Cell Biochem Biophys (2007) 48:115–125
DOI 10.1007/s12013-007-0016-7

ORIGINAL PAPER

Does IGF-I stimulate pancreatic islet cell growth?

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Published online: 25 April 2007
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Abstract Both IGF-I and its receptor (IGF-IR) are specifically expressed in various cell types of the endocrine pancreas. IGF-I has long been considered a growth factor for islet cells as it induces DNA synthesis in a glucose-dependent manner, prevents Fas-mediated autoimmune β-cell destruction and delays onset of diabetes in non-obese diabetic (NOD) mice. Islet-specific IGF-I overexpression promotes islet cell regeneration in diabetic mice. However, in the last few years, results from most gene-targeted mice have challenged this view. For instance, combined inactivation of insulin receptor and IGF-IR or IGF-I and IGF-II genes in early embryos results in no defect on islet cell development; islet β-cell-specific inactivation of IGF-IR gene causes no change in β-cell mass; liver- and pancreatic-specific IGF-I gene deficiency (LID and PID mice) suggests that IGF-I exerts an inhibitory effect on islet cell growth albeit indirectly through controlling growth hormone release or expression of Reg family genes. These results need to be evaluated with potential gene redundancy, model limitations, indirect effects and ligand-receptor cross-activations within the insulin/IGF family. Although IGF-I causes islet β-cell proliferation and neogenesis directly, what occur in normal physiology, pathophysiology or during development of an organism might be different. Locally produced and systemic IGF-I does not seem to play a positive role in islet cell growth. Rather, it is probably a negative regulator through controlling growth hormone and insulin release, hyperglycemia, or Reg gene expression. These results complicate the perspective of an IGF-I therapy for β-cell loss.

Keywords Reg family proteins · Insulin · Tissue-specific gene targeting · IGF-I receptor · Islet β-cells · Growth · Secretion · Apoptosis · Overexpression · Diabetes.

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Introduction

Islet β-cell mass is a key element in the development of autoimmunity-induced type 1 diabetes (T1D) and in compensating insulin resistance in type 2 diabetes (T2D) [1–5]. It has been known for years that insulin-like growth factors (IGF-I and -II) stimulate islet cell growth and promote the survival of transplanted islet cells in rodents [6–13]. Recently, this established view is challenged by several prominent reports of tissue-specific gene targeting. They indicate that IGF-I is not involved in islet cell growth and undermine its application in islet regeneration, protection and transplantation. It is thus necessary to re-evaluate what we know about the role of a prominent growth factor such as IGF-I in a crucial aspect against onset of diabetes, namely the islet cell growth. While I focus on IGF-I acting through its cognate receptor (IGF-IR), highly homologous insulin receptor (IR) also plays a role [14–17]; the intracellular signal mechanisms of IGF-I actions has been frequently reviewed [18, 19]; as well, the role played by key signal molecules (such as IRS-2, PI3K, Erk1/2, Akt1/PKBα, Foxo1, mTOM, and Pdx1) will not be discussed in length [20–23].

IGF-I is a members of the insulin/IGF family

From alignment of their primary peptide sequences, IGFs are highly homologous to proinsulin and perhaps derived from gene duplications of a common ancestor. Together, they form a family of 3 hormones, 3 trans-membrane receptors, and specific binding proteins for IGFs (IGF-BPs) [24]. Within this family, insulin is produced from β-cells of the pancreatic islets and plays a central role in fuel metabolism. IGF-I is expressed in many cells and tissues during embryonic and postnatal development as well as in adult animals. Although this growth factor is also synthesized extrahepatically and promotes postnatal growth in bone, muscle, fat, and other tissues, it has been well established that the primary source of circulating IGF-I is the liver [25–27]. IGF-II is expressed widely in early development and is important for intrauterine development. Unlike insulin that stays free in the circulation, most IGFs are tightly bound to IGF-BP proteins that prolong their half-lives and limit access to receptors [28]. These three peptide hormones and/or growth factors act through IR, IGF-IR, and perhaps IR-related receptor (IRR), in a highly specific fashion, to regulate a broad range of biological activities. Through specific ligand-receptor interactions, factors of insulin/IGF family affect cell growth and metabolism through seemingly common pathways intracellularly, as reviewed elsewhere [18].

