Growth Factor Mediated Regulation of Beta Cell Survival

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Abstract: Numerous studies have shown that a reduction in the endogenous functional β-cell mass is a major cause of every form of diabetes. Therefore, the development of therapies to preserve and regenerate endogenous β-cell mass and function holds great promise for both Type 1 and Type 2 diabetes. The importance of understanding the mechanisms and signaling pathways regulating beta cell death is key in our ability to prolong beta cell survival in the future. One potential way to protect beta cells would be to use growth factors that can enhance their survival against different damaging insults. In this review, we describe the current knowledge on the effects of several beta cell growth factors that have been shown to increase beta cell survival *in vitro* and/or *in vivo* against varied cell death inducers. Understanding the mechanisms that mediate the pro-survival effects of these factors will enhance the potential to use these factors, or develop drugs that target their downstream pathways, as therapeutic agents for improved beta cell survival *ex vivo* or *in vivo* in the future.

INTRODUCTION

Diabetes is one of the most prevalent and fastest growing diseases not only in the western world but surprisingly also in under-developed countries [1]. This epidemic is creating a phenomenal toll, both on the economics and on the health of nations worldwide [2]. Therefore, finding ways to combat, treat and cure this disease has become a priority in the health-care field. One of several approaches taken towards this goal is to better understand the etiology and cause of the disease, so that innovative cures can be found.

It is now well accepted that diabetes occurs due to a deficiency in the endogenous functional pancreatic beta cell mass, resulting in inadequate insulin supply and consequently, hyperglycemia. The two major forms of diabetes, Type 1, which results from an autoimmune attack on the beta cells, and Type 2, which encompasses components of insulin resistance as well as insufficient insulin production, both converge on the beta cell as the focal point of their phenotype [3-7]. The loss of functional beta cells in diabetes can result from an imbalance in proper beta cell differentiation, function, proliferation or death. Therefore, understanding the mediators, the cellular and molecular pathways, as well as factors that modulate these processes is extremely important in the ultimate treatment of the disease.

Pancreatic beta cell death is a key player in the pathogenesis of Type 1 diabetes, which is characterized by early stages of insulitis, islet infiltration by inflammatory cells, followed by a specific and progressive destruction of the beta cell. Both the extrinsic (cell-surface death receptors) as well as the intrinsic (mitochondrial) apoptotic pathways

are involved in this process [6-11]. There is increasing evidence that beta cell death also plays a role in the etiology of Type 2 diabetes. Reduced beta cell mass as well as increased apoptosis in pancreatic sections from patients with Type 2 diabetes relative to normal non-diabetic subjects suggests that beta cell death may be directly involved in the reduction in functional pancreatic beta cell mass in these patients [3,4,11-14]. Furthermore, several animal models of Type 2 diabetes, such as the ob/ob and db/db mice on a C57BL/KS background, transgenic rodents overexpressing the human islet amyloid polypeptide (hIAPP), the Zucker diabetic fatty rats, or Psammomys obesus, all show increased beta cell death with a resultant reduction in functional beta cell mass [15-20]. Thus, it is now well accepted that pancreatic beta cell death is a major regulator of the decreased beta cell mass observed in both Type 1 and Type 2 diabetes. Therefore, an understanding of the mediators and mechanisms of beta cell death, and more importantly, finding ways to enhance beta cell survival is critical in the treatment of this disease.

Numerous insults lead to beta cell death in the pathogenesis of diabetes. Immune-mediated inflammation and cytokines are vital mediators of beta cell destruction in Type 1 diabetes. Whereas in Type 2 diabetes, excessive nutrients leading to glucolipotoxicity (GLT), as well as toxicity due to accumulation of islet amyloid polypeptide (IAPP) oligomers are the major cause of beta cell loss [3-7,19-26]. There is accumulating evidence that the boundaries distinguishing the mediators of beta cell loss in Type 1 and Type 2 diabetes are becoming increasingly blurred. For example, recent evidence suggests that inflammation and cytokines are also involved in the loss of beta cells in Type 2 diabetes, and glucotoxicity in Type 1 diabetes [27,28]. The lack of nutrients and hypoxia are two other key effectors of beta cell death, besides glucotoxicity, that likely play a significant role in the massive loss of beta cells that occurs in the initial stages of islet transplantation [29], a procedure

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used for the treatment of severe cases of Type 1 diabetes [30]. Finally, glucocorticoids such as dexamethasone (DEX) [31,32], and chemical beta cell toxins such as alloxan and streptozotocin (STZ) [32-35], also induce beta cell destruction, and the latter have been widely used to induce diabetes in animal models.

Depending on the nature and extent of the insult, it can induce necrotic, caspase-independent or caspase-dependent mitochondrial apoptotic, or cell-death receptor-mediated extrinsic apoptotic beta cell death pathways [36-39]. Beta cell death inducers mediate their action through common as well as distinct signaling pathways and molecular mechanisms. For example, synthesis of nitric oxide (NO), accumulation of reactive oxygen species (ROS), intracellular calcium, and activation of the endoplasmic reticulum (ER) stress pathway are common mediators downstream of a number of beta cell death effectors. The pro-apoptotic effects of cytokines are mediated through the activation of all three mitogen activated protein kinase (MAPK) pathways, with the c-Jun N-terminal kinase (JNK) pathway being the most important mediator of this process. The JNK pathway is also activated by the excessive production of ROS in beta cells as a consequence of prolonged exposure to hyperglycemia. The role of nuclear factor kappa B (NFκB) activation, on the other hand, is somewhat more controversial in cytokine-induced beta cell death. There is *in vitro* evidence indicating a pro-apoptotic role for NFκB downstream of cytokines interleukin 1-β (IL-1β) and interferon-γ (IFN-γ), however, other studies suggest that NFκB activation is protective against other cytokines such as IFN-γ and tumor necrosis factor-α (TNF-α). There are numerous excellent reviews on the causes and mechanisms of beta cell death that the reader is referred to, as it is beyond the scope of this review [3-7,19-28,40].

There are numerous studies describing ways to inhibit beta cell death under different pathophysiological insults. These include use of growth factors, hormones, modulation of specific intracellular signaling pathways, and regulation of molecules involved in the apoptotic pathways. The current review specifically describes the role of growth factors and hormones in the regulation of beta cell survival, focusing on glucagon-like peptide-1 (GLP-1), parathyroid hormone-related protein (PTHrP), lactogens, hepatocyte growth factor (HGF), insulin, and insulin-like growth factors (IGFs). In particular, we will emphasize what is known about the effects of these factors on beta cell survival in the context of (i) *in vitro* experiments; (ii) *in vivo* setting of transgenic overexpression or acute administration; (iii) *in vivo* setting of conditional gene deletion of the growth factor or its corresponding receptor; (iv) their action against varied cell death inducers; (v) and the signaling and molecular mechanisms involved (see Table **1** for summary).

