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New Insights into the Roles of Insulin/IGF-I in the Development and Maintenance of β-Cell Mass

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Key Words. **insulin, IGF, islet beta-cells, insulin resistance, replication, knockout**

Abbreviations. **IRS, insulin receptor substrates; IGF-I, insulin-like-growth factor; PI 3-kinase, Phosphatidyl inositol 3-kinase**

Introduction

Insulin and insulin-like-growth-factor-I (IGF-I) constitute two members of the growth factor family, which play important roles in the regulation of metabolism and growth of virtually all tissues in mammals. The receptors for insulin and IGF-I are expressed ubiquitously and mediate the growth and metabolic effects of the hormones in virtually all tissues in the body [1–3]. Most of the information we currently know regarding insulin/IGF-I signaling pathways is derived from studies underlying the defects in insulin action in type 2 diabetes [4,5]. Insulin and IGF-1 bind to distinct receptors that in turn transmit signals by phosphorylating insulin receptor substrates (IRS) including the four IRS proteins, Shc, Gab-1, FAK, Cbl, and potentially other substrates [1,4,6–9]. These insulin receptor substrates play different but crucial roles in cellular processes that are important for the metabolism and growth of tissues including glucose transport and utilization, protein synthesis, cell growth, proliferation and anti-apoptosis. Several reviews provide an excellent update on these signaling networks [1,6–8]. Over the last decade, several laboratories have created global and tissue-specific knockouts of genes that code for protein(s), which are considered potentially important in regulating the effects of insulin and/or IGF-I. The pleiotropic signaling effects of insulin and IGF-I family of growth factors have been studied in great detail in classic insulin sensitive tissues including skeletal muscle, liver and adipose [4,5]. While IGFs have been studied for their contributions to islet development, a role for insulin during growth of the endocrine cells in the embryonic and post-natal periods is not fully understood.

Although the presence of functional insulin receptors in β-cells is now undisputable (reviewed in [10]), it has been a challenge to study the signaling pathways activated by insulin in β-cells for several reasons. First, the precise localization of receptors on apical and/or basolateral surfaces of different islet cells using immunohistochemistry, has been limited due to lack of a robust anti-insulin receptor antibody. Second, the continuous secretion of insulin by β-cells via the regulated and constitutive pathways allows potential internalization and downregulation of insulin receptors and confounds the effects of added ligand. Thus, several studies have used experimental protocols wherein islets/beta cells are either treated with exogenous insulin [11,12,146] or with inhibitors of regulated insulin secretion such as somatostatin or diazoxide followed by examining the consequences on insulin secretion or synthesis due to direct or indirect effects. A confounding factor in the latter approach is the inability to completely inhibit insulin secretion since most secretory cells possess regulated and constitutive secretion pathways [13,14]. Thus, very small amounts of insulin secreted by the constitutive pathway likely maintain downregulation of insulin receptors in the presence of inhibitors of regulated secretion such as somatostatin and diazoxide, which in turn, can lead to erroneous interpretation of data. The development of powerful genetic engineering techniques has circumvented several disadvantages discussed above and allow for disruption of the gene(s) coding for a given protein and enable direct evaluation function of the targeted protein(s) [10]. Recent studies in humans provides further evidence for a role for insulin action in the β-cells [140]. Thus, this review will focus mostly on direct evidence provided by these techniques for a role for insulin and IGF-I during early growth and development of islets and in the maintenance of adult β-cell mass.

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Role of Insulin/IGF-I During Embryonic and Early Post-Natal Development of the Endocrine Pancreas

The pancreas develops from the fusion of two diverticula of primordial gut tissue to form the distinct endocrine and exocrine components observed in adulthood [15]. Several recent reviews provide insight into the development of the endocrine cells [16,17] and the role of numerous transcription factors that are considered essential for the development of the different endocrine cell types [18,19]. Several elegant studies provide compelling evidence to disprove old dogmas. For example, based on irreversible tagging of progeny through the activity of *Cre* recombinase it is now accepted that α- and β-cell lineages raise independently from a common precursor expressing the pancreatic homeodomain protein, PDX-1, and not from glucagon-expressing progenitors as was originally suggested [16]. Furthermore, direct lineage tracing studies indicate that NGN3+ cells are islet precursors and are distinct from ductal precursors [20]. It would be informative to use similar techniques to explore the role(s) of insulin and IGF-I signaling during embryonic development of islet cells.

