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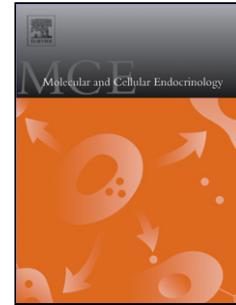
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Regulation of beta cell replication

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Abstract

Beta cell mass, at any given time, is governed by cell differentiation, neogenesis, increased or decreased cell size (cell hypertrophy or atrophy), cell death (apoptosis), and beta cell proliferation. Nutrients, hormones and growth factors coupled with their signalling intermediates have been suggested to play a role in beta cell mass regulation. In addition, genetic mouse model studies have indicated that cyclins and cyclin-dependent kinases that determine cell cycle progression are involved in beta cell replication, and more recently, menin in association with cyclin-dependent kinase inhibitors has been demonstrated to be important in beta cell growth. In this review, we consider and highlight some aspects of cell cycle regulation in relation to beta cell replication. The role of cell cycle regulation in beta cell replication is mostly from studies in rodent models, but whether these findings can be extended to human beta cells remains to be shown.

1. Regulation of beta cell mass

The understanding of factors and mechanisms responsible for pancreatic islet mass maintenance and integrity is of crucial importance in the face of prevalence of type 2 diabetes. Recent World Health Organization (WHO) data show approximately 180 million people worldwide have diabetes at present, and predict this number may well be more than double by year 2030; and type 2 diabetes accounts for over 90% of all cases (WHO Fact Sheet No 312, September 2006). The most worrying aspect is the rise of type 2 diabetes and obesity among children. Type 2 diabetes is characterized by lack of insulin secretion, insufficiency of beta cells mass or beta cells survival defects, and peripheral insulin resistance. Obesity is a major risk factor for the onset of insulin resistance. Beta cell mass increases in conditions such as obesity (Brüning et al., 1997; Cho et al., 2001; Kulkarni et al., 2004; Ogino et al., 2006; Okada et al., 2007) or during pregnancy (Parsons et al., 1992; Parsons et al., 1995; Gupta et al., 2007) when there are increased requirements for insulin in response to metabolic changes. Type 2 diabetes may develop when adaptation of the beta cells fails to compensate for the increased demand for insulin due to peripheral insulin resistance (Prentki and Nolan, 2006). Morphometric analyses of pancreata from a large number of patients have revealed a marked reduction of the beta cell area in both obese and lean patients with type 2 diabetes as compared with age and weight matched non-diabetic individuals (Butler et al., 2003).

Recent data available from man show beta cell replication capacity is highest in infancy coinciding with the postnatal beta-cell mass expansion, and the ability of beta cell regeneration declines soon after infancy (Meier et al., 2008). Several studies in mice and rats have documented an age-dependent decline in beta cell proliferation (Hellerström et al., 1988; McEvoy 1981; Finegood et al., 1995). Recent studies in adult mice (one-year-old) have shown that beta cells have low capacity to replicate and only limited turnover coupled with minimal beta cell apoptosis (Teta et al., 2005). Using mathematic modelling of available data of changes in beta cell number, size, proliferation, neogenesis and apoptosis it has been estimated that the life span of adult rat beta cells is between one and three months (Finegood et al., 1995). The life span of human beta cells is not known but *in vitro* data suggest that the life span of beta cells could be much longer. We have shown that human islets can maintain their capacity to release insulin in response to glucose for at least nine months in culture without signs of beta cell loss (Nielsen et al., 1979). Measurement of ^{14}C content in DNA may reveal a direct determination of beta cell turnover in adult man (Spalding et al., 2008).

Figure 1 is a general schematic illustration of the changes in beta cell mass under physiological and pathological conditions. A marked increase occurs under conditions with an increased insulin demand i.e. the neonatal period, in obesity and during pregnancy. Destruction of beta cells results in development of type 1 diabetes with loss of beta cells due to autoimmunity early in life or with late onset (LADA or type 1½ diabetes). The reduced beta cell growth in type 2 diabetes may be related to impaired fetal development of the endocrine pancreas combined with postnatal obesity and/or loss of beta cells due to gluco-lipotoxicity. In pregnancy the beta cell mass is normally increased in response to the increased demand. If not, gestational diabetes that affects 4-14% of pregnant women in the US (Lawrence et al., 2008) may occur. Interestingly, the beta cell

mass is reduced by apoptosis and decreased replication and cell size to normal levels after delivery in rats (Scaglia et al., 1995).