GLUCAGON-LIKE PEPTIDE-1 (GLP-1)

Introduction

GLP-1 is a 30-amino acid peptide hormone produced by differential processing of proglucagon in the intestinal epithelial endocrine L-cells in response to meal intake. The biologically active forms of GLP-1 consist of amino acids 7-37 or 7-36-amide. GLP-1 is rapidly metabolized and inactivated by the enzyme dipeptidyl peptidase IV with a

resultant half life of less than two minutes in the circulation. The GLP-1 receptor is a class 2, G protein-coupled receptor which is widely distributed in pancreatic islets, brain, heart, kidney, and the gastrointestinal tract. The receptor typically couples *via* a stimulatory G protein to adenylate cyclase resulting in the formation of cAMP, and activates many other signaling pathways including MAPK, phosphoinositol 3-kinase (PI3K), Akt and atypical protein kinase C-zeta (aPKCζ). The main reported actions of GLP-1 are to: stimulate insulin secretion and increase insulin gene expression, inhibit glucagon secretion, inhibit gastrointestinal motility and secretion, regulate appetite and food intake, promote insulin sensitivity, and increase beta cell mass through enhanced proliferation and neogenesis [41-44].

In Vitro Effects

Another important function of GLP-1, especially relevant to this review, is its pro-survival effect on the pancreatic beta cell. GLP-1 protects beta cells against varied cell death inducers, demonstrated *in vitro*, in rodent insulinoma cell lines, in primary rodent and primary human beta cells. Human islets cultured for five days in the presence of GLP-1 maintained a better morphology and showed reduced beta cell death measured by condensed beta cell nuclei and active caspase-3 levels compared to control islets [45]. Studies from different groups have shown that GLP-1 can protect beta cells against cell death inducers relevant to Type 1 and Type 2 diabetes. Both GLP-1 and liraglutide, a long-acting analogue of GLP-1, protect neonatal rat islets from cytokine and free fatty acids (FFA) mediated cell death in a dose-dependent manner. The maximal anti-apoptotic effects of GLP-1 occurred at 10 to 100 nM, a concentration of GLP-1 that is physiologically relevant, as 10 nM corresponds to the plasma concentration of the incretin following a high-carbohydrate meal. Liraglutide was more potent in its pro-survival effects with maximal effects at 100 to 1000 nM. Pre-incubation with an excess of a GLP-1 antagonist exendin(9–39) completely blocked the protective effect of liraglutide against both cytokine and FFA-induced cell death demonstrating that its antiapoptotic effects are mediated *via* the GLP-1 receptor [46]. A separate study showed that 10 nM of GLP-1 also protects human islets as well as the rat insulinoma cell line INS832/13 against lipotoxicity, glucotoxicity and GLT [47]. Several groups have established the protective effect of GLP-1 against cytokine-mediated cell death in adult rodent and human islets [48-51]. The study from Kieffer's group took a different interesting approach. They induced GLP-1 production directly in the alpha cells of mouse islets through adenoviral (Ad) transduction of the cDNA for the processing enzyme prohormone convertase (PC) 1/3. This enzyme, which is normally absent in islet cells, is required for the processing of the proglucagon precursor to produce GLP-1. Bioactive GLP-1 released from Ad-PC 1/3 transduced islets increased in a dose-dependent manner, resulting in enhanced islet cell survival in response to IL-1β treatment [50]. GLP-1 and the GLP-1 receptor agonist, exendin-4, also protect beta cells against other cell death mediators including hydrogen peroxide (H_2O_2), a potent generator of ROS, thapsigargin, an ER-stress inducer, a cocktail of immunosuppressive drugs (ISD) used to prevent rejection in islet transplantation and the glucocorticoid DEX.

The protective effect of GLP-1 against H_2O_2 was observed in the mouse insulinoma cell line, MIN6, only when the cells were pre-treated with the peptide [52]. Also, GLP-1-transfected MIN6 cells were protected against the toxicity induced by ISD [53]. Exendin-4 improved cell survival in INS-1 cells, and in rat and human islets, following induction of ER stress [54,55], and in mouse islets and INS-1 cells against DEX [31].

Of the various signaling pathways, PI3K/Akt and cAMP induction mediate the cytoprotective effects of GLP-1 in beta cells. Use of inhibitors demonstrated that GLP-1-induced activation of PI3K, but not MAPK, was required to inhibit the pro-apoptotic events in MIN6 cells exposed to H_2O_2 [52], as well in neonatal rat islets exposed to cytokines or FFA [46]. Similarly, overexpression of a dominant-negative protein kinase B mutant suppressed the anti-apoptotic action of GLP-1 against GLT in INS832/13 cells [47], substantiating the importance of the PI3K/Akt pathway in the pro-survival effect of GLP-1. The importance of the cAMP pathway in mediating the anti-apoptotic effect of GLP-1 was demonstrated using pharmacological inhibitors of this pathway in MIN6 cells against H_2O_2-induced cell death [52], in INS-1 cells against DEX-mediated cell death [31], as well as in primary rat beta cells against ER-stress induced beta cell death [54]. Exendin-4 significantly potentiated the induction of ER-stress response gene, activating transcription factor (ATF)-4, and accelerated recovery from ER stress-mediated translational repression in INS-1 cells in a PKA-dependent manner [54]. Exendin-4 also protected insulinoma cells and rat islets from cytokine-induced cell death by inhibiting the activation of the JNK pathway elicited by IL-1β. The inhibition of JNK by exendin-4 was found to be PKA- but not PI3K-dependent [51]. A potential candidate signaling pathway that has not yet been explored in mediating the pro-survival effects of GLP-1 in beta cells is the atypical PKCζ pathway, which although activated by GLP-1 in beta cells [56] and known to be a pro-survival signal in many cell types [57], has yet to be examined for its pro-survival effects in beta cells.

A very recent study showed that the anti-apoptotic actions of one growth factor, GLP-1, are mediated indirectly through regulating the signaling of another growth factor, IGF-II/IGF-I receptor. The study performed comparative transcriptomic analysis of islets from control and gastric inhibitory peptide (GIP) and GLP-1 double knockout mice and found that IGF-I receptor expression was markedly reduced in the mutant islets, which are more sensitive to cytokine-induced cell death. They demonstrated, both in MIN6 cells and in primary beta cells, that GLP-1 robustly stimulated IGF-I receptor expression and Akt phosphorylation. Furthermore, GLP-1-induced protection against apoptosis was shown to be mediated through an IGF-II/IGF-I receptor autocrine loop, where GLP-1-induced IGF-I receptor was activated by glucose-mediated IGF-II secretion and subsequent Akt phosphorylation [58].

Inhibition of beta cell apoptosis by GLP-1 against varied cell death inducers is associated with down-regulation of pro-apoptotic proteins including active caspase-3, caspase-12 poly-ADP-ribose polymerase cleavage, and thioredoxin interacting protein, and up-regulation of pro-survival factors including Bcl-2, Bcl-xL, islet-brain-1, JunB, inhibitor of apoptosis protein-2, and BiP, the major chaperone protein of the ER [31,45-55,58]. The regulation of some of these genes by GLP-1 was found both at the mRNA and protein level. Buteau *et al.* found that GLP-1 enhanced NFκB DNA binding activity to the promoters of two anti-apoptotic genes, inhibitor of apoptosis protein-2 and Bcl-2, thus stimulating their expression. Inhibition of NFκB abolished the protective effect of GLP-1 against GLT in insulinoma cells [47]. On the other hand, Kang *et al.* found that exendin-4 inhibited IL-1β-induced inducible nitric oxide synthase (iNOS) expression in insulinoma cells at the post-translational level and not at the transcriptional or post-transcriptional levels [59]. The role of the forkhead transcription factors of the O subclass (FoxO) in mediating the protective effect of GLP-1 was apparent when a constitutively nuclear FoxO1 prevented the anti-apoptotic actions of GLP-1 against oxidative stress in insulinoma cells. GLP-1 inhibited FoxO1 binding to the promoter of the transcription factor pancreatic and duodenal homeobox gene-1 (Pdx1), thus promoting Pdx1 transcription. GLP-1 inhibited FoxO1 through phosphorylation-dependent nuclear exclusion, which was dependent on EGF receptor and PI3K activation, in INS832/13 cells [60].