Global and Conditional Knockouts of Insulin, IGF-I, IGF-II, and Proteins in Their Signaling Pathways

The significance of IGF-I and insulin during early development and growth of islet cells has been a major focus of study for several decades and several important insights have emerged from these experiments [21–24]. The fetal pancreas expresses IGF-I, IGF-II, and IGF-binding protein 3 during late gestation [21,25,26]. IGF-II levels are higher than IGF-I during fetal development and IGF-II has been localized to islets and duct epithelial cells by immunohistochemistry and *in situ* hybridization techniques [21,25,27]. Together, these studies indirectly implicate a role for IGF-I and IGF-II during the post-natal development of the endocrine pancreas [28].

The development of genetic engineering techniques over the last decade, to create gain of function or loss of function mutations [29,30], has been used successfully to examine the function of specific proteins in different cell types in the islet. Further, the ability to "turn off" a gene encoding for a particular protein in a time-dependent manner provides a tool to simulate the gradual dysfunction that is usually observed in chronic diseases [30]. Although adaptation to the creation of a genetic mutation during embryonic life is a natural consequence of this method the information obtained in studies in multiple biological disciplines has been extremely useful to unravel potentially novel and unexpected functions of proteins. These observations are comparable to those made from humans bearing naturally occurring genetic mutations, who of necessity adapt to the mutation, but nevertheless provide important clues to understand the function of the proteins encoded by the gene(s). A partial list of global and conditional knockouts/transgenics of insulin, IGFs and proteins in their signaling pathways is provided in Tables 1 and 2. Unfortunately, many references could not be cited due to space limitations.

To directly evaluate the role of growth factors, several investigators have utilized homologous recombination in mice. Thus, global knockout of genes coding for

Table 1. *Partial list of phenotypes and references of global knockout/transgenics of insulin and IGF-1 genes and proteins in the insulin receptor/IGF-1 signaling pathway.*

Protein	Phenotype	Reference
Insulin	Intrauterine growth retardation,	[31]
	Neonatal lethality, Ketoacidosis,	[121]
	Liver steatosis	
IGF-1	Dwarfism,	[66]
	Variable survival	[42]
IGF-II (overexpressor)	Islet hyperplasia,	[37]
	organ overgrowth	
Insulin receptor	Neonatal lethality,	[122]
	Ketoacidosis	[123]
IGF-1 receptor	Dwarfism	[124]
	Neonatal lethality	
IRS-1	Post-natal growth retardation	[55]
	Insulin resistance	[56]
	Islet hyperplasia	[57]
	Insulin secretory defect	[59]
		[58]
		[60]
		[61]
IRS-2	Insulin resistance	[46]
	Diabetes	[58]
	Islet hypoplasia	
IRS-3	Relatively normal	[66]
IRS-4	Mild glucose intolerance	[67]
PI 3-kinase isoforms		
p85α	Increased insulin sensitivity,	[69]
	Hypoglycemia	[70]
p85β	Increased insulin sensitivity,	[68]
	Hypoglycemia	
p50α/p55α	Increased insulin sensitivity	[125]
Akt1	Growth retardation,	[126]
	Increased apoptosis,	
	Normal glucose tolerance.	
Akt2	Insulin resistance in liver and muscle,	[127]
	Increased islet mass.	
P70S6kinase	Hypoinsulinemia,	[72]
	Glucose intolerance, and	
	Reduced beta-cell size.	
Insulin receptor-related receptor		[128]
	Normal phenotype	

Table 2. *Partial list of phenotypes and references of tissue-specific knockout/transgenics of insulin and IGF-1 genes and proteins in the insulin receptor/IGF-1 signaling pathway.*

