ligation of the duct in the pancreatic tail resulted in some beta cell proliferation but, more strikingly, a large upregulation of *Ngn3* expression that was required at least in part for the accompanying beta cell hypertrophy. The *Ngn3* was in cells very closely associated with ducts, quite possibly in the ductal lineage itself. These cells could be isolated and used to reconstitute hormone-positive cell-types in explant cultures of *Ngn3*^{-/-} embryonic pancreas; thus, taken together, pressing the case that there are endocrine precursor cells within (or very close to) the pancreatic duct in adult mice (Xu et al., 2008).

2.2. The potential for non-ductal islet stem cells / progenitors

A variety of other cell-types within the pancreas, or cells for which no clear lineage is apparent, have been described as potential precursors for islet cells. For most of these cell-types, the data arise from *in vitro* culture and expansion of cells isolated from the adult pancreas. Some of these sources

seem unlikely to be significant contributors *in vivo*. Ngn3 is expressed only in epithelial cells that previously expressed Pdx1 (Gu et al., 2002; Schwitzgebel et al., 2000). The total loss of the endocrine lineage in the absence of Ngn3 (Gradwohl et al., 2000) makes it unlikely that mesenchymal-to-epithelial (MET) transition is significant *in vivo* as a mechanistic source of new beta-cells. Others have identified cells capable of *in vitro* differentiation that to date have lacked obvious markers of recognizable pancreatic cell-types. Termed pancreatic multipotent precursors (PMPs), these cells were able to differentiate into progeny that secreted insulin in response to a glucose challenge *in vitro* (Seaberg et al., 2004). However, seemingly at odds with normal pancreas development, PMP cells also formed neural cell lineages. Although emanating from different germ layers, there is a clear transcriptional overlap between neural and islet cell differentiation (Edlund, 2002; Wilson et al., 2003). There are convincing data on neural differentiation from progenitor cells via expression of the intermediate filament protein, Nestin (Lee et al., 2000). Nestin-positive cells have also been identified within adult rat islets and were capable of differentiating into insulin-positive cells *in vitro* (Zulewski et al., 2001). Although differing results have been published on Nestin expression during mouse pancreas development (Bernardo et al., 2006; Selander and Edlund, 2002), our group did not observe its expression during development of the human pancreatic epithelium (Piper et al., 2002) and lineage tracing experiments in mice imply that Nestin-positive cells contribute to the vasculature but not the endocrine lineage (Treutelaar et al., 2003). For other tissues, bone marrow cells have been advocated as a source of replenishable progenitors. Insulin positive cells have been differentiated *in vitro* from cells isolated from the umbilical cord (Yoshida et al., 2005) while a more provocative report details the appearance of green fluorescent protein (GFP)-positive beta-cells in the pancreas of mice post-bone marrow transplant (Ianus et al., 2003). Such studies require corroboration by others as, presently, significant cynicism remains regarding non-ductal, non-beta-cell stem cells or progenitors residing

within the pancreas and giving rise *in vivo* to functional beta-cells (Choi et al., 2003; Taneera et al., 2006).