In Vivo Effects

The pro-survival role of GLP-1 has also been well demonstrated *in vivo* in different animal models of diabetes. Endogenous GLP-1 receptor signaling directly modifies the susceptibility to apoptotic injury. GLP-1R-/- mice exhibited increased beta cell death relative to wild type controls, 48hrs after multiple low dose STZ (MLDS) treatment, a surrogate model of Type 1 diabetes. Conversely, normal C57Bl6 male mice pre-treated with exendin-4 for 3 days prior to MLDS administration were significantly protected from apoptosis resulting from this treatment [48]. Similarly, continuous infusion of recombinant GLP-1 at 1.5 pmol/kg/min for 4-8 weeks in another model of Type 1 diabetes, the NOD female mouse, led to a delay or suppression of the onset of diabetes, with a concomitant reduction in beta cell apoptosis [61]. Exendin-4 was also protective in animal models of Type 2 diabetes. Six week old female db/db mice on a BKS background showed reduced beta cell death and prevention of hyperglycemia when injected daily intraperitoneally with 1 nmol/kg of exendin-4 for two weeks [62]. Similarly male Zucker diabetic fatty rats showed reduced cell death and caspase-staining in the beta cells of their pancreas when harvested at day 7 after a continuous infusion of recombinant GLP-1 at 30 pmol/kg/min for two days [63]. Exendin-4 also reduced beta cell apoptosis in pancreatectomized (Px) rats treated with or without STZ, partly by attenuating the expression of ER-stress response genes such as *X-box-binding protein-1 (XBP-1), ATF-4, ATF6, and C/EBP-homologous protein (CHOP)* [64]. The role of Pdx1 in mediating the pro-survival effect of exendin-4 *in vivo* was demonstrated using beta cell-specific Pdx1-/- mice. Treatment with exendin-4 for 3 days inhibited endogenous beta cell death in wild-type mice, but did not inhibit the increased beta cell apoptosis in beta cell-specific Pdx1-/- mice [65]. Surprisingly, in the setting of syngeneic islet transplants under the kidney capsule of STZ-induced diabetic mice, exendin-4 treatment did not improve beta cell survival in these grafts at 1, 3, or 10 days post-transplant.

Possible explanations for this surprising finding are that either the precise regimen used in these experiments was not ideal, or that pre-treatment with GLP-1 agonists may be required to mediate its protective effect [66]. Pre-treatment prior to the delivery of an insult has been employed in most models demonstrating GLP-1 receptor agonist-mediated β-cell cytoprotection *in vitro* or *in vivo* [48,52,65].

There is convincing evidence to demonstrate that GLP-1 is a pro-survival factor in rodent beta cells under stress-induced conditions relating to both Type 1 and Type 2 diabetes *in vitro* and *in vivo*. PI3K/Akt and cAMP signaling pathways are the major mediators of the protective effect of GLP-1 in rodent beta cells. A number of specific molecular regulators of apoptosis including the Bcl family of proteins and ER-stress response genes are involved in this process. More focus on the human beta cell is required to better understand the specificity, mechanism and *in vivo* pro-survival effect of GLP-1 in human islets.

PARATHYROID HORMONE-RELATED PROTEIN (PTHrP)

Introduction

PTHrP was initially discovered as a tumor peptide that causes humoral hypercalcemia of malignancy, but subsequently was found to be expressed in almost all tissues of the body. PTHrP undergoes post-translational processing to give rise to several mature forms of the peptide, including the amino-terminal(1-36), mid-region(38-94), carboxyl-terminal(107-139) peptides, and a segment unique to primates, PTHrP(140-173). PTHrP is targeted to the secretory pathway by the signal peptide at its amino-terminus, and can also be targeted to the nucleus through its nuclear localization signal at amino acids 87-106. PTHrP functions as a potent smooth muscle relaxant, plays a role in transepithelial calcium transport, regulates proliferation, survival, differentiation and development in several tissues, and regulates epithelial-mesenchymal transition. The majority of the differentiation and developmental roles of PTHrP have been attributed to amino-terminal PTHrP(1-36), which interacts with a seven transmembrane G-protein coupled receptor, PTH/PTHrP receptor-1 (PTH1R), also found in all tissues where PTHrP is made, consistent with the known autocrine/paracrine functions of PTHrP [67-69].

In Vitro Effects

PTHrP is expressed in the pancreas during development [70,71], in the adult islet in all four endocrine cell types [72], and in insulinomas [72-74]. PTH1R is also expressed on the pancreatic beta cell [75,76]. In rodent beta cells, PTHrP(1-36) stimulates the PKA/cAMP pathway resulting in increased expression of MAP kinase-specific phosphatase-1 (MKP-1) which causes dephosphorylation and inactivation of the JNK pathway [74,77]. PTHrP also activates the Ca/PKC, novel and atypical PKC pathways in rodent pancreatic beta cells [78,79]. PTHrP enhances beta cell proliferation and function [74,77,79,80], and relevant to this review, is a survival factor for the rodent beta cell. Islet cell cultures from transgenic mice over-expressing PTHrP in their beta cells (RIP-PTHrP) are less prone to beta cell death induced by STZ or glucose and serum-deprivation, measured by TUNEL or propidium iodide co-staining with insulin,

compared to normal littermates [81]. To determine which specific PTHrP peptide is responsible for the protective effect, normal mouse islet cell cultures deprived of glucose and serum were treated with either the amino(1-36), mid-region(38-94), or the carboxyl(107-139) terminal PTHrP peptides. Only the amino-terminal(1-36) peptide significantly inhibited beta cell death, suggesting the importance of the PTHrP/PTH1R interaction for its pro-survival effects. There is a significant and specific increase in the level of anti-apoptotic *Bcl2* mRNA in RIP-PTHrP transgenic islets; however, this increase did not clearly translate to the protein level [81]. The signaling and molecular pathways through which PTHrP mediates its anti-apoptotic effects have yet to be delineated.