Protein/Tissue	Phenotype	Reference
IGF-I		
Liver	Normal growth and development,	[129]
	Muscle insulin sensitivity.	[124] [130]
Islets	Islet hyperplasia, Resistance to diabetes	[36]
IGF-II		
β-cell (overexpressor)	Islet hyperplasia, diabetes	[38]
Insulin receptor		
Muscle (transgenic)	Increased adiposity, Dyslipidemia and Glucose intolerance.	[131]
	Normal glucose tolerance, Elevated triglyceride and FFA levels	[132]
β-cell	Glucose intolerance, Loss of acute phase insulin secretion, Reduced β-cell mass.	[48]
Liver	Severe glucose intolerance, Hepatic dysfunction	[112]
Brain		[133]
	Increased food intake and obesity in female	
	Impaired spermatogenesis and ovarian follicle maturation	
Adipose		[134]
	Protect against obesity and glucose intolerance	
	Heterogeneity in white adipose cell size	
Insulin receptor		
Muscle + Adipose	Impaired glucose tolerance Insulin resistance	[135]
IGF-1 receptor		
β-cell	Impaired glucose tolerance	[49]
	Reduced glucose-stimulated insulin secretion	[50]
IRS-2		
β-cell	Mild diabetes, obesity	[63] [62] [64]
Insulin receptor + IGF-1 receptor		
Muscle	Insulin resistance, β-cell dysfunction and diabetes	[136]
Akt		
β-cell (overexpressor)	Islet hyperplasia, hyperinsulinemia	[137] [138]

the insulin gene in mice leads to growth retardation, and death due to diabetes mellitus with ketoacidosis and liver steatosis [31,32]. Interestingly, pancreas examination during the post-natal period revealed large islets and prompted the authors to suggest that insulin is a negative regulator of islet growth [31,32]. However, since IGF-II levels are reported to be elevated during the immediate post-natal period [28], it is possible that lack of insulin allows for unopposed action of IGF-II at both insulin and/or IGF-1 receptors to promote islet hyperplasia. Alternatively, the enhanced vascularization in the absence of insulin may lead to an increase in local concentrations of morphogens, derived from the circulation and/or endothelial cells, to promote islet cell growth [33–35]. A recent study in which the IGF-I gene was inactivated in islets using the PDX-1 promoter described hyperplastic islets that are resistant to streptozotocin-induced diabetes [36]. The increase in the size of islets was disproportionate to the mild hyperglycemia suggesting that IGF-II or insulin acting via insulin and/or IGF-1 receptors enhanced islet growth in the absence of locally produced IGF-I. In this context, it is worth noting that over-expression of IGF-II in β-cells has also been reported to lead to hyperplastic islets [37] and intriguingly the mice develop diabetes [38]. While the islet growth effects induced by IGF-II could be mediated via the insulin receptor, the creation of a model of IGF-II overexpression in a mouse lacking insulin receptors in β-cells will directly address whether the IGF-II/insulin receptor pathway is indeed critical in islet/β-cell growth.

Not surprisingly, mice with null mutations of the IGF-I and IGF-II genes show similar but milder defects compared to mice lacking the insulin gene (reviewed in [32,39–41]. IGF-I null mice show growth defects similar to IGF-II null mutants [39,41], and depending on the genetic background, some of the IGF-I knockouts die, while others survive into adulthood [40,41]. Mice lacking the IGF-I gene exhibit postnatal lethality, growth retardation, infertility, and defective development of bone and muscle [41,42]. Similar findings were reported in a human with homozygous partial deletion of the IGF-I gene [43]. Taken together these global knockouts underscore the crucial importance of insulin and IGF-I and their cognate receptors in the overlapping regulatory functions of metabolism and growth in mice and humans.