2.3. The potential for acinar transdifferentiation to beta-cells

Transdifferentiation is the conversion of one mature cell phenotype to another (Li et al., 2005). The majority of the adult pancreas is composed of acinar cells that are responsible for synthesizing and secreting the exocrine juices by which the pancreas aids digestion within the intestinal tract. It has been investigated whether these cells are capable of transdifferentiating into islet cell-types, specifically beta-cells. Certainly, it appears possible that acinar cells can switch phenotype to that of functional hepatocytes (Shen et al., 2000). The potential for foci of pancreatic metaplasia in the liver and vice versa has long been recognized (Li et al., 2005). However, there is also support for *in vivo* transdifferentiation of exocrine cells into islet cell-types following ligation of the pancreatic duct in rats (Lardon et al., 2004) and as a minor source of beta cell regeneration in humans (Si et al., 2001). *In vitro*, this process in rats has been enhanced by the addition of leukaemia inhibitory factor (LIF) and EGF (Baeyens et al., 2005). Persuasively, expression of Ngn3 was increased via signalling from the JAK-STAT pathway (Baeyens et al., 2006). Lineage tracing has also been used *in vitro* to strengthen the conclusion that endocrine cells can be generated from exocrine cells (Minami et al., 2005). Others have shown the presence of Nestin-positive intermediaries during acinar-to-endocrine transdifferentiation (Means et al., 2005). However, it is interesting that the same group has more recently conducted *in vivo* lineage tracing experiments in mice. Post-pancreatectomy, acinar cells supported their own regeneration but did not contribute to the appearance of new endocrine cells (Desai et al., 2007; Ferrer et al., 2007). This latter study implies that transdifferentiation from acinar cells is not a major mechanism *in vivo* for maintaining postnatal beta-cell mass, at least in mice. Such illustration that *in vitro* phenomena do not necessarily translate clearly into *in vivo* physiology is

perhaps not surprising as cells taken into a foreign environment commonly behave differently than when residing in their natural niche.

2.4. New beta cell formation via the proliferation of pre-existing beta-cells

Having considered ductal and other cell-types within the pancreas as potential beta-cell precursors, what about the beta-cell itself as a resource from which new beta-cells might be derived?

Some pancreatic regeneration studies that supported a role for ductal cell progenitors also noted an important contribution from beta-cell proliferation (Waguri et al., 1997). Indeed, by assessing cell numbers, it has been argued that sustained beta-cell proliferation in a small subset of cells could in fact be responsible for a marked increase in beta-cell mass (Wang et al., 1996). Such proliferation in postnatal mice increases beta-cell mass four-fold by weaning (Georgia and Bhushan, 2004). More recently, murine genetic manipulation has facilitated ever more detailed analyses proving a critical role for beta-cell proliferation (Heit et al., 2006). Many of these studies have manipulated genes that regulate the cell cycle. For instance, the Checkpoint dependent kinase (Cdk) inhibitor (Cki) p27 (also known as Kip1) blocks cell cycle progression (Nakayama et al., 1996); when expressed under the influence of the rat insulin promoter mice develop diabetes, implying that non-insulin expressing stem or progenitor cells were unable to ensure adequate beta-cell mass (Uchida et al., 2005). Ordinarily p27 is inhibited by the transcription factor, Foxm1, which is also required for maintenance of beta-cell mass (Zhang et al., 2006). Similarly, deleting Cyclin-dependent kinase 4 (Cdk4) and CyclinD2, both of which are required for progression through the cell cycle, caused diabetes due to inadequate beta-cell provision (Georgia and Bhushan, 2004; Rane et al., 1999). The phosphoinositide 3-kinase/Akt-kinase pathway has been implicated in a Cdk4-dependent manner (Fatrai et al., 2006). CyclinD2, along with Pitx2, seems likely to mediate the effect of Wnt signalling on beta-cell proliferation (Rulifson et al., 2007). Akt can also be activated by signalling from the EGFR, which has been shown independently to

be necessary for postnatal beta-cell proliferation (Miettinen et al., 2006). Down-regulation of the EGFR in beta-cells irreversibly reduced their replication rate and diminished beta-cell mass.

By lineage tracing cells in mouse, the same conclusion was reached both during unperturbed adult life (Dor et al., 2004), and following two-thirds pancreatic resection (Dor et al., 2004) or transgenic expression of diphtheria toxin (Nir et al., 2007). These data rely upon faithful expression of promoter sequences driving transgene expression; something that cannot always be guaranteed. Most recently, Teta and colleagues used an unbiased approach to cell labelling, where label is retained over multiple rounds of cell division (Teta et al., 2007). The data conclusively demonstrated populations of stem cells in the gut and skin, however, they did not support such a population with the postnatal mouse pancreas. Brennan and colleagues used a similar line of experimentation (Brennan et al., 2007). They combined cell labelling with clonal analysis of dividing beta-cells by using tetracycline-induced histone 2B fused to GFP (Tumbar et al., 2004) and mosaic analysis of double markers (Zong et al., 2005). According to their results all beta-cells seemed to contribute equally to the growth and maintenance of the beta-cell population. Taken together, these experiments argue convincingly that under normal conditions, mouse beta-cells arise from beta-cells without significant contribution from an underlying stem or progenitor cell population.