In Vivo Effects

PTHrP also has pro-survival effects on the rodent beta cell *in vivo*. Adult RIP-PTHrP transgenic mice were resistant to the diabetogenic and cytotoxic effects of STZ [81-83]. Uptake of STZ by the beta cell which occurs through the Glut2 transporter is likely to be similar in transgenic and normal islets, as the level of Glut2 was unchanged. To confirm that the resistance to STZ is not simply a result of the two-fold increase in beta cell area observed in adult transgenic mice, one-week old neonatal transgenic mice, which do not have increased islet mass, were treated with a single high dose of STZ for 24hrs. Neonatal RIP-PTHrP mice showed decreased beta cell death and lower blood glucose levels compared to age-matched littermates, demonstrating an anti-apoptotic effect of PTHrP in rodent islets *in vivo* [81]. To examine whether PTHrP overexpression affected the increased wave of apoptosis reported in neonatal pancreas, pancreata from 11, 13, 15 and 17 day old normal and RIP-PTHrP transgenic mice were analyzed for beta cell death by insulin and TUNEL co-staining. Surprisingly, in these studies there was no increase in beta cell turnover in normal mice at any of these ages, nor was there a difference in the transgenic mice [Vasavada *et al*, unpublished results]. Thus, further studies are needed to determine whether PTHrP protects beta cells against other pathophysiologically relevant cell death inducers, and more importantly, whether it is a pro-survival factor for human beta cells.

LACTOGENS

Introduction

Prolactin (PRL), placental lactogen (PL) and growth hormone (GH) are three of the major peptides that make up the family of lactogenic hormones. The major site of synthesis of PRL and GH is the lactotroph and the somatotroph cell of the anterior pituitary respectively, whereas PL is synthesized in the giant trophoblast cell of the placenta during pregnancy. These proteins consist of ~200 amino acids including a signal peptide, range in molecular weight from 22 to 25Kd, and undergo post-translational modifications including glycosylation, phosphorylation and proteolytic cleavage. All three peptides signal through receptors (R) which belong to the class 1 cytokine receptor superfamily. PRL and PL signal through a common PRL-R, whereas GH signals through a distinct GH-R. Both receptors are expressed in several different cell types including the beta cell, and can activate numerous downstream signaling

pathways. GH is involved in the regulation of postnatal growth and metabolism, with its actions often mediated by IGF-I. PRL has a much broader spectrum of activities than GH, and these are classified as reproduction, metabolism, osmoregulation, immunoregulation, and behavior. PL regulates maternal carbohydrate and lipid metabolism [84-87].

In Vitro Effects

There is ample evidence demonstrating that lactogens can enhance beta cell function as well as proliferation [87-90]. Surprisingly, despite numerous studies indicating a pro-survival role of these peptides in other cell types [91-93], the effect on beta cell survival was not examined till recently. The first evidence suggesting a protective role of lactogens in beta cells is the study by Sorenson and colleagues, demonstrating decreased cell death in DEX-treated rat islets cultured in the presence of PRL [94]. More recently, our group has shown that 200ng/ml of ovine PRL protects rat insulinoma cells INS-1 from STZ- and DEX-induced cell death, measured by cell viability assays and induction of cleaved caspase-3 [32]. Furthermore, primary mouse beta cells either overexpressing mouse placental lactogen 1 (mPL1) or treated exogenously with PRL are also protected from STZ- and DEX-induced cell death *in vitro* as measured by TUNEL and insulin co-staining (Fig. **1A**, **B**) [32]. More recently, human islets cultured with PRL for 48 hrs showed a relative increase in islet beta cell content and viable beta cell mass compared to control non-treated islets [95].

To examine the signaling pathways responsible for the pro-survival effect of PRL, three different approaches, pharmacological inhibitors, siRNAs specific to Janus-activated kinase-2 (Jak-2), and a dominant negative (DN) mutant of signal transducer and activator of transcription-5 (Stat5), were used either in INS-1 cells or primary mouse beta cells. Neither the activation of PI3K/Akt nor the extracellular regulated kinase 1/2 (ERK 1/2) pathways were accountable for the pro-survival effects of PRL against DEX-induced beta cell death. Rather, the Jak-2/Stat5 pathway was shown to mediate the anti-apoptotic effects of PRL in INS-1 cells and primary mouse beta cells (Fig. **1C**) [32]. One of several transcriptional targets downstream of Stat5 is the anti-apoptotic molecule Bcl-xL. PRL significantly increased the expression of Bcl-xL protein in INS-1 cells by 6hr, reaching a peak induction at 18hr and maintaining the increase for up to 24hrs (Fig. **1D**, **E**). Similarly, primary mouse islets overexpressing mPL-1 in their beta cells also showed an increase in Bcl-xL expression, compared to normal islets. Using Bcl-xL-specific siRNA, lactogen-induced up-regulation of Bcl-xL was shown to be required to mediate its protective effect in INS-1 cells against DEX-induced cell death (Fig. **1F**, **G**). However, Bcl-xL-specific siRNA significantly reduced, but did not completely abolish, the protective effect of PRL suggesting that there could be additional molecular pathways through which PRL mediates its pro-survival effect in beta cells (Fig. **1G**) [32]. In this regard, a study examining the transcriptional modulation of genes by PRL in rat islets by DNA microarray analysis identified pro-apoptotic genes such as *clusterin*, *NFKBIA*, and *TNFRSF1A* as being down-regulated by PRL [96].

The role of GH in beta cell survival has barely been examined. Bovine GH inhibits cytokine-induced nitric oxide production by reducing iNOS gene induction in INS-1 cells. To elucidate the underlying mechanism, activation of transcription factors implicated in the induction of iNOS was examined, and only Stat1 tyrosine phosphorylation and DNA binding was inhibited by GH, but not that of NFκB. GH was found to induce suppressor of cytokine signaling (SOCS)-1 and -3, both of which are able to inhibit cytokine-activation of Stat1, suggesting that they are likely to mediate the inhibitory action of GH [97]. A more recent study showed that human (h) GH reduces the apoptotic effects of cytokines in INS-1 cells. hGH only slightly reduced cytokine-induced iNOS gene expression, and unlike the other study, hGH further increased cytokine-induced nitric oxide production and had no effect on either Stat1 or NFκB binding in INS-1 cells, despite enhanced SOCS-3 expression. Finally, the anti-apoptotic effect of hGH was mediated through Stat5 activation resulting in an increase in the Bcl-xL/Bax ratio in INS-1 cells [98]. One caveat of the latter study [98] is that human GH was used on rodent beta cells, and it has been previously demonstrated that hGH can interact and signal through the rodent PRL-R [87,99]. Therefore, it is unclear whether GH acting through its own receptor indeed has anti-apoptotic effects on the rodent beta cell. Although the former study [97] used bovine GH which signals through the GH-R, they did not directly demonstrate an anti-apoptotic effect of GH against cytokine-induced cell death.

In Vivo Effects

Whether lactogens have a protective effect on beta cells *in vivo*, was examined in transgenic mice, RIP-mPL1, overexpressing mPL-1 in their beta cells. Adult transgenic mice were completely protected from the diabetogenic effects of a single high dose of STZ, compared to normal littermates, up to 4 weeks after STZ injection [76, 90]. Furthermore, the RIP-mPL-1 mice were also protected from the cytotoxic effects of STZ *in vivo*, as demonstrated by reduced beta cell death 14 hrs after an STZ injection, as well as a 10-fold increase in the remnant beta cell mass 4 weeks after STZ treatment, relative to normal littermates [76]. One other study found that daily injections of PRL for 13 weeks in pre-diabetic NOD mice resulted in a lower incidence of Type 1 diabetes compared to the saline treated control group, although the results were not significant. Also, this study did not examine the contributions of increased proliferation versus decreased cell death by PRL in these mice [100]. Future studies will examine the anti-apoptotic role of lactogens against pathophysiologically relevant cell death inducers *in vitro* and *in vivo*, as well as in human islets.