Insulin and IGF-I mediate their effects via the insulin and IGF-1 receptors respectively. Considering the high degree of homology between the insulin and IGF-1 receptors it is likely that the ligands can also act via their cognate receptors [39]. Thus, one would predict either similar phenotypes when either of the receptor is lacking or alternatively one receptor could compensate for the absence of the other receptor in an effort to maintain normal signaling in target tissues. Mice homozygous for a null mutation of the insulin receptor show normal intrauterine growth but die within 48 to 72 h after birth due to severe hyperglycemia and diabetic ketoacidosis [44,45]. On the other hand, IGF-1 receptor null mutants show severe growth

deficiency and die at birth due to respiratory failure [41] and manifest a phenotype similar to the IGF-1 null mutants [40]. Although both mutants die early, these studies clearly indicate the mice are born with β-cells. While β-cells in insulin receptor null mutants show degranulation, which likely occurs due to severe hyperglycemia, mice lacking functional IGF-1 receptors show small [46] or relatively normal islet/β-cell mass [47]. These studies provide evidence that neither receptor is critical for the early development and formation of β-cells. Furthermore, these findings have been confirmed in conditional knockouts of insulin or IGF-1 receptors. Thus, β-cell-specific insulin receptor knockouts (βIRKO) [48] or β-cell specific IGF-1 receptor nulls [49,50] are both born with a normal complement of islets/β-cells. Considering the overlapping signaling pathways shared by insulin and IGF-I stimulation, both these knockouts develop secretory defects manifested by blunted glucose-stimulated insulin release secondary to poor glucose sensing [48–51,141,142,147]. However, one notable difference between the two mutants is the effect on maintenance of β-cell mass in adults. Thus, follow-up studies indicate an increased susceptibility of βIRKO mice to develop age-dependent diabetes consequent to a reduced β-cell mass [51,52]. In contrast, β-cell mass in 12 month-old βIGFRKO mice is relatively normal [49] (R.N. Kulkarni unpublished observations). Together, these studies point to similar phenotypes related to secretory function when the insulin or IGF-1 receptors are disrupted selectively in β-cells but suggest a prominent role for insulin signaling in maintenance of adult β-cell mass (see below).

Humans bearing mutations of the insulin receptor (leprechaunism), however, manifest quite a different phenotype characterized by intrauterine growth retardation and only mild hyperglycemia and display large islets [53,54]. It is unclear whether the receptor is devoid of all signaling capability or whether the mutated protein continues to transmit some signals that allows selective growth pathways to be active [54]. Thus, it is possible that the islet hyperplasia in humans with leprechaunism is due to selective activation of proteins critical for mediating growth effects in the β-cells. The lack of reports describing mutations in insulin or IGF-1 receptors that are restricted only to β-cell in humans makes it difficult to resolve the issue of whether insulin signaling plays a role in modulating β-cell growth and function *in vivo*. The creation of mouse models bearing mutations in β-cells, similar to mutations that occur in humans, perhaps using a knock-in strategy, is one way to gain insight into this question.

Gene deletion of proteins downstream to the insulin and IGF-1 receptor, including the IRS proteins, leads to different phenotypes compared to those observed in the receptor mutants. Thus, IRS-1 knockouts exhibit post-natal growth retardation and hyperinsulinemia but a relatively normal lifespan [55,56] and interestingly, the IRS-1 null mice show hyperplastic but dysfunctional islets [57–61]. In contrast, IRS-2 knockouts show only mild growth retardation, and depending on the genetic background of the founder mice either manifest a mild phenotype [58] or develop β-cell hypoplasia leading to overt diabetes [46]. It must be noted, however that β-cell-specific loss of IRS-2 does not prevent development and growth of β-cells during the embryonic and early post-natal periods [62–64]. Notwithstanding the observation that a few β-cells "escape" *Cre* recombination, it is intriguing that lack of IRS-2 in a "majority" of β-cells during the early post-natal period does not lead to a phenotype as severe as that observed in global mutants despite being created on similar genetic backgrounds. This indicates that signals independent of IRS-2 are likely necessary during early growth and development of β-cells. Thus, it is prudent to exercise caution when interpreting and extrapolating data from global mutants to alterations in function and growth of specific cell types.