The concept of beta-cell proliferation as the long-term adaptation to increased insulin need extends to several physiological and pathological scenarios. Beta-cell mass increases during pregnancy when *Hnf4a* is required and activates the MAPK signalling pathway (Gupta et al., 2007). Beta-cell mass is also increased in pathological states of insulin resistance, such as obesity. Elevated demand for insulin leads to mouse beta-cell proliferation mediated in significant part via the insulin receptor (Okada et al., 2007), downstream signalling from the PI 3-kinase pathway (Hashimoto et al., 2006) and requires the action of Pdx1 (Kulkarni et al., 2004).

3. Maintaining beta-cell mass in the human postnatal pancreas

The evidence from the preceding sections arises close to entirely from studies in rodents and is persuasive that under normal circumstances and under some pathological scenarios, beta-cell mass can be maintained or regenerated from both ductal cells and by beta-cell proliferation. The data from human tissue are limited by comparison. Genetic manipulation is not possible *in vivo* although clinical data can be obtained. Thus, we know that islet mass increases in pregnancy and under conditions of generalised insulin resistance (Bernard-Kargar and Ktorza, 2001; Butler et al., 2003). It is tempting to assume that the mechanisms of regeneration or maintenance of beta-cell mass are the same across species. In pigs, similar beta-cell hypertrophy was seen as a compensatory response to streptozotocin (Dufrane et al., 2006). However, there are histological differences between humans and mice. The mouse islet contains central beta-cells surrounded by peripheral glucagon-secreting alpha cells. Human islet structure is less clear, possibly being organised in a similar manner but within smaller subunits, several of which make up the whole islet. In addition, a significant proportion of human beta-cells, estimated at approximately 15%, are scattered within the pancreas as single cells (Bouwens and Pipeleers, 1998; Bouwens and Rومان, 2005), circumstantial evidence against beta-cells emanating from their own proliferation within existing islets. *In vitro* data have suggested that beta-cells can be generated from human ductal material, in general support of the potential ‘stem cell’ or precursor status of at least some ductal cells (Bonner-Weir et al., 2000) although it is always difficult to categorically exclude contamination by a few ‘rogue’ beta-cells in such material. Careful elimination of such contaminating cells as well as BrdU pulse-chase experiments clearly indicate differentiation of beta-cells from ductal cells, or at least cells phenotypically resembling duct cells based on their expression of cytokeratin isoforms (Gao et al., 2003; Hao et al., 2006). However, it remains conceivable that at least some of the duct-like cells in these *in vitro* experiments represent dedifferentiated endocrine or acinar cells (Gao et al., 2005).

More recently, there has been interest in whether the pancreata of patients with type 1 diabetes continue to show new beta-cell formation (Meier et al., 2005; Meier et al., 2006c). Aspects of the later of these two studies have proven controversial (Meier et al., 2006a; Zhao, 2006). However, the identification of single insulin-positive cells in ductal or peri-ductal locations is suggestive of regenerative efforts, which could serve as ‘fuel’ to the ongoing autoimmune reaction. Consistent with this insinuation of stem or progenitor cell mediated regeneration in humans, under conditions of diabetic ketoacidosis, the same authors recently found no evidence to support beta-cell proliferation as the means of maintaining beta-cell mass (Butler et al., 2007).