IR has widespread distributions and is particularly abundant in insulin targets such as the liver, skeletal muscles, and adipose tissues. The receptor has two isoforms, type A and B which differ in the presence of exon 11-encoded sequence in IR-B protein [29]. IR-A is expressed during development and in tumor cells and mediates actions of insulin and IGF-II, through high affinity binding to the ligands. IR-B is expressed in adult insulin target cells and normally responds only to insulin itself. IGF-I has much lower affinity to either IR subtypes. The highly homologous IGF-IR is a transmembrane heterotetrameric tyrosine kinase and is ubiquitously expressed in cell types derived from all three embryonic lineages, in a tissue-specific manner. It can be activated by both IGF-I and IGF-II, as well as insulin albeit with much lower affinity. Upon ligand binding, the receptor undergoes autophosphorylation on intracellular tyrosine residues and activation of its intrinsic tyrosine kinases [25]. In recent reports, human and murine IGF-I deficiency causes severe intrauterine growth retardation, perinatal lethality, postnatal growth retardation, and defects in the development of major organ systems [30–33]. In contrast, overexpression of IGF-I in transgenic mice leads to widespread tissue hypertrophy in brain, heart, muscle, and intestine [34–37]. Therefore, IGF-I is essential for normal cell growth, intrauterine development, and postnatal growth.

IRR is a new member of the family and highly expressed in the pancreatic islets as well as in differentiated β-cell lines. As an orphan receptor, IRR exhibits preferential binding to neither insulin nor IGFs. To study its signaling pathway, a chimeric receptor consisting of the extracellular domain of IR and the intracellular domain of IRR was created and proved functional because insulin caused tyrosine phosphorylation at the catalytic domain [38]. Insulin also stimulated the tyrosine phosphorylation of insulin receptor substrate (IRS)-1 and IRS-2, indicating that both proteins serve as substrates of IRR in intact cells [39, 40]. Since IRR engages in heterodimer formation with both IR and IGF-IR, it probably functions by modulating IR and/or IGF-IR signaling [38]. To address whether it plays a physiological role, mice lacking IRR were found to have normal islet morphology, β-cell mass, and insulin secretion. Moreover, lack of IRR did not impair compensatory β-cell hyperplasia in insulin-resistant $IR^{+/-}$ mice, nor did it affect β-cell development and function in $IR^{-/-}$ mice. It seems that glucose-stimulated insulin secretion and embryonic β-cell development occur normally in mice lacking IRR [41], despite its higher level expression in β-cells than either IR or IGF-IR. In fact, metabolic analyses and insulin release studies from perifused islets of IRR knockout mice have thus far failed to demonstrate any abnormalities [42]. Lack of phenotype might be explained by gene redundancy; or possibly by an ineffective gene inactivation because it was achieved by inserting a neomycin cassette into the exon 3 rather than disrupting the endogenous gene entirely.

IGF-I Inhibits insulin secretion but stimulates islet cell growth

IGF-I could influence islet cell function either as a paracrine/autocrine factor or as a liver-derived hormone as both IGF-I and the IGF-I receptor are expressed in islet cells [43]. IGF-I mRNA and immunoreactivity have been variably detected in islet cells in the fetus and neonate [10]. By Northern blot analysis, IGF-II is the major IGF expressed in the fetal and neonatal rat pancreas, localized in islet cells as well as in some ductal epithelial cells. By the 2nd postnatal week, IGF-II expression is replaced by IGF-I. Isolated rat islet α- and β-cells express IGF-IR, so do islet-derived cell lines that synthesize insulin, glucagon, or somatostatin [13]. In adult rat islets, IGF-I is localized to all three major cell types, the β-, α-, and δ-cells [11, 12].