It is increasingly clear that pancreatic beta cells have the capacity to replicate themselves, albeit there is still much debate regarding the sources of the newly formed islets (Nielsen et al., 2001, Nielsen and Serup, 2004, Dor et al., 2004; Bonner-Weir and Weir, 2005; Teta et al., 2007; Brennand et al., 2007, Butler et al. 2007; Hanley et al., 2008; Xu et al., 2008). The mechanisms underlying the regulation of beta cell mass growth are complex, which includes cell cycle regulation that is central to cell replication. As a comprehensive review of this topic was published in 2006 (Cozar-Castellano et al., 2006a), the present review is focused on the most recent advances in cell cycle regulation concerning beta cell replication.

2. The role of cyclin-dependent kinases in beta cell replication

Normal cell replication and growth are dependent on and governed by the precise control of entry, passage, and exit through the cell cycle. This series of events is initiated by the complexing of cyclins (D-type cyclins) to cyclin-dependent kinases (Cdk4 or Cdk6). D-type cyclins are the first to emerge upon mitogenic stimulations while Cdks are constitutively expressed and are the cell cycle entry regulators. Distinct and tissue specific induction of different cyclin Ds may result in different cyclin D-Cdk complexes formed, which can be considered to be the checkpoint of mitogenic stimulations that lead to phosphorylation and deactivation of the retinoblastoma protein (pRb), thereby allowing the progression of the cell cycle cascade. The role of cyclin D 1 in association with Cdk 4 in beta cell proliferation following mitogenic stimulation was suggested over a decade ago (Dunlop et al., 1996); and the involvement of Cdk4 in beta cell replication was first demonstrated by *in vivo* knockout and knock-in Cdk4 mouse models (Rane et al., 1999; Tsutsui et al., 1999); and subsequently confirmed by genetic Cdk4^{-/-} rescue experiments (Martin et al., 2003). These studies showed that complete ablation of Cdk4 expression affected only a few specific organ (ovary, testis, and pancreas) and led to growth retardation, reproduction dysfunction and impairment of post-natal development of the endocrine pancreas with reduced islet area. Among the five cell types of pancreatic islets, only beta cell-specific gene expression was affected and the Cdk4^{-/-} mice exhibited phenotypic characteristics of insulin deficient diabetes. In contrast, the knock-in genetic mice with a mutated form of Cdk4 (Cdk4R24C mice) that has no affinity to p16 (a member of the INK4a of Cdk inhibitors) showed islet hyperplasia, and insulin immunostaining demonstrated that the pancreatic islets mainly consisted of beta cells (Rane et al., 1999). The findings represent the first indication of the potential implication of Cdk in islet growth through cell cycle progression. When the endogenous Cdk4 expression as restored in these Cdk4^{-/-} animals, beta cell proliferation returned, resulting in normglycaemic and fertile animals, however, the genetic rescued mice remained small in size (Martin et al., 2003).

Pancreatic beta cells from Cdk4R24C mice showed similar characteristics to those of differentiated beta cells, with intact pro-insulin biosynthesis and conversion rates comparable to those of wild-type controls. Furthermore, these mice acquired the ability

for a faster rate of glucose clearance that probably was due to their higher insulin contents per islets relative to their control islets. When human islets were infected with a lentiviral vector containing CdkR24C cDNA, a higher rate of proliferation (increased [³H] thymidine incorporation) was also observed (Marzo et al., 2004). Adenoviruses expressing Cdk4 and cyclin D1 in isolated rat and human islets also gave rise to enhancement of beta cell proliferation and retinoblastoma protein phosphorylation (Cozar-Castellano et al., 2004). Studies of transgenic mice expressing mutated Cdk4 (CDK4 R24C-Tg mice) revealed marked increases of beta cell proliferation, and the mice showed no sign of pancreatic islet malignancy over the study period of 18-month (Hino et al., 2004). Results from these studies clearly indicate that cell cycle regulation is involved in beta cell replication and Cdk4 plays a key role in murine beta cell proliferation. Moreover, the data suggest that Cdk inhibitors may also play a contributing role in beta cell proliferation. Cdk6, the functional analogue of Cdk4, can also complex with D-cyclins, but there is an apparent total lack of, or only minimum, Cdk 6 expression in murine islets.

3. The role of cyclin Ds in beta cell replication

There are three D-type cyclins, 1, 2, and 3 and they are responsive to extra-cellular stimulation (for example, growth factors). Distinct mitogenic stimulations can bring about and cause differential utilization of cyclin Ds, depending on the circumstances and cell types. Thus different cyclin D-Cdk complexes can be assembled which render the bound Cdks active for cell cycle progression. In human, it has been reported that cyclin D1 is highly and more frequently expressed in most pancreatic endocrine tumours (Chung et al., 2000). In pancreatic islets, there is a general consensus that cyclin D1 and D2 are expressed across different species, however, it is less clear for cyclin D3, as both presence and absence of cyclin D3 in pancreatic islets have been reported (Heit et al., 2006).