HEPATOCYTE GROWTH FACTOR (HGF)

Introduction

HGF is a mesenchyme-derived protein originally identified as a circulating factor that promotes hepatic regeneration after liver injury. Since then, many studies have demonstrated that HGF has mitogenic, motogenic, anti-apoptotic, angiogenic, and morphogenic activities in a wide variety of cell types. To exert its functions, HGF needs to be activated through proteolysis by serine proteases, and forms a disulfide-linked heterodimer composed of a 69-kDa alpha-
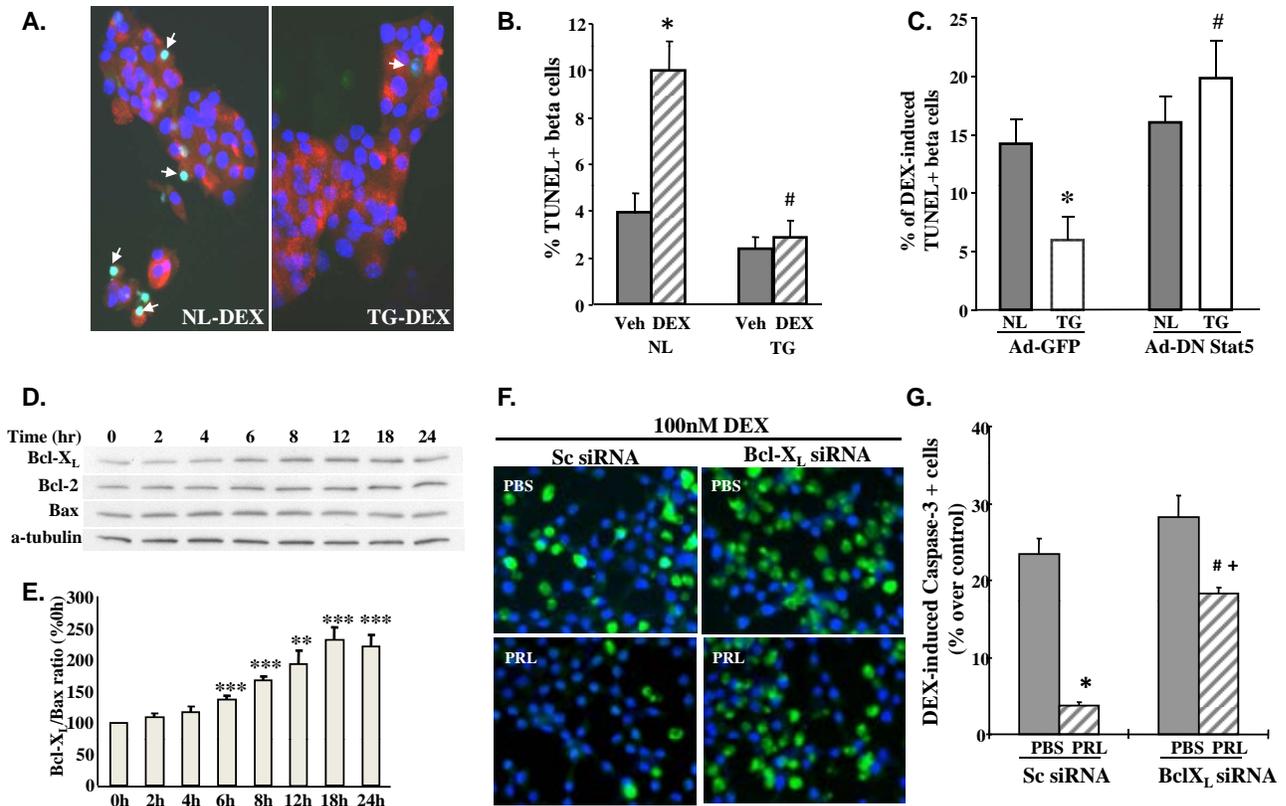
Fig. (1). Lactogens protect mouse primary beta cells and INS-1 cells against DEX-induced cell death *in vitro*. A) Primary islet cell cultures from normal (NL) and RIP-mPL1 transgenic (TG) mice stained for insulin (red), TUNEL (green), and Hoechst 33258 (blue) after 48 hrs of 500 nM DEX-treatment. Arrows indicate TUNEL-positive beta cells. **B)** Quantitation of the percentage of TUNEL-positive beta cells in NL and TG islet cell cultures treated with vehicle (Veh, gray bars) or DEX (stippled bars) shows a significant 2.5-fold increase in DEX-induced beta cell death in NL cells which is completely and significantly inhibited in TG islet cells (* $p < 0.001$ versus veh, # $p < 0.001$ versus DEX.) **C)** Transduction with Ad-DN-Stat5 completely blocks the protective effect of lactogens on DEX-induced beta cell death in primary mouse islet cells. (* $p < 0.05$ versus NL Ad-GFP, # $p < 0.05$ versus TG Ad-GFP). **D)** Western blot analysis of Bcl-xL, Bcl2, Bax, and tubulin expression in INS-1 cells treated with PRL for different times. **E)** Densitometric analysis of the ratio of Bcl-xL/Bax depicted as percentage of untreated cells. (* $p < 0.05$). **F)** INS-1 cells transfected with scrambled (Sc) or Bcl-xL siRNA, treated with DEX ± PRL, were stained for cleaved caspase-3 (green) and Hoechst 33258 (blue). **G)** Quantitation of the percentage of caspase-3-positive cells shows Bcl-xL siRNA significantly diminished the protective effect of PRL against DEX-induced INS-1 cell death. (* $p < 0.0001$, Sc siRNA-transfected cells PBS versus PRL; + $p < 0.0005$, PRL-treated cells Sc versus Bcl-xL siRNA-transfected; # $p < 0.05$, Bcl-xL siRNA-transfected cells PBS versus PRL). [*"This research was originally published in the Journal of Biological Chemistry. Fujinaka Y, Takane K, Yamashita H, Vasavada RC. Lactogens promote beta cell survival through JAK2/STAT5 activation and BclXL upregulation. J Biol Chem 2007; 282: 30707-17. © the American Society for Biochemistry and Molecular Biology."*].

chain and a 34-kDa beta-chain. HGF binds with high affinity to, and induces the dimerization of, a membrane-spanning tyrosine kinase receptor encoded by the proto-oncogene, c-met. The receptor and ligand have widespread distribution, including in the pancreatic islet [101-103].