Further, global IRS-2 knockouts also show defects in the CNS that indicate a potential role for the substrate in neuronal function [65]. Pituitaries of female IRS-2 deficient mice are small and have a reduced number of gonadotrophs and the mutants show hypothalamic resistance to leptin. By contrast, IRS-3 null mice develop normally and have normal glucose tolerance [66]. IRS-4 deficient mice manifest mild growth defects and glucose intolerance and this is evident only in males because the IRS-4 gene is located on the X chromosome [67]. Knockout of the p85 regulatory subunit of PI 3-kinase in mice leads to increased insulin sensitivity and hypoglycemia [68–70], while null mutants for Akt-2 (PKB-β) show insulin resistance in muscle and liver and an increased islet mass [71]. Mutants for p70S6 kinase show reduced β-cell size, lower insulin secretion and reduced pancreatic insulin content [72].

The lack of a defect in the early growth and development of islet/β-cells in mice lacking insulin or IGF-1 receptors indicates that other growth factors are likely important for the development of the insulin-secreting cells (Fig. 1). Indeed, several reports indicate a role for placental lactogen and GH during the early development of the pancreas (reviewed in [73,74,143,144]). Furthermore, over-expression of placental lactogen [75], PThRP [76] and HGF [77] individually in β-cell using the rat insulin promoter lead to an increase in β-cells mass and resistance to strepotozotocin-induced β-cell death. It will be useful to examine the potential cross-talk between signaling pathways activated by the receptor tyrosine kinases for these growth factors and the insulin/IGF-I signaling pathway to dissect the mechanisms underlying the growth and apoptosis of islet β-cells.

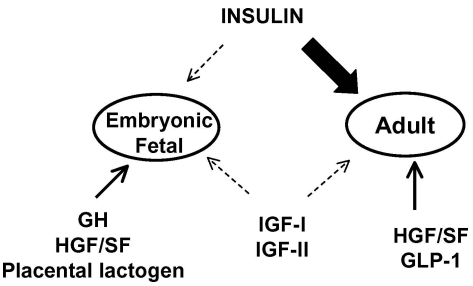

Fig. 1. *A schematic showing relative significance of growth factors on growth of β-cells during embryonic and adult periods. Data supported by direct evidence is indicated by arrows with solid lines and indirect evidence by dotted lines.*

Role of Insulin and IGF-I in the Maintenance of Adult β-Cell Mass

The mechanisms and signaling pathways that maintain adult β-cell mass are currently intense areas of research in stem cell biology and especially in type 1 and type 2 diabetes. Several mechanisms have been proposed to influence adult β-cell mass including neogenesis from ductal cells [78,79] and apoptosis [80,81]. Recent studies using lineage trace analysis provide compelling evidence for β-cell replication as a major pathway for the renewal of adult β-cells in mice [82]. Whether a similar mechanism is operative in humans is not known and impossible to prove by lineage trace analysis. Some evidence for DNA duplication is available from two recent studies suggesting dedifferentitaion and differentiation of human islet precursor cells [83,84]. Nevertheless, it is conceivable that all three processes are occurring to maintain an appropriate number of β-cells for glucose homeostasis, and identifying the major pathway that contributes to β-cell regeneration will be key to plan strategies to intervene therapeutically. Therefore, until lineage trace analyses or a similar technique can conclusively prove that neogenesis from duct or periductal cells is also a major source of β-cells in rodents, or studies in humans conclusively show β-cell regeneration does not involve mitosis, efforts must be targeted to understand the basic mechanisms regarding β-cell replication. Some immediate questions include—what are the signals that promote β-cell replication and how many times can a single β-cell divide over its life span? Answers to these questions, though not trivial, will likely provide therapeutic targets to enhance β-cell growth.

A recent study examining the role of cyclin D1 D2 supports the replication hypothesis [85,145]. Mice lacking cyclin D2 showed a selective decrease in β-cell expansion while maintaining normal ductal cells suggesting that the cell cycle protein is important for proliferation of β-cells independent of influencing duct cells.