3.1. Regulation of cyclin Ds by hormones and growth factors

Growth hormone, prolactin, glucagon-like peptide 1 (GLP-1), and exendin (GLP-1 receptor agonist), are known insulinotropic factors as well as having positive effects on pancreatic islet growth. Furthermore, their effects on endocrine pancreatic functions have been corroborated by the respective receptor-knockout studies (Freemark et al., 2002; Liu et al., 2004; Flamez et al., 1999). We have previously shown that growth hormone and prolactin signalling via the Janus kinase 2 (JAK2)/Signal Transducer and Activator of Transcription 5 (STAT5) induce cyclin D2 expression (Friedrichsen et al., 2001; Friedrichsen et al., 2003) and documented that incretins (GLP-1, GIP) cause transcriptional activation of the cyclin D1 promoter activity, resulting in induction of cyclin D1 expression (Friedrichsen et al., 2006). Kim et al have shown that exendin-4, a GLP-1 receptor agonist, increased cyclin D1 expression in the pancreatic beta-cell line, INS-1, and acted predominantly via the cAMP/PKA signalling pathway (Kim et al., 2006). In addition, activation of the EGF (Epidermal Growth Factor) receptor by GLP-1 has been suggested (Buteau et al., 2003) and inhibition of phosphatidylinositol 3'-kinase (PI3K) reduced the effect of GLP-1 on beta cell replication and cyclin D1 expression

(Friedrichsen et al., 2006). Furthermore, EGF receptor-mediated signalling has been suggested to be involved in postnatal beta cell growth in mice (Miettinen et al., 2006).

3.2. Signalling pathways involved in cyclin D expression

Regarding beta cell proliferation, negative regulators of GLP-1 action has also been shown recently. Using RNA interference to knockdown CREM α , an inhibitor of the cAMP/PKA/CREB pathway, or DUSP14, an inhibitor of the MAPK/ERK1/2 pathway, the results showed increased induction of beta cell proliferation by GLP-1 (Klinger et al., 2008). Interestingly the IRS-2 mutant mice can be rescued in the short term by GLP-1 but still depend on IRS-2 later in life (Park et al., 2006), further stressing the extensive cross-talk between the signalling pathways in the regulation of beta cell growth.

Calcineurin/nuclear factor of activated T-cells (NFAT) signalling has been implicated in the regulation of pancreatic beta cell growth and function. Islets of mice with a beta-cell specific deletion of calcineurin b1 (β Cnb1KO mice) showed a significant decrease of cyclin D2 mRNA expression (Heit et al. 2006).

GH and PRL stimulate beta cell replication via STAT5 activation of cyclin D2 expression and this was not affected by inhibition of PI3K with wortmannin. We have shown that overexpression of a dominant negative STAT5 prevented GH stimulated replication but not serum induced replication of INS-1 cells (Friedrichsen et al., 2001) confirming the use of different pathways. Overexpression of a constitutive active STAT5 was also able to increase replication and cyclin D2 expression in neonatal rat beta cells and INS-1 cells (Friedrichsen et al., 2003) but simultaneous exposure to GH resulted in complete inhibition of INS-1 cell growth that may be due to the induction of SOCS-3 (suppressors of cytokine signalling) (Y. C. Lee, unpublished). GH also has an anti-apoptotic effect on beta cells exposed to the proinflammatory cytokines interleukin-1 β , interferon- γ and tumor necrosis factor- α that is mediated via STAT5 (Jensen et al., 2005). This effect is probably mediated by increased expression of Bcl-xL. Studies in transgenic mice expressing STAT5 mutants in the beta cells confirmed the protective effect of STAT5 against high fat diet induced diabetes and multiple low dose streptozotocin induced diabetes (Jackerott et al., 2006). The role of GH regulated calcium metabolism in beta cell is discussed in another paper in this issue (Zhang et al., 2008) and the role of STAT5 in beta cells was recently reviewed (Dalgaard et al., 2008).

That distinct signals lead to activation of cyclins D1 and D2, respectively, is supported by our finding that combinations of GH (or PRL) and GLP-1 (or GIP) resulted in an additive, or potentiation of the mitogenic effect in rat islets (Friedrichsen et al., 2006). Under similar condition no mitogenic effects of GLP-1 and hGH on adult human beta cells were observed (Parnaud et al., 2008), stressing the species variation in beta cell response to various growth factors.