In Vitro Effects

Several studies have demonstrated that HGF is a mitogen and an insulinotropic agent for fetal and adult islet cells *in vitro* and *in vivo* [104-109]. The role of HGF on pancreatic beta cell survival has been examined more recently. HGF enhances survival of porcine neonatal pancreatic cell clusters (NPCC) transfected with Epstein-Barr virus (EBV)-based HGF plasmid [110], and reduces beta cell death in islets of non-human primates (NHP) transfected with Ad-mouse HGF *in vitro* [111]. HGF also confers protection against STZ *in*

vitro. Cell viability of INS-1 cells 24 hrs after STZ treatment was significantly improved with the addition of 25 ng/ml of HGF [112]. HGF activated the PI3K/Akt signaling pathway in both INS-1 cells and mouse islets. An inhibitor of this pathway abrogated the pro-survival effect of HGF against STZ in INS-1 cells, indicating the importance of the PI3K pathway in mediating the anti-apoptotic effect of HGF [112]. Another study found that HGF protects the rat insulinoma cell line, RINm5F, against FFA-mediated apoptosis. Treatment with 50 ng/ml of HGF every 8 hrs significantly inhibited cell death in RINm5F cells exposed to a mixture of FFAs (oleate:palmitate 2:1) for 72 hrs. HGF exerted its pro-survival effect by counteracting, the increased intracellular oxidative stress and decreased expression of the anti-apoptotic molecule Bcl-2, induced by prolonged FFAs

exposure. As in the previous study this pro-survival effect is mediated through activation of PI3K [113].

Contrary to the pro-survival effects of HGF demonstrated thus far, a rather surprising finding was uncovered in a very recent study [114]. Garcia-Ocaña's group showed that HGF can be detrimental for beta cell survival in an environment with excessive fatty acid supply. They found that normal mouse islet cell cultures treated with HGF peptide, or transgenic mouse beta cells overexpressing HGF (RIP-HGF) display enhanced sensitivity to apoptosis mediated by incremental concentrations of palmitate *in vitro*. The pro-apoptotic effect of HGF peptide to palmitate-induced beta cell death was dose-dependent, peaking at 10 ng/ml of HGF. HGF-overexpressing islets show significantly decreased AMP-activated protein kinase (AMPK)-α and acetyl-CoA carboxylase (ACC) phosphorylation, diminished fatty acid oxidation (FAO), increased serine palmitoyltransferase (SPT) expression and enhanced ceramide formation compared with normal islets. Importantly, they found that human islets overexpressing HGF are also more susceptible to palmitate-induced beta cell apoptosis. Inhibition of de novo ceramide synthesis abrogated beta cell apoptosis induced by HGF and palmitate treatment in both mouse and human islet cells [114]. Thus, the authors surmise that the normal action of HGF, which is to induce glucose metabolism and glycolysis, is detrimental in the presence of palmitate, as it could lead to elevated malonyl-CoA which would inhibit carnitine palmitoyltransferase-I activity thereby reducing fatty acid oxidation, increasing ceramide synthesis, and ultimately resulting in enhanced beta cell death. As discussed in their study, the potential explanations for the opposite pro- and anti-apoptotic effects of HGF against FFAs observed in their study [114] versus that from Anastasi's group [113] are: i) the use of primary beta cells versus insulinoma cells which could exhibit very different glucose and lipid metabolic profiles, and ii) the composition of FFAs used, palmitate versus a mixture of oleate and palmitate, where oleate has been shown previously to have pro-survival effects on the beta cell.

In Vivo Effects

The protective effect of HGF on the beta cell has also been demonstrated *in vivo*. RIP-HGF transgenic mice that overexpress HGF in their beta cells are resistant to the diabetogenic effects of STZ for up to a month, as well as to its cytotoxic effects *in vivo* [108,112]. Administration of naked DNA containing HGF prior to the induction of diabetes by STZ, also protected beta cells from cell death, and conferred resistance to the diabetogenic effects up to 7 days [115]. Interestingly, intravenous injection of an adenovirus containing HGF cDNA into mice previously rendered diabetic by MLDS also resulted in amelioration of hyperglycemia and increased insulin-positive cells/islet after two weeks; however, the effect of HGF on beta cell death was not directly measured in this study [116]. Another *in vivo* situation where HGF was protective of beta cells was in the setting of islet transplants. The first few hours and days after islet transplant are characterized by substantial islet dysfunction and death [29]. RIP-HGF transgenic and Ad-HGF-transduced mouse islets transplanted under the kidney capsule of STZ-induced diabetic severe combined immunodeficient (SCID) mice substantially improved their

blood glucose [109,112]. The number of condensed, pyknotic beta cell nuclei was significantly reduced and the insulin content was significantly increased 24 hrs after the transplant in Ad-HGF grafts compared with control Ad-beta galactosidase (LacZ) grafts [112]. Similar results were obtained when islets from another species, NHP, transduced with Ad-mouse HGF were transplanted under the kidney capsule of diabetic SCID mice [111]. Using Ad-HGF in a different islet transplant model that more closely resembles human islet transplantation protocols, i.e., an intraportal allogeneic rat islet transplant using the same immunosuppressants employed by the Edmonton group, also showed markedly improved islet transplant outcomes. The number and size of islets remaining in the liver 25 days after transplant were significantly higher in the Ad-HGF-transduced versus Adv-LacZ-transduced islets, suggesting improved survival, although beta cell death was not directly measured in this study [117]. As with the *in vitro* studies, the only condition in which HGF seems to be potentially detrimental to the beta cell *in vivo* is in the state of obesity. The improved glucose homeostasis and increased beta cell mass and proliferation observed in RIP-HGF transgenic mice relative to normal littermates fed with standard chow [108,109,114] is completely lost following 15 weeks of high-fat feeding [114]. Furthermore, there is an increase, although not significant, in beta cell apoptosis *in vivo* in RIP-HGF mice compared to normal littermates put on a high-fat diet [114]. Future studies will determine whether HGF is protective against cytokine-induced cell death and whether endogenous HGF/c-met signaling is critical for beta cell survival in different situations of stress.

INSULIN AND INSULIN-LIKE GROWTH FACTORS (IGFs)

Introduction

Insulin, IGF-I and IGF-II belong to the IGF family and share at least a 50% homology in their amino acid sequence. All three peptides are expressed in distinct cell types of the pancreas at different stages of development. Insulin first detected very early in pancreatic development (E8.5), continues to be expressed and secreted from the beta cell through adulthood with a short half-life in minutes, circulating at picomolar concentrations. IGFs, on the other hand, are synthesized and secreted mainly from the liver, but are also made locally in almost every tissue, including the pancreas, with a half-life in hours determined by interaction with the IGF binding proteins (IGFBPs) and the acid-labile subunit, and circulate at nanomolar concentrations. Insulin and IGF-I mediate their action through two distinct tyrosine kinase receptors, the insulin receptor (IR) made in beta cells, and the IGF-I receptor (IGF-IR) present on alpha, beta and delta cells, of the pancreas respectively. At higher concentrations the peptides can also cross-react with the complementary receptor. IGF-II can activate both the IGF-IR and the IR (A-subtype) and also binds to a third receptor, IGFII- mannose-6-phosphate receptor, which likely regulates the degradation of IGF-II [118-121].