In humans, determining whether neogenesis occurs from a non-beta-cell source carries therapeutic implications. Recent interest has been generated from the approval of two new classes of drug for the treatment of type 2 diabetes: glucagon-like peptide 1 (GLP-1) analogues and dipeptidyl peptidase IV (DPP-IV) inhibitors, which inhibit degradation of endogenous GLP-1. Collective data from rodents imply that these agents are capable of inducing beta-cell proliferation, inhibiting apoptosis and also stimulating beta-cell neogenesis (Drucker, 2006). For instance, the differentiation process following streptozotocin can be enhanced by administration of GLP-1 (Thyssen et al., 2006), however, it also increases beta-cell proliferation mediated by FoxO1 (Buteau et al., 2006). Some have identified cross-over with the EGF signalling pathway (Buteau et al., 2003), a regulator of islet growth, although down-regulation of the EGFR did not impact upon the insulintropic effect of GLP1 analogue, Exendin4 (Miettinen et al., 2006). Similar interest exists for the potential of other hormones and growth factors such as gastrin, INGAP, EGF and betacellulin, to induce new beta-cell formation (Bonner-Weir and Weir, 2005). Phase 1 clinical trials have been initiated to explore the safety and potential beneficial effect of GLP-1 analogues and similar agents on residual beta-cell mass in recent-onset type 1 diabetes.

4. Could there be a species difference between rodents and humans in maintaining beta cell mass?

The preceding sections support both beta-cell proliferation and differentiation from pancreatic ductal cells as major mechanisms for maintaining beta-cell mass *in vivo* in both rodents and humans (Table 1). However, the relative balance between the two mechanisms is less clear and may vary across species; for instance, the relative proliferative tendencies of islet cell-types. A high predilection for proliferation might be expected to translate into increased tumour risk. This appears true. Impaired function of cell cycle checkpoint proteins and tumour suppressor genes or overactivity of pro-proliferative proteins can lead to inappropriate beta-cell expansion with the concomitant risk of hypoglycaemia from hyperinsulinism. For instance, overexpression of C-myc with blocked apoptosis (Pelengaris et al., 2002) or loss of the tumour suppressor gene, *PTEN*, (Stiles et al., 2006) leads to islet hyperplasia. Islet hyperplasia and insulinomas are well recognized in humans with the autosomal dominant tumour predisposition syndrome, multiple endocrine neoplasia type 1 (MEN1), due to loss of MENIN, a tumour suppressor protein and a negative regulator of beta-cell cycle (Akerstrom et al., 2005). However, even when the incidence from MEN1 is combined with sporadic cases, insulinomas are very rare in humans. They make up the majority of islet cell tumours, yet they only occur in 2-4 individuals per 1,000,000 population each year (Grant, 2005; Service et al., 1991). In contrast, islet cell tumours are much more common in rodents. The data arise largely from the control animals of drug toxicity screening programmes, with survival commonly reaching approximately two years. In CD rats of this age 11.7% of males and 5.5 % of females possessed islet adenomas. Islet cell carcinoma was less frequent, 2.4% for males and 1% in females, but still much greater than the total incidence of benign and malignant islet cell tumours in humans (Majeed, 1997). Rates of islet cell tumourigenesis are similar for other strains of rat, such as Wistar (2-6 %) (Walsh and Poteracki, 1994), Fischer 344 (6.5%) (Chandra and Frith, 1992a) and Sprague Dawley (3.7%) (Chandra et al., 1992). In mice, the islet cell

tumour frequency is below 1% (Chandra and Frith, 1992b, c), but still identifiable amongst colonies of less than 2,000 animals. Thus, it is in excess of comparable data in human. In fact, the lower value in mice compared to rats may reflect their very high susceptibility to earlier death from other more aggressive tumours. 90% of mice kept in captivity die of cancer (Lipman et al., 2004): hepatocellular or bronchoalveolar malignancies being the most common (Chandra and Frith, 1992b, c).