3.3. Effects of cyclin D expression on beta cell mass

Overexpression of cyclin D1 in pancreatic beta cells revealed a more than 3-fold increase of beta cell mass as determined by morphometric analysis in transgenic mice when compared with their littermates. Significantly more proliferating cell nuclear antigen (PCNA) positive staining was present in the transgenic mice islets, while TUNEL-positive cells were identical between the transgenic and wild type, suggesting the hyperplasia observed in the transgenic animals was primarily due to beta cell proliferation. No differences was found in blood glucose levels between the two groups (Zhang et al, 2005).

Genetic loss of function mouse models of cyclin D2 showed that the loss of cyclin D2 expression caused a marked reduction in postnatal (animals of 14-day old) beta cell mass, glucose intolerance and the animals develop diabetes (Georgia and Bhushan, 2004). Lack of cyclin D 2 expression was associated with a considerable reduction of beta cell replication rate as shown by BrdU incorporation in the cyclin D2^{-/-} adult mice when compared with aged matched wild type controls. The results showed that the deleterious effect on beta cell proliferation was further worsen by an additional lack of cyclin D1; and the combined loss of both cyclin D2 and D1 expressions resulted in beta cell deficiency characteristically similar to the cdk4 null mice (Kushner et al., 2005; Kushner, 2006).

4. Autocrine regulation of beta cell replication.

4.1 The dual role of glucose in beta cell survival

It has long been suggested that exposure to glucose (short-term and doses within physiological ranges) play a stimulatory role in the pancreatic islet growth in rodent model systems (Swenne, 1985; Bonner-Weir et al., 1989; Guillemain et al., 2007); whereas continual elevated glucose exposure and hyperglycemia are deleterious to beta cell function including the beta cell differentiation process (Olson, et al., 1995, Jonas, et al. 1999, Poitout and Robertson, 2002). *In vitro* studies have shown that glucose increases the number of beta cells entering the cell cycle rather than the cell cycle rate (Swenne 1985). Recent studies suggest that glucose (50%) infusions over 4-days cause an increase of cyclin D2 at the protein level and promote cyclin D2 nuclear localization in pancreatic mouse islets. Alonso et al. showed that glucose infusions induced beta cell replication but that significant differences in the BrdU staining were only observed on the last day of the infusion period. Moreover, no changes in mouse beta cell mass, islets number, size or death were observed (Alonso et al., 2007). More recently, Jetton et al reported that continuous glucose (20%) infusions in rats for 1 to 4 days induced a 2 to 3-fold increase of beta cell mass at 48 and 96 hours, respectively, over the controls. This led to increased Akt activation in rat islet beta cells but no effect on beta cell proliferation was detected, and comparable cyclin D2 immunostaining patterns were observed in pancreatic islets of the saline- or glucose-infused rats (Jetton et al, 2008).

These contrasting results between mice and rats are puzzling and the role of cyclin D2 in glucose-mediated beta cell growth remains unclear. It is noteworthy that contrasting beta-

cell responses to glucose infusions in the rats have also been shown to be due to experimental designs and animals strains used. For example, studies of alternating 4-hour glucose (50%) infusions over a 72-hour period showed a total lack of effect of glucose on beta cell mass and proliferation in Wistar rats (Hagman et al., 2008), whereas, as mentioned above, continuous glucose infusion (20%) increased beta cell mass significantly after 48-hour in Sprague-Dawley rat (Jetton et al., 2008). These results suggest considerations regarding infusion protocols, animal strains, dose-dependency, must be taken into accounts when comparing data between studies, and also their interpretations.

4.2 Positive feed-back of insulin signalling on the beta cell growth

It has previously been shown that in INS1 cells (rat insulinoma cells) that the for maximal [³H] thymidine incorporation, the most effective glucose concentration range was 15- 18 mM, alone, or together with insulin-like growth factor 1 (IGF-1) (10 nM). These treatment regimes also caused an increase of protein phosphotyrosin phosphorylation of the insulin receptor substrate 2 (IRS-2), leading to phosphatidylinositol 3'-kinase (PI3K) activation (Hügl et al., 1998). PI3K signalling pathway is of importance in cellular functions including cell growth, proliferation, survival; and the survival signal provided by PI3K activation is mediated by Akt /PKB family of serine/threonine protein kinases (see review: Downward, 2004). Activation of Akt/PKB in cultured murine insulinoma cells (MIN6) and mouse islets by glucose has also been shown, however, unlike studies in rats as mentioned above, a considerable higher glucose concentration (25 mM) was employed in these studies (Srinivasan et al., 2002), giving rise to the possibility that differential glucose responsiveness exists between rat and mouse beta cells.