In Vitro Effects

As with the other growth factors, insulin and IGFs also potentiate the function and growth of the pancreatic beta cell [122-125]. Several studies have now shown that insulin and

IGFs are important pro-survival factors as well for the beta cell. *In vitro* culture experiments found rodent insulinoma cell lines as well as primary rodent and human beta cells are protected from glucose and/or serum-deprived cell death in the presence of exogenous insulin or IGFs [126-131]. Interestingly, the pro-survival effect of insulin on mouse and human beta cells was manifested only at low, more physiological concentrations, in the picomolar to low nanomolar range, but not at higher concentrations [130]. Similarly, IGF-II exhibited a dose-dependent effect at 50-500 ng/ml concentrations to increase the viability and decrease the apoptotic index of rat islets cultured *in vitro* [127]. Importantly, glucose was shown to mediate its pro-survival effects on the beta cell through the secretion of insulin. This was demonstrated in MIN-6 cells using insulin antibody or through deletion of the IR in mouse beta cells [129,132]. Insulin and IGFs also protect insulinoma cells against other cell death inducing agents including STZ [133], DEX [134], and ER-stress [135]. Several studies demonstrate the anti-apoptotic effects of insulin, IGF-I and IGF-II on cytokine-mediated beta cell death. Pretreatment of insulinoma cells, adult or 22-day old rat islets, and islets from pre-diabetic NOD female mice with insulin, IGF-I or IGF-II was effective in protecting against cytokine-mediated apoptotic cell death [136-142]. In this regard, Hill's group found that IGF-I was present *in vivo* in islets from pre-diabetic NOD mice that did not demonstrate insulitis, but not in islets with extensive immune infiltration, as seen by immunohistochemistry [139]. Similarly, IGF-II was expressed and present in fetal and neonatal islet cells, but declined rapidly 2 weeks after birth, with little IGF-I associated with fetal or postnatal islets [138]. They further demonstrated that the endogenous IGF-II was protective against cytokine-mediated cell death *in vitro*, since islets from 5-day old rats were not susceptible to cytokine-induced cell death unless treated with an IGF-II antibody [138]. Finally, human islets were also protected against IL-1β-induced, Fas-mediated apoptosis after adenoviral gene transfer of human IGF-I [140].

The anti-apoptotic effect of IGF-1/insulin against serum deprivation-induced apoptosis in RINm5F cells is prevented with inhibitors of NO generation, PI3K and Akt, and the dominant negative form of the tyrosine kinase c-Src [131]. However, in mouse islets physiological levels of insulin did not increase Akt phosphorylation, and the protective effect of insulin was only partially altered in serum-deprived islets lacking 80% of normal Akt activity, suggesting the presence of additional insulin-regulated anti-apoptotic pathways [130]. Similarly, inhibition of DEX-induced apoptosis in INS-1 cells by IGF-I was mediated through ERK phosphorylation and not through PI3K activation, as demonstrated with specific inhibitors of these pathways [134]. A general mechanism of IGF-I action in insulinoma cells, rodent islets and human islets subjected to cytokines is a reduction in cytokine-induced NOS expression and NO production. This effect appears to be mainly dependent on the activation of PI3K and an inhibition of NFκB activation [133,136,140-142]. IGF-I elevated expression of Bcl-2 and Bcl-xL proteins, and diminished caspase-9 activity, in cytokine-treated insulinoma cells and islets from NOD mice [142]. Finally, Pdx1 seems to play an important part in the pro-survival effect of insulin. Mouse islets treated with insulin showed nuclear localization of Pdx1, and more importantly, the pro-survival effects of insulin were largely absent in islets 50% deficient in Pdx1. Also, proteomic analysis of insulin-treated human islets revealed significant changes in a Pdx1-binding partner and regulator of beta cell survival, Bridge-1 [130].

In Vivo **Effects**

The pro-survival roles of insulin and IGFs have also been substantiated *in vivo* in the beta cell. These factors have been proven to protect against induction of Type 1 diabetes in the MLDS and NOD mouse models using both transgenic and acute peptide administration approaches. Several transgenic mouse models overexpressing IGF-I, either using the metallothionein promoter [143] or the rat insulin promoter in two different genetic backgrounds (C57BL/6-SJL and CD-1) of mice [125] displayed resistance to MLDS-induced diabetes and insulitis, and specifically reduced beta cell death in STZ-treated mice. Similarly, double transgenic mice that simultaneously overexpress IFN-γ and IGF-I in their beta cells had reduced 2-microglobulin expression, decreased Fas expression, and reduced islet infiltration, with a parallel decrease in STZ-induced apoptotic cell death relative to single IFN-γ overexpressing transgenic mice [144]. Acute administration of IGF-I, IGF-I derived peptides, or IGF-I/IGFBP-3 complex reduced the severity of insulitis and delayed the onset of diabetes in NOD mice [142,145,146]. The IGF-I/IGFBP-3 complex was more stable and afforded more efficient protection from insulitis and beta cell destruction than IGF-I [142]. IGF-II was also found to have pro-survival effects on the beta cell *in vivo*. Persistent circulating levels of IGF-II, in a keratin promoter driven IGF-II transgenic mouse model, suppressed the normal wave of beta cell apoptosis observed in neonatal pancreas [147]. Also, transplantation of IGF-II pre-treated rat islets in diabetic mice was much more effective in making mice normoglycemic compared to untreated islets, however, beta cell death was not directly measured in this study [127].

To assess the physiological role of locally produced IGF-I, pancreatic specific IGF-I gene deficient (PID) mice were generated by crossing Pdx1-Cre and IGF-I/loxP mice. Surprisingly, when challenged with MLDS the PID mice were more resistant to STZ-induced hyperglycemia and beta cell death compared to normal littermates, suggesting that endogenous locally produced IGF-I is detrimental for beta cell survival under conditions of MLDS [148]. Similarly, the role of endogenous insulin on beta cell survival was examined in mice with targeted disruption of the two nonallelic insulin genes. As these mice develop severe diabetes and die within 48 hrs of birth, the endocrine pancreas in late gestation was examined, and found to have reduced beta cell apoptosis, again suggesting that endogenous insulin is detrimental to beta cell survival [149]. Whether the detrimental phenotypes in these two knock out mouse models is due to compensatory changes in other growth factors, or cross-reactivity of the receptors with other members of this family, is not known. In contrast, mouse models lacking functional receptors for either insulin or IGF-1 only in the beta cell, did not exhibit any major phenotype. However, when both receptors were simultaneously deleted from the beta cell to create the double knock out mouse (bDKO), they developed diabetes 3 weeks after birth.

Interestingly, bDKO mice manifested increased apoptosis in their beta cells even at 2-weeks of age when these mice were normoglycemic, indicating that the endogenous peptides have a pro-survival function in the beta cell [150].

OTHER FACTORS

The factors described above are by no means a comprehensive list of growth factors and hormones that affect pancreatic beta cell survival. There are previous reports and some more recent studies describing anti-apoptotic actions of several other growth factors on the pancreatic beta cell. These include, and again may not be limited to, nerve growth factor (NGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), transforming growth factor (TGF), stromal cell derived factor, ghrelin, thyroid hormone, leptin and estradiol. However, studies on the effects of these factors on beta cell survival are limited, and therefore, have not been included in the current review.

CONCLUSION

The field describing the effects of various factors on beta cell survival and their mode of action is rapidly expanding. It is clear from a review of this literature (see Table **1** and Fig. (**2**) for summary) that the state of knowledge in this regard varies greatly between the different factors. Some obvious patterns that emerge are: i) the same factor can have differential effects on beta cell survival depending on the insult; ii) there is often interplay between factors to mediate their action, for example, GLP-1 mediates its pro-survival function through activation of the IGF or the EGF signaling pathways; iii) activation of PI3K/Akt and cAMP pathways most commonly mediate the pro-survival effects, with activation of ERK1/2 and inhibition of JNK pathways also playing a role; and, iv) inhibition of apoptosis is mediated most commonly through regulation of the bcl family of molecules, ER-stress response, and NO production for cytokine-mediated cell death.