The reason underlying the increased islet cell tumour risk in mice compared to humans potentially lies in inter-species differences in replicative aging (Wright and Shay, 2002). Mice do not use replicative aging to limit cell proliferation (Forsyth et al., 2002). In contrast, replicative aging in humans is regarded as a critical anti-tumour defence selected under evolutionary pressure as a consequence of a much larger body and far greater lifespan. Indeed, it has been estimated that humans need to be approximately 100,000 times more protected against cancer than mice (Shay and Wright, 2001). In line with this, telomerase expression persists in mouse somatic cell-types leading to the maintenance of very long telomeres, compared to human cells where telomerase is extinguished early in fetal development in all but the germline (Wright and Shay, 2002). Consequently, human somatic cell telomeres shorten and cells undergo replicative aging leading to senescence and lower cell proliferation. If this argument is extended to organ maintenance, then it perhaps becomes feasible that, when demands are made on maintaining euglycaemia, mouse beta-cells might tend to respond by proliferation, whereas humans, as a by-product of evolutionary anti-tumour selection pressure, favour differentiation from stem cells or precursor cell-types.

5. Conclusions

Understanding how beta-cells are maintained postnatally is important. The underlying mechanisms offer promise for pharmacological enhancement as new strategies to ameliorate and potentially cure different forms of diabetes. To bring about these advances in the clinic will require further effort in the

laboratory. Firm data in rodents are appearing based on the detailed studies described here, but man still remains a mystery. For therapeutic benefit, there is an urgent need for answers in human pancreas.

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Table legends

Table 1. Selected studies showing evidence for beta-cell replication, neogenesis or both as the major mechanism of beta-cell regeneration.

IHC, immunohistochemistry

Figure legends

Figure 1. Schematic illustration of potential cell sources for postnatal beta-cell regeneration.

Table 1.

Species	Experimental design	Conclusions	Reference
A. Studies showing evidence for beta-cell replication as the major mechanism			
Mouse	RIP-CreER/HPAP mice, lineage tracing	New beta-cells arise from pre-existing beta-cells	(Dor et al., 2004)
Mouse	Ins-rtTA/tet-DTA mice, diphtheria toxin	Beta-cells regenerate through proliferation	(Nir et al., 2007)
Mouse	Multiple thymidine analogue labeling	Extensive beta-cell proliferation throughout life	(Teta et al., 2007)
Mouse	TetO-H2BGFP, MADM mice, clonal analysis	All beta-cells contribute equally to mass maintenance	(Brennand et al., 2007)
Mouse	CyclinD2 (-/-) mice, morphometry	Beta-cell proliferation is responsible for mass maintenance	(Georgia and Bhushan, 2004)
Human	Immunohistochemical morphometry	Proliferation of beta-cells in an old patient with T1DM	(Meier et al., 2006c)
Human	Immunohistochemical morphometry	Proliferation of beta-cells close to gastrin-producing tumor	(Meier et al., 2006b)
B. Studies showing evidence for beta-cell neogenesis as the major mechanism			
Rat	Duct ligation	Appearance of small islets in close association to ducts	(Boquist and Edstrom, 1970)
Rat	90% pancreatectomy	Ductal proliferation and formation of new islets	(Bonner-Weir et al., 1993)
Rat	Duct ligation and gastrin Rx	Extra-insular beta-cells and small beta-cell clusters close to transdifferentiated ducts	(Rooman et al., 2002)
Human	IHC in normal pancreas	Beta-cells in ductules suggestive of islet neogenesis	(Bouwens and Pipeleers, 1998)
Human	IHC in type 2 diabetic pancreas	Beta-cell replication very low, but signs of neogenesis and apoptosis increased	(Butler et al., 2003)
C. Studies showing evidence for contribution from both beta-cell neogenesis and replication			
Rat	STZ between birth and weaning	Regeneration from beta-cell replication and neogenesis	(Thyssen et al., 2006)
Mouse	Alloxan selectively to pancreatic lobes	Regeneration from beta-cell replication and neogenesis	(Waguri et al., 1997)
Mouse	Alloxan + adenoviral betacellulin gene therapy	Regeneration from beta-cell replication and neogenesis	(Tokui et al., 2006)
Mouse	Alloxan + EGF and gastrin	Regeneration from beta-cell replication and neogenesis	(Rooman and Bouwens, 2004)
Mouse	Duct ligation	Regeneration from neogenesis and beta-cell replication	(Xu et al., 2008)

Figure 1.