Insulin receptor substrate 2 (IRS-2) is an intracellular signalling protein immediate downstream of cell surface receptors such as insulin and IGF-1, and coordinates IGF-1 receptor mediated actions on beta cells (Withers, et al 1999). Ablation of IRS-2 in mice (IRS-2^{-/-}) caused a marked reduction in beta cell mass (Withers, et al 1998). In addition, glucose up-regulated IRS 2 expression in rat primary beta cells, which was coinciding with protein kinase B (Akt/PKB) activation (Lingohr et al., 2006).

Akt overexpression studies have established that Akt promotes islet growth (Tuttle et al., 2001; Bernal-Mizrachi et al., 2001). The findings showed mice overexpressing constitutively active Akt (caAktTg) in beta cells increased both cell size and islet mass, resulting in an increased insulin content per islet for the Akt transgenic mice when compared with their littermates. The increase in islet mass was considered to be beta cell-specific as no difference in glucagon levels were indicated between the experimental and control groups. Upon streptozotocin-treatment, no elevation of glucose levels among the Akt transgenic mice was found throughout the experimental period while their littermates develop progressive hyperglycaemia. The de-regulation of glucose balance in control mice was due to the selective loss of beta cells by streptozotocin exposure, suggesting Akt plays a role in promoting beta cell survival (Tuttle et al., 2001).

The importance of Akt in beta cell proliferation is further supported by other studies of positive or negative mediators of PI3K signalling. For example, beta-cell-specific deletion of 3-Phosphoinositide-dependent protein kinase 1 (PDK1) caused reduction of beta cell mass leading to insulin deficiency (Hashimoto et al., 2006). In contrast, the beta cell specific deletion of phosphatase with tensin homology (PTEN), a negative regulator of PI3K signalling, showed an increase of islet cell mass but maintained beta cell function (Nguyen et al., 2006).

5. Developmental signals in beta cell replication

5.1 GSK3/Wnt and cyclin D expression in beta cells

Signalling factors and pathways implicating cell cycle regulation in beta cell replication are only emerging recently. The notable examples are the works by Fatrai et al. and Rulifson et al. By examining pancreatic islets from mice expressed both caAkt and Cdk4 (caAktTg /Cdk4^{+/+} mice), Fatrai et al showed there was a pronounced increase of cyclin D1, cyclin D2 (less relative to cyclinD1), and p21 expression at the protein level; while p27 expression remained unchanged but a suppression of p57 was observed (Fatrai et al., 2006). The induction of cyclin D2 and D1 seen in islets from caAktTg /cdk4^{+/+} mice was coinciding with increased phosphorylation of the islet glycogen synthase kinase (GSK3 β) as shown by immunofluorescence staining and immunoblotting. GSK3 is a downstream target for Akt, and its inactivation has been shown to protect beta cells from endoplasmic reticulum stress-induced apoptosis (Srinivasan et al., 2005), and has a negative effect on the stability of PDX1 (Boucher et al., 2006). Inactivations of GSK3 by small molecule inhibitors or siRNA to GSK have recently been shown to stimulate beta cell replication in INS-1E (rat insulinoma cells) and isolated rat islets (Mussmann et al., 2007). GSK3 inhibitors suppressed apoptosis induced by the combined treatment of high glucose (25 mM) plus palmitate (0.3 mM) in INS1-E cells.

Wnt signalling has been implicated in the regulation of beta cell proliferation. Data from studies of transgenic mice (Ipf1/Frz8CRD) expressing the dominant-negative form of mouse Frz8 (acting as an antagonist of Wnt signalling) showed a marked reduction in pancreatic mass (75%) and absolute beta cell numbers (50%). The adult beta- cell function was maintained as greater production and secretion of insulin were observed in the beta cells of the transgenic mice, but the mechanisms responsible for the compensatory adaptation remain unknown (Papadopoulou and Edlund, 2005). More recently, Wnt signalling has been suggested to be linking to cell cycle regulators. In MIN 6 cells or purified murine islets, Wnt3a caused a marked increase in the expression of Pitx2 (a target of Wnt signalling) and cyclin D1, and cyclin D2 as well as Cdk4, the increased expressions of which were abolished in the presence of Wnt3a inhibitor, Fz 8- cysteine-rich domain (Fz8-CRD). A 3- fold increase of BrdU incorporation was observed in MIN6 cells treated with purified Wnt3a (Rulifson et al., 2007). Interestingly, the GLP-1 induced beta cell replication was very recently reported to be mediated β -catenin and TCF7L2 activation (Liu and Habener, 2008). These observations further support the essential role of the Wnt-signalling pathway not only in the embryonic development but also in the adult beta cells.