It is generally accepted that IGF-I inhibits insulin secretion, stimulates β-cell growth and inhibits cell apoptosis, although conflicting results are sometimes reported. IGF-I inhibits insulin secretion from perfused rat pancreas in response to either glucose or arginine [44]. In conscious

rats under hyperglycemic conditions, IGF-I reduces plasma insulin levels probably by reducing blood glucose levels [45]. IGF-I has been proposed to directly inhibit insulin secretion by activating phosphodiesterase 3B (thus decreasing intracellular cAMP levels) and protein kinase B [46, 47]. Interestingly, in isolated rat islets under perifusion and in the presence of high-level glucose, low doses of IGF-I (e.g., 13.3 nM or 102 ng/ml) initially stimulated insulin secretion. At a higher concentration of 53 nM (405 ng/ml), however, the effect became significantly inhibitory [48]. It is also reported that IGF-I does not inhibit insulin secretion in cultured human islets [49]. Thus, normal low levels of IGF-I most likely stimulate insulin secretion, while an acute, high concentration of IGF-I becomes inhibitory, possibly through different mechanisms. Recent studies of β-cell-specific IGF-IR gene inactivation further strengthen the role of IGF-I in insulin secretion (see Section entitled, ''Cell-specific IGF-IR gene deficiency causes impaired insulin secretion without affecting β-cell growth''). These mice exhibit increased basal release of insulin but decreased response to glucose stimulation. Decreased GLUT-2 and glucokinase gene expression in β-cells further indicate that IGF signals are essential for normal insulin secretion.

Substantial evidence supports a role for IGFs in islet cell growth [50]. In early reports, DNA synthesis in neonatal rat islet cultures is stimulated by multiplication stimulating activity (MSA; an IGF), in the presence of high-level glucose. This effect seems independent of IR as it is 100-fold more potent than insulin and cannot be blocked by IR antibody [51]. In purified adult rat islets, both IGF-I and glucose stimulate DNA synthesis synergistically [10], demonstrated with IRS-2-mediated phosphorylation of PI3K and mSOS [11]. In neonatal rodents, the β-cell mass undergoes a phase of remodeling that includes a wave of apoptosis, which peaks at approximately 2 weeks of age and is probably caused by lack of IGF-I signaling during a dynamic interplay with IGF-II [52]. In more recent reports, IGFs are potent trophic factors for transplanted fetal pancreatic islets, helping to reverse the diabetic symptoms in streptozotocin diabetic rats [7–9]. IGF-I is shown to promote β-cell development and survival via the IRS-2 signaling pathway in intercrossed heterozygous mice of IGF-IR/IRS-1/IRS-2 knockouts [53]. Unlike other growth factors (TGF-α and EGF), IGF-I can cause a prolonged activation of Erk1/2 and PI3K via recruitment of specific docking proteins, especially IRS-2, and induction of β-cell mitogenesis [20]. IGF-I induces proliferation of rat insulinoma-1 cells, in a glucose-dependent manner, via IRS-induced activation of PI3K and the 70-kDa S6 kinase ($p70^{S6K}$) [11]. Also supporting the IGF-I effect, overexpression of active Akt1/PKBα (a substrate of IGF-I or insulin action) in the mouse β-cells substantially increases both β-cell size (hypertrophy) and total islet cell mass [22].

IGF-I prevents islet cell apoptosis and serves as a survival factor for autoimmune destruction of islets [54–57]. Administration of IGF-I prevented Fas-mediated autoimmune β-cell destruction and delayed the onset of diabetes in non-obese diabetic (NOD) mice by minimizing insulitis [54–57]. IGF-I-treated animals had a higher percentage of intact islets (49% vs. 2%), a lower percentage of lymphocytic infiltration and a higher overall β-cell mass, exhibiting a significant protection against autoimmune diabetes. When complexed with IGF-BP3, the IGF-I effect in preventing diabetes becomes more effective [13]. In purified human islets, adenoviral gene transfer of IGF-I prevents cytokine-induced β-cell death through diminished nitric oxide production [12]. Similarly, IGF-II significantly improved islet cell viability in 6-day cultured, microencapsulated rat islets and reduced the amount of islet cells required to achieve normoglycemia by transplantation in diabetic mice [7]. The anti-apoptotic effect of IGF-I in islet cells is dependent upon PI3K activation. In cultured β-cell lines (INS-1 and MIN6), IGF-I prevented cell death with increased phosphorylation of protein kinase B and other PI3K substrates such as glycogen synthase kinase 3β, BAD, FKHR, and $p70^{S6K}$ [58].