One potential mechanism that can contribute to β-cell expansion is a well-recognized pathway that has been studied in considerable detail in organogenesis and in cancer [86,87]. Epithelial-to-mesenchymal transition or EMT occurs in epithelial cells expressing tyrosine kinase receptors and involves disappearance of differentiated junctions, reorganization of cytoskeleton and redistribution of organelles, together transforming epithelial into mesenchymal cells [87–89]. Eventually the mesenchymal cells may regain a fully differentiated epithelial phenotype via a mesenchyme-to-epithelial transition (MET) or reverse EMT [90]. A characteristic feature of EMT is repression of epithelial markers including E-cadherin and α- and γ-catenins and induction of mesenchymal markers including vimentin, fibronectin and N-cadherin [87,91]. Although the term EMT has mostly been associated either with early development or neoplasia, it is possible that this process is occurring, albeit modified, in normal cells responding to physiological demands that require cell/tissue expansion. Indeed, the E-cadherin/catenin family of proteins can also act as master regulatory and signaling molecules for differentiation, proliferation and apoptosis [87,92] indicating that these proteins have the capacity to regulate growth in normal tissues [92]. Cell-cell adhesion, as mediated by the cadherin-catenin system, is a prerequisite for normal cell function and the preservation of tissue integrity in most tissues including islet cells. Both E-cadherin and β-catenin and several other members of the cadherin/catenin family are under the control of growth factors including epidermal growth factor (EGF), hepatocyte growth factor/scatter factor (HGF/SF) and insulin like-growth factors (IGF-I and IGF-II) [87,90]. Receptors for these growth factors are expressed in β-cells and proteins in their signaling pathways have been reported to play functional roles in β-cell growth and hormone secretion. Intriguingly, treatment of mouse embryonic stem cells or rat bladder carcinoma cells with IGF-II induces EMT and the withdrawal of IGF-II allows a reversal of the phenotype to an epithelial cell [90]. Direct association between insulin/IGF-1 receptors with the E-cadherin-catenin system forming a multi-element complex has been recently suggested based on co-localization of IGF-1 receptors with E-cadherin and β- and α-catenins at points of cell contacts [87]. The presence of IGF-1 receptors and its substrate proteins insulin receptor substrate-1 and SHC in the same complex with E-cadherin indicates potential cross talk between growth factor and catenin-cadherin signaling pathways [87]. Further evidence for a role for cadherin-catenin complex in islet growth is provided by mouse experiments in which dominant-negative expression of E-cadherin on the rat insulin promoter perturbed islet formation without increasing the incidence of tumor formation [87,93]. Together, these data provide a basis for