5.2 The role of insulin gene transcription factors in beta cell replication

As with Pdx-1 that originally was identified as a transcription factor for the insulin but later found to be essential for development and growth of the pancreas and the beta cells, other insulin gene transcription factors have been shown to be implicated in beta cell replication. Thus over-expression of Pax4 was found increase beta cell replication in rat islets and beta cell growth factors like glucose and GLP-1 increased the expression of Pax4 in both rat and human islets. However, no increase in replication was seen in the human (Brun et al., 2008). In a recent study it was demonstrated that over-expression of the beta cell specific the homeodomain transcription factor Nkx6.1 increased ³H-thymidine incorporation in both rat and human islets, and a host of cell cycle regulatory genes including cyclin D2 was increased in rat islets (Schisler et al 2008). However, Nkx 6.1 effect on glucose-stimulated insulin secretion was only increased in rat but not in human islets. These studies underscore the fundamental physiological differences between rodent and human beta cells.

6. The role of cell cycle inhibitors on beta cell senescence

Cyclin-dependent kinase inhibitors consist of two families of proteins: INK4/ARF (inhibitor of kinase 4/Alternative Reading Frame), p16INK4A, p15INK4b, p18INK4c, p19INK4d; and the CIP/KIP Cdk inhibitors family, p21CIP, p27KIP, p57KIP ; thereafter, the Cdk inhibitors are referred to without their respective superscripts. It is worth noting that under certain circumstances, p21 and p27 can also function as a assembly factor in promoting the binding of cdk4 with D-type cyclins (LaBaer et al., 1997; Cheng et al., 1999).

6.1 p27 inhibits beta cell replication in the adult

There was found an increased p27 expression in beta cells of IRS2^{-/-} and LepR^{-/-} mice, both animal models of type 2 diabetes and *in vitro* p27 overexpression in murine islets resulted in insulin insufficiency (Uchida et al., 2005). In contrast, loss of p27 expression increased pancreatic islet mass that is associated with increased islet numbers rather than size, and p27-deficiency improved markedly the metabolic defects in IRS2^{-/-} and LepR^{-/-} mice (Uchida et al., 2005). The data highlighted that the appropriate suppression of Cdk inhibitor activity under certain circumstances may be the key in beta cell mass maintenance, thus implicating the involvement of Cdk inhibitors in murine islet cell mass and pathophysiology.

Interestingly, Georgia and Bhushan have recently shown that loss of p27 allowed newly differentiated beta cell to proliferate, and the p27^{-/-} mice were less likely to develop streptozotocin-induced diabetes (Georgia and Bhushan, 2006). The studies suggest that p27^{-/-} mice have greater beta cell mass when compared with their littermates controls after birth, however, increased beta cell mass was observed in both the p27^{-/-} mice and the wild-type controls in the first 3-week after birth; indicating that during early postnatal periods, regulation of p27 has no major influence on beta cell proliferation. This is

consistent with the idea that beta cells have the highest replication capacity during the postnatal period.

6.2 The p21 paradox

Targeted expression of hepatocyte growth factor (HGF) or placental lactogen (mPL-1) under the control of the rat insulin promoter (RIP) in beta cells showed a marked increase in beta cell proliferation, islet mass, and insulin content leading to hypoglycaemia in these transgenic mice (RIP-HGF, RIP-mPL-1) (Garcia-Ocaña et al., 2000; Vasavada et al., 2000). Pancreatic islets from both these animals showed a dramatic increase of p21 expression, of which the increase was more pronounced in islets from RIP-mPL-1 than those from RIP-HGF mice (Cozar-Castellano et al., 2006c). This increased p21 was apparently specific as the overexpression of HGF or placental lactogen did not affect other cell cycle regulators expression in the pancreatic islets. Increase of p21 was also observed in rat insulin-producing cells (INS-1) when prolactin was acting as the stimulus. Adenoviral mediated transfer of cyclinD1 and Cdk4, alone or in combination, caused a marked up-regulation of p21 in human islets, accompanying by translocation of the p21 into the nucleus of beta cell (Cozar-Castellano et al., 2006c).

In a separate report, Cozar-Castellano et al. recorded that p21 null mice showed no effects on pancreatic islet proliferation or glucose balance, while the p21 ablation caused a dramatic reduction of p18 expression in the islets (Cozar-Castellano et al., 2006b). The absence of influence on islets physiology by the knockout of p21 was confirmed by studies of islets from RIP-mPL-1 crossed with the p21-null mice. While increased islet p21 expression was induced by mitogen stimulation (HGF or mPL-1), the lack of effects on murine islet growth by p21 deletion and the dramatic reduction of p18 expression as a result of loss of p21 underscore the complexity of the roles of the Cdk inhibitors in cell cycle progression.