Insulin stimulates its own secretion and islet β-cell growth

Pancreatic islet β-cells are known to express IR and IRSs. It has been proposed that secreted insulin from the islet β-cells stimulates its own synthesis and secretion [59–61]. Using amperometry on single β-cells, insulin causes an autocrine stimulation of insulin secretion, which only requires physiological levels of insulin (~4 nM) and can be blocked by IR antibody [62]. This effect seems to be mediated by IRS-1, PI3K, and the release of intracellular calcium stores [63]. Consistent with an positive, autocrine feedback mechanism, β-cell-specific IR gene deficiency (βIRKO) causes impairments in glucose-stimulated insulin release and glucose tolerance [15, 16]. In vitro evaluation using perfused pancreas also confirmed an islet secretory defect in response to a ''ramped'' increase in glucose concentration. At 8 weeks, the βIRKO mice exhibit a selective loss of glucose-stimulated acute insulin secretion, accompanied by reduced glucokinase expression in the β-cells [59]. Consequently, these mice develop progressive glucose intolerance and, in fact, some become overtly diabetic at 7–10 months. Thus, loss of the IR in the β-cells leads to altered glucokinase expression and impaired insulin secretion, a characteristic feature of T2D [15]. As for insulin biosynthesis, it was proposed that insulin

activates the transcription of its own gene by signaling through IR-type A, PI3K class Ia, and p70^{S6K} [59].

Different and even opposite results have been reported on the role of insulin on β-cell secretion. In isolated pancreatic islets, neither exogenously added nor secreted insulin affected insulin secretion, proinsulin translation, or insulin mRNA levels [64]. Using perforated patch clamp technique, insulin induces hyperpolarization in single β-cells and inhibits electrical activities. This PI3K-dependent effect is potent enough to abolish high glucose-induced elevation in intracellular calcium concentration [65]. Moreover, gene-deficient IR$^{-/-}$ mice are born with slight growth retardation (~10%) but without apparent metabolic abnormalities [66, 67]. After birth, metabolic control rapidly deteriorates: glucose levels increase upon feeding and insulin levels rise up to a thousand fold above normal, although the massively increased insulin output is most probably secondary to uncontrolled hyperglycemia, rather than to indicate that insulin inhibits its own secretion. β-cell failure occurred within days, characterized by the disappearance of insulin storage granules within the cytoplasm and followed by death of the animals due to diabetic ketoacidosis. These inconsistent results call for further studies using better tools.

The observation that insulin stimulates islet cell growth in diabetic rats and neonatal rat islet culture has been reported earlier [51, 68]. Insulin promotes islet regeneration (neogenesis and proliferation) in neonatal diabetic rats treated by streptozotocin [14, 69]. Consistently, evaluation of pancreatic sections of the βIRKO mice show a 18% reduction in β-cell mass, which becomes more severe (31% reduction) in the diabetic βIRKO mice vs. age-matched control littermates [15]. Given the nature of the morphometric quantification and limited sample sizes (n = 3~6), the 18~31% reductions do not seem very significant. The defects in β-cell secretion and growth are likely mediated via IRS-1 and IRS-2 respectively, since complete knockout of IRS-1 leads to defective insulin secretion in response to glucose and amino acids [70], whereas inactivation of IRS-2 leads to impaired β-cell neogenesis and proliferation [21]. Downstream of IRS-2, Foxo1 seems to act as a repressor to Pdx1 promoter, as partial inactivation of Foxo1 restores Pdx1 gene expression and β-cell proliferation in IRS-2$^{-/-}$ mice [23]. It is thus proposed that insulin/IGFs stimulate β-cell proliferation and neogenesis through IRS-2 by relieving Foxo1 inhibition of Pdx1 expression in a subset of cells embedded within pancreatic ducts [71]. On the other hand, morphometric analysis of mice lacking both non-allelic insulin genes reveal enlarged islets of Langerhans in the pancreas, suggesting that insulin might function as a negative regulator of islet cell growth [72]. Indeed, increased islet cell hyperplasia and decreased apoptosis enabled enlarged islets without changing individual cell size. The relative hyperplasia of the islets in embryos of late gestation might be related to an increased vascularization of the pancreas [73]. As insulin deficiency also causes hyperglycemia and neonatal lethality, one has to be cautious in interpreting the role of insulin itself on β-cell growth and to design better experiments to clarify the situation.