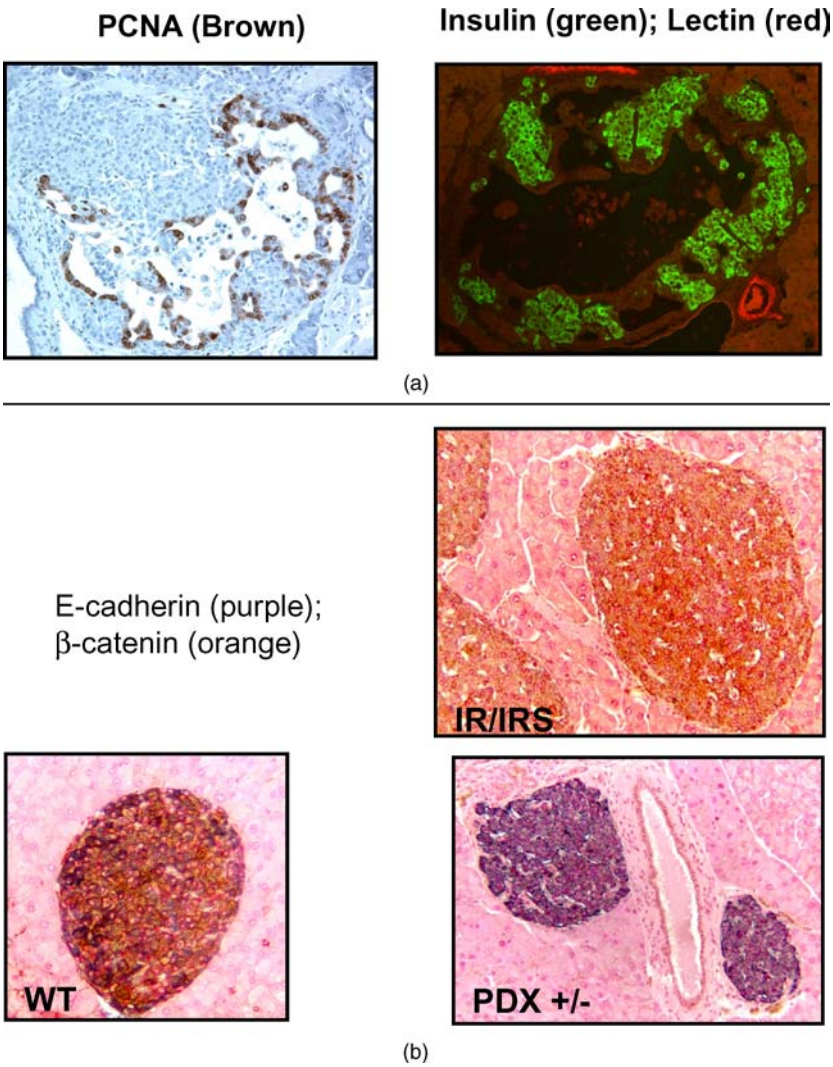

Fig. 2. *(a) Representative islet from a mouse model of islet hyperplasia (insulin receptor/insulin receptor substrate-1 double heterozygous (DH) mouse) showing multiple PCNA+ cells. A serial section from the same pancreas shows that PCNA+ cells are independent of a pancreatic ductal marker (lectin). (b) Pancreas sections from wild-type (WT), IR/IRS-1 double heterozygotes (IR/IRS-1) and PDX-1 haploinsufficient mice (PDX-1+/−). The WT islet shows immunostainng for both E-cadherin and β-catenin, while E-cadherin is down-regulated in DH islet and β-catenin is down-regulated in PDX+/− islet. Figures 2a and 2b reproduced with permission from Kulkarni et al., J Clin Invest 114(6):828–836, 2004.*

a link between growth factor signaling and the potential for EMT in islet/β-cell growth.

Recently, features suggestive of EMT were described to occur *in vivo* in a mouse model of insulin resistance manifesting robust islet hyperplasia [94]. The presence of PCNA+ cells within the islets, which also showed down regulation of E-cadherin provided evidence for alterations in adhesion properties and an ability of the cells to replicate (Fig. 2(a)) [94]. Furthermore, the lack of close association of replicating cells with lectin (a ductal marker), in multiple pancreas sections, suggests the cells are independent of pancreatic ducts and are likely replicating β-cells that have undergone metaplastic changes. We also observed down-regulation of β-catenin, another adhesion protein, in islet cells from a mouse which is haploinsufficient for PDX-1, indicating a potential role for the homeodomain protein in the EMT process (Fig. 2(b)). In fact, growth factor signaling has been linked to PDX-1-mediated regulation of β-cell growth [139] providing additional evidence for a role for PDX-1 in β-cell regeneration. Thus, it is possible that EMT or an EMT-like process, may promote β-cell expansion under the appropriate stimulatory conditions induced by insulin resistance. The report that EMT also occurs in human islet cell precursor cells [83,84] suggests that this process is a common response across species. Whether this indeed occurs *in vivo* in humans in early stages of diabetes is an important but difficult question to address.

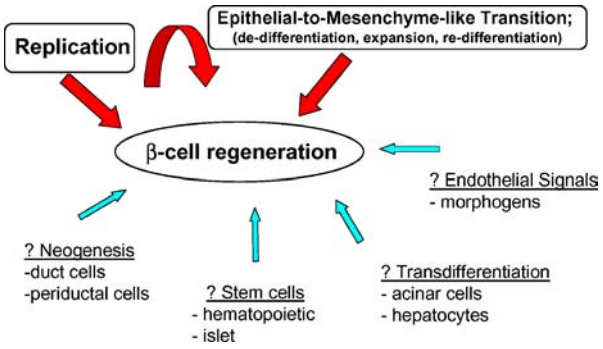

Fig. 3. *A schematic of potential mechanisms contributing to β-cell regeneration.*