The lack of influence on islet growth in some single- Cdk-inhibitor-knockout *in vivo* models (including p21 mentioned above) may, in part, be explained by functional dependence, cooperativity, tissue-specificity, and redundancy between different Cdk inhibitors. For example, previous studies have shown that mice lacking either p18 and p27 or p18 and p21 are distinctly different from each other (Franklin et al., 2000). Double mutant mice of p18^{-/-}/p27^{-/-} died early (3-4 month of age) and developed tumours in a wide spectrum of endocrine tissues including the endocrine pancreas (islet hyperplasia). The multiple types of tumours that occur within the same animal closely resembles human multiple endocrine neoplasia syndromes (MEN 1 and 2). In contrast, p18^{-/-}/p21^{-/-} have a normal lifespan with limited tissue tumorigenesis (Franklin et al., 2000). Moreover, mice deficient in p21 have also been shown to develop normally without any obvious indications of tissue malignancies observed throughout life (Deng et al, 1995).

6.3 Does p16 promote beta cells aging?

p16 is widely expressed including in human pancreatic islets with an increased expression level in adult tissues as compared with young tissues (Nielsen et al.,1999)

Because of its unique expression pattern, p16 has been proposed to be as a biomarker of aging (Krishnamurthy et al., 2004). In line with this notion, interestingly, Krishnamurthy et al have found that only p16 expression (mRNA) was increased significantly with aging in pancreatic islets, but not other Cdk inhibitors examined, including p15, p18, p19, p21, p27. In addition, overexpression of p16 in transgenic mice caused a reduction of islet proliferation in younger more than in older animals (Krishnamurthy et al., 2006).

When exposed to the beta cell-specific toxin streptozotocin, the older genetically modified p16^{-/-} and p16^{+/+} mice had greater beta cell survival (regeneration) capacity than the younger mice. Recently, studies of beta cell turnover in rat and human islets have shown that aging may have a negative effect on beta cell regeneration, and is associated with a decreased pancreatic duodenal homeobox (PDX)-1 expression and older islets were more sensitive to high glucose-mediated apoptosis (Maedler et al., 2006). Aging is a normal physiological event associated with a progressive decline of bodily functions over time. A paradox to the effect of aging affecting beta cell regeneration potential and capacity is the increase incidence of type 2 diabetes in children. Does it mean that the components that are pivotal in glucose balance have “aged” biologically in the case of the young? p16 has an almost undetectable level of expression in the young pancreata. It would thus be interesting to examine the effect of caloric restriction on p16 expression in aged pancreatic tissues since caloric restriction is known to affect the endocrine pancreas, and longevity (Mattison et al., 2001).

Another question is whether aging may promote islet quiescence or senescence? A quiescent cell, at any given time, can be stimulated to undergo repeated cell division, unlike senescent cells that are considered to be in a permanent state of growth arrest. Aging has long been postulated to have an impact on beta cells regeneration potential (Swenne, 1983). Recent human data have shown that there was a decreased parenchymal volume in type 2 diabetes when compared with that of non-diabetic controls. Interestingly, pancreatic fat increased with aging and obesity, and it was identical between diabetic and non-diabetic groups (Saisho et al., 2007). Thus, the role of aging on beta cell replication and the incidence of type 2 diabetes remains controversial.

7. Menin prevents beta cell hyperplasia

7.1 Menin as a tumour suppressor

Menin is a tumour suppressor, encoded by the Men1 gene and mutation of which causes multiple endocrine neoplasia type 1 (MEN1) that is characterized by abnormalities present primarily in parathyroid glands, anterior pituitary, and endocrine pancreas. In insulinoma cells (INS-1), menin suppressed insulin promoter activity, glucose-induced insulin secretion and ³H thymidine incorporation (Sayo et al. 2002). Since the homozygous knockout of the Men1 gene is lethal, conditional knockout mouse models have been established to investigate its possible functional implications. Targeted heterozygous Men1 deletion mice displayed phenotypes close to the human MEN1, resulting in pancreatic hyperplasia that coincided with the elevation of insulin and reduction of blood glucose level (Crabtree et al., 2003).