In vivo overexpression of IGF genes affects β-cell growth

Studies from IGF-I and -II transgenic models reveal specific effects of IGF-IR activation, although IGF-II is also known to activate IR-type A [29]. Using a transgene driven by the metallothionein-I and other promoters, mice overexpressing IGF-I in multiple tissues display elevated serum IGF-I levels and increased body mass without a significant increase in linear growth [74–76]. The increase in weight is due to tissue hypertrophy in many organs, including brain, heart, muscle, and intestine [34–37]. The pancreas of MT-I/IGF-I transgenic mice is enlarged 2-fold without affecting islet histology [74, 77]. While this may reflect an influence of endocrine IGF-I, specific overexpression of IGF-I in the pancreatic islets has been reported. In vivo, β-cell-specific overexpression of IGF-I (in RIP-IGF mice), unlike IGF-II, causes no change in normal islet growth and β-cell mass [6, 78]. Upon streptozotocin treatment, these mice on C57BL/6-SJL background exhibit delayed onset of T1D. More importantly, in both C57BL/6-SJL and CD-1 strains, RIP-IGF mice exhibit a quick recovery from diabetes to normal glycemia in 4–6 months, in contrast to wild-type controls that never recover [6]. Upon close examination, RIP-IGF mice after streptozotocin treatment have elevated rate of neogenesis and decreased rate of apoptosis of the β-cells, both of which increase the overall β-cell mass. Thus, IGF-I plays a role in β-cell neogenesis when most β-cells are damaged. In a similar approach, we have demonstrated a partial rescue in islet cell growth by IGF-I overexpression in growth hormone receptor gene-deficient mice [78].

On the other hand, IGF-II overexpression affects islet cell growth profoundly. In a mouse model of Beckwith-Wiedemann syndrome, overexpression of IGF-II in fetal life causes profound islet hyperplasia and a reduction in the attrition of islet cells through apoptosis [79, 80]. Specifically, at the end of gestation, the fetal islets of transgenic offspring are of irregular shape and ~5-fold enlarged (in area) than that in wild-type controls. The islet density and average size of individual cells are unaffected. Further, significant increases in cell proliferation and decreases in apoptosis within the islets of these mice cause an increase in α-cell and decrease in β-cell populations. These changes were accompanied by reduced endogenous

IGF-I expression in the pancreatic islets and normal circulating insulin and serum glucose levels. In another report, overexpression of IGF-II in the pancreatic β-cells increases β-cell mass and insulin mRNA levels. However, the architecture of islet cells is altered in such a way that α-cells are randomly distributed throughout the core of the pancreatic islets. As a result, these mice display hyperinsulinemia, mild hyperglycemia, altered glucose and insulin tolerance tests and about 1/3 of them develop overt diabetes on a high-fat diet [81].