It has been recognized for over a decade that β-cells compensate in order to overcome the ambient hyperinsulinemia [52,95]. However, few studies have examined the pathways and proteins underlying the islet hyperplastic process. Since circulating insulin levels are significantly elevated in insulin resistance, an obvious candidate for β-cell proliferation is insulin itself [23,96] (Fig. 1). In fact, insulin has been shown to enhance islet β-cell replication in neonatal rat monolayer cultures [97] and to increase the regenerative ability of β-cells in transplantation models of fetal rat pancreas [98,99]. A role for insulin as a growth factor is also supported by studies in βIRKO mice, which display an age-dependent decrease in β-cell mass [48,51] and by reports that treatment of MIN6 β-cells with insulin receptor siRNA leads to altered expression of cell cycle proteins and proliferation [100]. In addition to its nutrient role, glucose has been shown to increase β-cell mass in several models (reviewed in [73])[101]. Whether the effects of glucose are mediated by the secreted insulin acting in an autocrine manner to promote growth and/or prevent apoptosis in β-cells requires further study in models lacking insulin receptors in β-cells. Similarly, it is possible that the effects of GLP-1 on β-cell proliferation [19,102] are mediated, in part, by secreted insulin acting in an autocrine manner.

Other mechanisms that have been reported to contribute to regeneration of β-cells include transdifferentiation from acinar cells [103,104] and the ability of transcription factors to induce hepatocytes to differentiate into insulin-secreting cells [105,106]. Figure 3 shows a schematic of current concepts on potential pathways of β-cell regeneration.

The Liver-Pancreas Connection

The liver and the pancreas are known to share a common developmental pathway [107,108] and express several common transcription factors, which are essential for their growth [108]. Therefore, it is not surprising that even in the adult organism there is evidence suggestive of communication between the two metabolic tissues. For example, mice with a hepatic glucokinase knock-out manifest impaired insulin secretion in response to glucose suggesting that loss of hepatic glucose sensing impacts on islet function [109]. Furthermore, the liver has long been recognized as a source of circulating growth factors including IGF and HGF/SF, both of which are known to especially influence islet growth (reviewed in [52]) [28,77,110,111]. In this context, it is interesting that mice lacking insulin receptors in hepatocytes develop large islets [112]. One interpretation of these observations is that in pathophysiological states, the insulin-resistant liver may potentially transmit signals to the islets via secreted growth factors to allow for β-cell compensation [52]. Although studies in hepatocytes implicate a role for insulin in regulating the hepatocyte nuclear transcription factor 3β (Foxa2) [113], and PDX-1 has been linked to growth factor signaling in the context of β-cell growth [139,142], further studies to define the proteins linking upstream signals such as insulin with transcription factors in islets/β-cells are worth exploring [114].

Growth and Development of Islet α-Cells

Insulin and IGF-1 receptors are also expressed in islet α-cells [115,116] and their role in early development and growth of glucagon-producing cells is not fully explored. However, a relative increase in α-cell number is a recognized feature in adult patients with type 2 diabetes [117]. Whether potential stimulation of α- cell proliferation is in fact mediated by high circulating levels of insulin in established cases of type 2 diabetes is not clear. Several studies implicate a role for intra-islet insulin to suppress glucagon release as an important factor in poor recovery from hypoglycemia in patients with long-standing type 1 diabetes and in advanced stages of type 2 diabetes on insulin therapy [118,119]. The signaling proteins and pathways that mediate this inhibitory effect are not fully defined. A recent study, in which mice were treated with glucagon receptor inhibitors, reported a significant increase in α-cell hyperplasia providing evidence for therapeutic intervention at the receptor level in modulating the ability of islet cells to grow [120].

Future Insights

Genetic engineering techniques have revolutionized the understanding of the role of insulin and IGFs in the development and maintenance of β-cell mass. An understanding of the link between insulin/IGF-I/FoxO1 signaling and PDX-1 with molecules that regulate β- and α-cell cycle control will be crucial for the development of therapeutic strategies aimed at promoting β-cell regeneration.

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