6.2 Menin as regulator of beta cell replication and survival

Since these reports on menin in islet hyperplasia and their possible implication in glucose balance regulation, (Sayo et al., 2002; Crabtree et al., 2003), progress has been swift in linking menin and islet proliferation, and its possible implication in beta cell function. Milne et al have shown increased cell growth rate in menin- and mixed lineage leukaemia (MLL) - deficient fibroblast cells when comparing with normal fibroblasts; the increase was accompanied by decreased p18 and p27 expression. The transcriptional regulation of MLL on p18 and p27 expression was menin-dependent. Human pancreatic islet histological sections showed menin, MLL and p27 positive staining in normal tissue while a remarkable reduction of all three in islet tumour sections (Milne et al, 2005). The phenotypic characteristics of Men1^{+/-} mice islets were not different from their wild type controls postnatally (Karnik et al; 2005). However, at 7-month of age, increased BrdU incorporation was observed in Men1^{+/-} beta cells, and occurrence of islet hyperplasia with detectable hypoglycaemia in the experimental animals. In pancreatic islets of Men1^{+/-} mice, there was a progressive reduction of mRNA expression for p18, p27, p15 by week 40, and p21 mRNA expression was also absent, except for p19 mRNA expression that remained comparable to Men1^{+/+} mRNA level. Protein (western blot) analysis showed a total absence of p18 and p27 expression but a marked increase of Cdk4 in Men1^{+/-} pancreatic islets. The regulation of p18 and p27 by menin is through histone methylation (Heit et al., 2006).

When Schnepf et al. set out to examine the effect of menin on cell cycle regulation, they found that ablation of Men1 accelerated S phase entry in mouse embryonic fibroblasts, which was accompanied by increased cyclin-dependent kinase 2 (Cdk2) activity and a reduction of the Cdk inhibitors p18 and p27 in the pancreas. In addition, they showed that soon after deletion of Men1, increased islet proliferation occurred, leading to enlargement of the pancreatic islets and no effect on the exocrine part of the pancreata (Schnepf et al., 2006).

Menin has been shown to transcriptionally increase caspase 8 expression, and there was a marked decrease of menin expression in insulinoma cells (La et al. 2007). Caspase 8 is known to be involved in cell death induced by Fas that itself is depending FLIP (FLICE inhibitory-protein) activity (Irmeler et al., 1997); and FLIP can reverse Fas signalling from apoptosis to proliferation in beta cells (Maedler et al 2002). Interestingly, caspase 8 has recently been suggested to have distinct context-specific effects on pancreatic beta cells (Liadis et al., 2007).

6.3 A role for menin in beta cell adaptation to pregnancy

More recently, menin has been linked to gestational diabetes. During pregnancy, levels of menin, p18, p27 drop in maternal islets, thus lead to islet mass expansion to meet the increased metabolic demand, and after birth, menin returns to normal. During the gestation period, menin is regulated by prolactin via STAT 5 signalling (Karnik et al.,

2007). It will be interesting to see if menin also regulates beta cell expansion in human pregnancy.

8. Concluding remarks and remaining challenges

In summary, data have emerging in the last several years regarding the functional implications of cell division cycle in beta cell regeneration. The various genetic mouse models have shown that the regulations are complex. For example, growth factors and mitogens have influence on the cyclin Ds expression levels, the regulations of which involve multiple intracellular signalling pathways including cAMP/PKA, PI3K/Akt, JAK/STAT and Wnt/GSK. How is the cross-talk between these pathways integrated? Could the stimulation of beta cell replication by insulin gene transcription factors be mediated by autocrine effects of insulin?

The interplay between the Cdk inhibitors in the regulation of beta cell replication is intriguing and remains unresolved. How is p21 involved in both growth arrest and proliferation of beta cells? Does p16 plays a role in early “aging” of beta cells in type 2 diabetes? How can the paradoxical regulation by menin on Cdk4, p18, and p27 expression be explained. It will be interesting to elucidate the role of menin in beta cell adaptation in human pregnancy and gestational diabetes. The maintenance of beta cell mass is depending on the balance between beta cell survival and death, and several of the factors discussed in this review are involved in beta cell differentiation, proliferation, survival and apoptosis. A model for these interactions is presented in Fig. 2.

Much information regarding the impact of cell cycle regulation in beta cell replication is gained from mouse genetic models; although the data are useful for the consideration in the development of beta cell preservation strategies and regeneration therapies for diabetes, the findings need validations in humans.

Induction of regeneration of beta cells after partial pancreatectomy or partial duct-ligation in mice and rats is well documented (Lee et al., 1989; Bonner-Weir et al., 1993; Wang et al., 1995; Peshavaria et al., 2006; Lee et al., 2006; Xu et al., 2008), however, most recently, it has been reported that, unlike in rodents, no beta cell regeneration was observed in partial pancreatectomy in adult humans (Menge et al., 2008) and no replication was observed in isolated human beta cells in vitro (Parnaud et al., 2008). Although beta cell expansion have been described in human pregnancy and obesity the mechanism whereby it occurs still remains a major challenge

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