Liver-and pancreatic-specific IGF-I gene deficiency increases β-cell growth

As an essential growth factor, a total deficiency of IGF-I causes high rate of neonatal death and severe defects in development. Although a small percentage of IGF-I$^{-/-}$ offspring does survive, study of their islet phenotype might not be truly representative of IGF-I function. Nevertheless, studies on the affected embryos and the minority surviving IGF$^{-/-}$ mice reveal no change in normal islet development and growth [33, 82]. Thus, more efforts have been made to determine the effects of tissue-specific, e.g., liver- or pancreatic-specific, IGF-I gene deficiency using a Cre/loxP system [83]. Following an initial assessment of their growth phenotype, we have studied the metabolic consequences of a liver-specific IGF-I-deficiency (LID) [26, 27, 84, 85]. The LID mice show a marked reduction in serum IGF-I and concurrent elevation in growth hormone levels, due to lack of feedback inhibition by IGF-I. Consequently, adult LID mice also show a 4-fold increase in serum insulin level and apparent insulin resistance, although the blood glucose level and glucose tolerance are unaffected. Interestingly, the average size of the pancreatic islets is increased by 2-fold in LID mice, as compared to control mice [86]. The hyperplasia seems to contribute to the high level of circulating insulin and enables the LID mice to maintain euglycemia. At the molecular level, insulin-stimulated autophosphorylation of the IR and tyrosine phosphorylation of IRS-1 are abolished in muscle, but normal in liver of the LID mice compared to control mice. Replacement therapy with IGF-I in LID mice normalizes insulin and growth hormone levels and insulin sensitivity [86, 87]. These observations suggest that circulating IGF-I is important in maintaining insulin sensitivity and may serve an inhibitory function on islet cell growth.

In a follow-up study, we demonstrate that hyperinsulinemia in LID mice is age-dependent and late in onset, whereas insulin resistance begins much earlier, at the age of 1 month. Only adult LID mice exhibit islet β-cell hyperplasia. To help to understand the cause-and-effect relationship of insulin resistance and islet hyperplasia, LID mice develop accelerated diabetes in response to streptozotocin administration, as compared to control littermates [88]. Islet hyperplasia seems to be a secondary change thus provides no protection to islet cells, while insulin resistance is the root cause of the islet change and insulin hypersecretion. Our studies on the islet phenotype of LID mice indicate that endocrine IGF-I inhibits growth hormone release and indirectly islet cell growth, as growth hormone stimulates islet growth [89, 90]. On the other hand, lack of IGF-I and resulting growth hormone hypersecretion cause insulin resistance, which further causes compensatory islet cell growth. It is well known that IGF-I increases and excess growth hormone decreases insulin sensitivity [91, 92]. Further supporting a growth hormone effect, when its action is abolished in LID mice by crossing to a transgenic strain that overexpresses a growth hormone antagonist (GHa), insulin resistance and hypersecretion (indication of islet hyperplasia) are both corrected [93].

In order to assess the physiological role of locally produced IGF-I, we have further developed pancreatic-specific IGF-I gene deficiency (PID) by crossing Pdx1-Cre and IGF-I/loxP mice [94]. Under normal conditions, PID mice exhibit enlarged (rather than diminished) pancreatic islets and reduced blood glucose level. Significant islet hypertrophy and increased islet density per pancreatic area, an indication of islet neogenesis, are likely the causes of increased islet cell mass. Interestingly, serum insulin and pancreatic insulin mRNA levels were not elevated. The pro-islet effects in PID mice have been further reinforced under streptozotocin-induced islet β-cell toxicity, i.e., islet cell death, islet insulin staining, serum insulin and pancreatic insulin mRNA levels were mostly protected from damage. To exclude the possibility that islet cells in PID mice have reduced uptake or altered metabolism/degradation of streptozotocin, we extended this islet-protection experiment to an obesity-induced, T2D model. Once again, PID mice show delayed onset of the diabetes and preserved insulin sensitivity and insulin production. GLUT-2 is normally localized at the cell membrane, essential for glucose recognition in islet β-cells and its level is reduced in several animal models of diabetes [95, 96]. High-fat diet (HFD)-induced T2D is accompanied with diminished GLUT-2 expression and disrupted membrane localization [97]. In PID mice fed a HFD, the GLUT-2 localization in the islet cells is significantly preserved together with the capacity of glucose sensing and transportation. These results suggest that, under basal conditions as well as in types 1 and 2 diabetes, pancreatic islet cell growth and neogenesis are promoted while islet cell apoptosis is prevented under a local IGF-I gene deficiency, thus challenging the dogma that IGF-I is an islet growth factor.

As the phenotype is unlikely a direct consequence of IGF-I deficiency and no compensatory changes in the

levels of IGF-II or insulin have been detected, we have explored activation of pro-islet genes in PID mice, using oligonucleotide DNA microarray [98]. As a result, multiple new members of the Reg family genes, previously not known to promote islet growth, were significantly upregulated in the pancreas, results subsequently confirmed by Northern blot and/or real-time PCR. Reg family proteins have been known for years. Among them, Reg1 is clearly linked to islet cell proliferation/regeneration [99, 100]; islet neogenesis-associated protein (INGAP; Reg3δ) promotes β-cell regeneration from pancreatic ductal cells [101, 102]. Consequently, five new members of the family (Reg2, 3α, 3β, 3γ, and Reg4) have been discovered [103–106]; three of them exhibited elevated expression in the pancreas of our PID mice. In contrast, the responses of Reg1 and INGAP in our system were only marginal. Our results revealed a novel mechanism of islet growth and protection in PID mice through Reg proteins. It remains to be determined how IGF-I affects Reg gene expression and whether these new Reg proteins indeed stimulate β-cell growth.

Cell-specific IGF-IR gene deficiency causes impaired insulin secretion without affecting β-cell growth

Compared to its ligand IGF-I, the role of IGF-IR is even more crucial because it mediates the actions of not only IGF-I but also IGF-II, especially during early development. Thus, IGF-IR gene deficiency is completely lethal with more severe defects in vital organ systems [30, 31]. In order to study the role of IGF signals, the IGF-IR gene has been inactivated specifically in islet β-cells using the Cre/loxP approach in two independent reports [107, 108]. These βigf1r$^{-/-}$ mice exhibit normal glucose but increased insulin levels, glucose intolerance, and normal insulin sensitivity. There is no defect in islet cell architecture or β-cell mass, and no sign of any change in the rates of islet cell replication or apoptosis, indicating that IGFs, acting through IGF-IR, are not involved in islet development and maintenance of β-cell mass. A significant defect in β-cell function is on glucose-stimulated insulin secretion, where the first phase of insulin release is abolished. Yet the response to arginine is normal. The secretory defect is perhaps related to diminished GLUT-2 or glucokinase gene expression in β-cells. Electron microscope analysis of β-cells revealed depleted insulin-storage granules and increased rough endoplasmic reticulum and Golgi stacks, consistent with a state of constitutively active release of insulin [107]. These observations indicate a requirement of IGF-IR-mediated signaling for normal insulin secretion but not for β-cell growth [107, 108].

Insulin and IGFs were well-known growth factors that stimulate islet cell growth and inhibit cell apoptosis [11, 50, 54, 56, 109, 110], a view that has been held for decades and supported by recent studies of transgenic overexpression [6, 81]. However, except 2 reports that remain supportive [16, 53], most gene-targeted models and our own research have challenged this view (Table 1), suggesting that IGFs are not involved in normal islet formation and cell growth. For instance, combined inactivation of IR and IGF-IR genes in early embryos, but not of either receptor alone, results in a 50% decrease in the size of the exocrine pancreas without affecting development of endocrine α- and β-cells; Combined ablation of IGF-I and IGF-II creates an identical phenotype [82]; Islet β-cell-specific inactivation of IGF-IR gene caused no change in β-cell mass, suggesting that IGF signaling is not essential for normal growth and development of the pancreatic islets [107, 108]; Our own research of liver- and pancreatic-specific IGF-I gene deficiency suggests that IGF-I exerts an inhibitory effect on islet cell growth albeit indirectly [86, 88, 93, 94]. Most recently, Ueki et al. have created a β-cell-specific double knockout of IGF-IR and IR, i.e., βDKO [111]. In contrast to the mild phenotypes observed in single knockouts, βDKO mice manifest reduced β-cell mass by 2 weeks and develop frank diabetes by 3 weeks of age. It provides a stronger evidence of a role for insulin and IGF signaling in regulating β-cell growth and function, with a dominant role for insulin [111].

In my opinion, these models still have considerable limitations. Given the complexities of the insulin/IGF family, permanently gene-targeted mice, being born with the deficiency, might have been compensated through elevated activities of other homologous members. In future studies, a broad range, dominant negative ablation might be able to avoid this gene redundancy effect [112]. In both reports of β-cell specific IGF-IR gene targeting (βigf1r$^{-/-}$), an insulin promoter has been used to drive Cre expression [107, 108]. Activation of insulin gene expression is relatively late during embryonic development and the promoter is fully activated only in adult islets (model limitation). Insulin production in rodents begins at E15.5, its promoter would be inactive in islet precursor cells where IGF-I might play a role [113]. Both reports are therefore inadequate in evaluating the role of IGF-IR during islet formation and early development [109]. It is very possible that IGF-IR gene was spared from β-cells during a major portion of the early development. Moreover, both models would have spared IR on β-cells, which could well compensate for the loss of IGF-IR; likewise, IR and IGF-IR on other islet cells are unaffected. On a technical note, I have noticed that the negative results in β-cell mass were reported from only 2~4 mice in each group, which might overlook subtle changes.

Table 1 Highlight of islet phenotype of some transgenic and knockout mice

Animal model	Islet growth/mass	Insulinemia/secretion	References
Gene knockout			
$IR^{-/-}$	Normal	Massive increase	[66, 67]
	Normal		[82]
$IGF\text{-}IR^{-/-}$	Decreased		[53]
	Normal		[82]
$IRR^{-/-}$	Normal	Normal	[41]
$IR^{-/-}$ & $IGF\text{-}IR^{-/-}$	Normal		[82]
$Ins1^{-/-}$ & $Ins2^{-/-}$	Increased	Undetectable	[72, 73]
$IGF\text{-}I^{-/-}$ & $IGF\text{-}II^{-/-}$	Normal		[82]
$IGF\text{-}I^{-/-}$	Normal	Normal	[33] & Unpublished
Tissue-specific gene knockout			
βIRKO	Decreased	Hyper-/Decreased	[15, 16]
$\beta igf1r^{-/-}$	Normal	Hyper-/Decreased	[107, 108]
βDKO	Decreased	Decreased, diabetic	[111]
LID: liver $IGF\text{-}I^{-/-}$	Increased	Hyper-	[86, 88, 93]
PID: pancreas $IGF\text{-}I^{-/-}$	Increased	Normal	[94]
Islet β-cell-specific overexpression			
IGF-I	Normal/regeneration	Normal	[6, 78]
IGF-II	Increased	Hyper-/Increased	[81]

Summary

Although the direct evidence that IGF-I stimulates islet cell growth (DNA synthesis) is clear, it needs to be verified in a more cutting edge system given the current controversies; we need to explore and differentiate its precise roles in β-cell replication, neogenesis (transformation), or hypertrophy that all contribute to changes in β-cell growth; even if IGF-I is not required for the formation and normal growth of islet cells, it might still be useful as a pharmaceutical agent (e.g., for β-cell apoptosis or insulin secretion); there is no doubt that IGF-I prevents β-cell apoptosis, which can be potentially used to prevent human diabetes; in an in vivo setting, future applications of IGF-I therapy have to consider its indirect effects on growth hormone, insulin and glucose and its paracrine effects. To address the question from the title of this article, I still believe a direct, immediate stimulation of IGF-I on β-cell growth. While in an in vivo setting, especially during some pathophysiological processes, IGF-I might play different roles. The role of IGF-I in diabetes has been pursued for years, e.g., T1D is often associated with reduced IGF-I level [114]; MODY3 diabetes, caused by a mutation of HNF-1α gene, exhibits reduced IGF-I expression and β-cell growth [115]; IGF-I treatment is effective in T2D [91]. Future studies will no doubt provide a clearer guideline on its appropriate applications in various human conditions.

Acknowledgments This work was supported by a Career Development Award (2–2000–507) from the Juvenile Diabetes Research Foundation International, New York, NY, operating grants (MOP-53206, NMD-83124) from Canadian Institutes of Health Research, John R. & Clara M. Fraser Memorial fund, and the Shanghai Education Commission (China). Research contributions were made by Y. Lu, K. Roberston, Y. Guo, R. Yu, Z. Tang, and Y. Liu of McGill University.

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