The importance of knowing which factor can have pro-survival effects against specific cell death inducers, and understanding the pathways and mechanisms of their action, is obvious. It provides the potential to use these factors as therapeutic agents for improved beta cell survival *ex vivo* or *in vivo*. Specifically, one can envision using a combination of factors for maximal benefits on beta cell survival under conditions of varying susceptibility. It is of interest that all
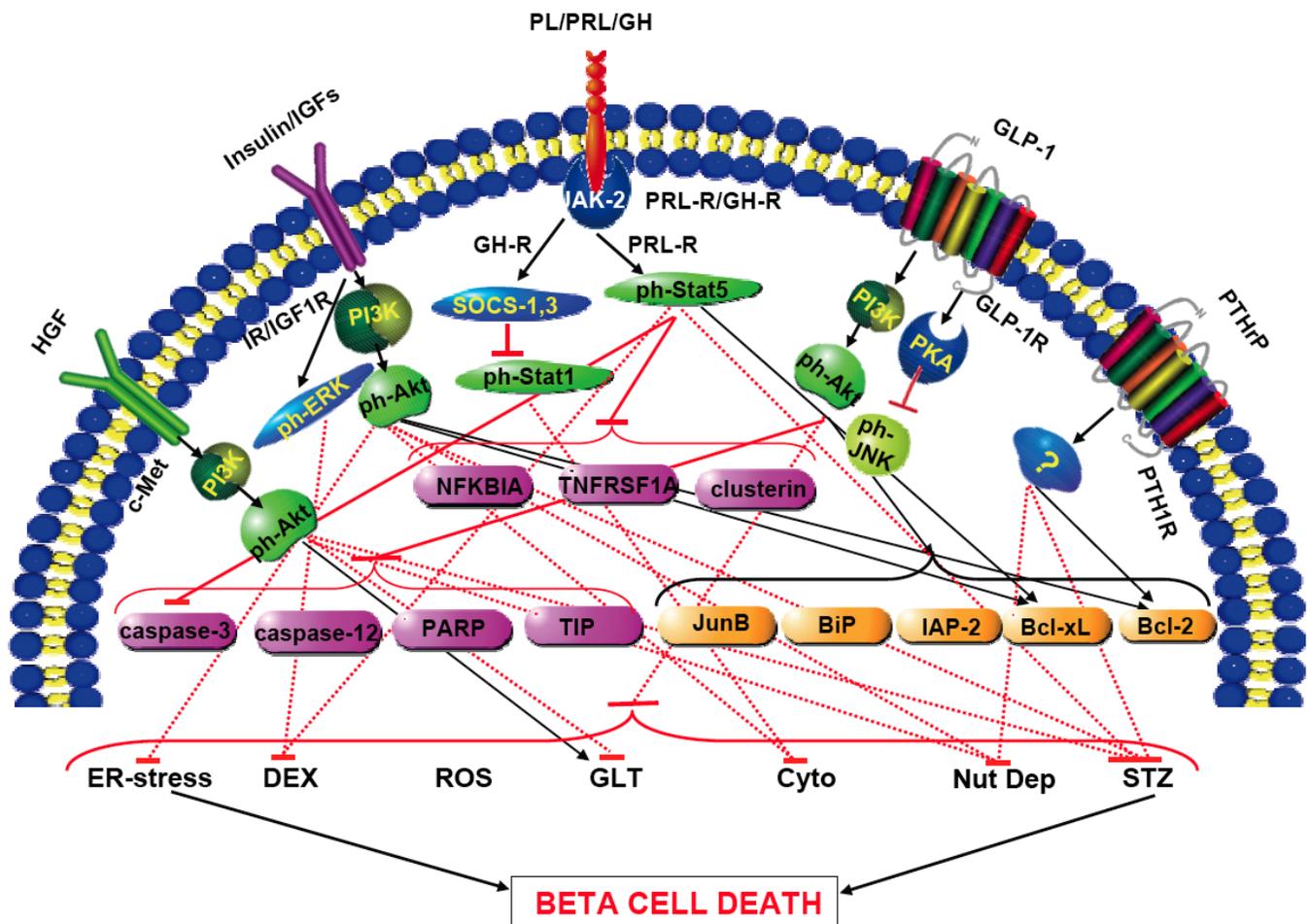
Fig. (2). Schematic representation summarizing the signaling pathways and molecular mediators through which growth factors modulate beta cell death against specific cell death effectors. Activation (black arrows) and inhibition (red lines) of targets downstream of the growth factors, and their effect on beta cell death against specific cell death stimuli. See text for abbreviations; TIP (thioredoxin interacting protein), Nut Dep (Nutrient Deprivation).

Table 1.   Summary of Effects Induced by the Different Growth Factors on Beta Cell Death Described in this Review

Growth Factor	Cell Death Inducers	Approach	Effect	Intracellular Effectors	Ref.
GLP-1					
	*In vitro***:**				
	Culture	Human islets/GLP-1	↓β-cell death		[45]
	Gluco/Lipotoxicity	Neonatal rat islets/GLP-1 & liraglutide	↓β-cell death	↑PI3K	[46]
		INS832/13 cells; human islets/GLP-1	↓β-cell death	↑PI3K; ↑NFκB binding; ↑Bcl-2, IAP-2	[47]
	Cytokines	Neonatal rat islets/GLP-1 & liraglutide	↓β-cell death	↑PI3K	[46]
		Rat islets/exendin-4	↓β-cell death		[48]
		INS-1E cells; Rat islets/exendin-4	↓β-cell death	↑phAkt; ↓caspase-3, iNOS, ↓ROS	[49]
		Mouse islets/Ad-PC1/3	↓β-cell death		[50]
		INS-1E cells; Rat islets/exendin-4	↓β-cell death	↑PKA; ↓JNK; ↑islet-brain1 transcription	[51]
		MIN6 cells; mouse islets/GLP-1	↓β-cell death	↑phAkt; ↑IGF-IR, IGF-II	[58]
		GIPR$^{-/-}$/GLP-1R$^{-/-}$ double knockout mouse islets	↑β-cell death	↓IGF-IR	[58]
		RINm5F cells/exendin-4		↑cAMP; ↓iNOS post-translationally	[59]
	ER-stress	Rat islets; INS-1 cells/exendin-4	↓β-cell death	↑PKA; ↑ATF-4 translation	[54]
		Rat islets; INS-1E cells/exendin-4	↓β-cell death	↑BiP, JunB, XIAP; ↓caspase-12	[55]
	ROS	MIN6 cells/GLP-1	↓β-cell death	↑PI3K, cAMP; ↑Bcl-2, Bcl-xL; ↓PARP cleavage	[52]
		INS832/13 cells/GLP-1	↓β-cell death	↑PI3K, EGFR; ↓FoxO1	[60]
	DEX	INS-1 cells; Mouse islets/exendin-4	↓β-cell death	↑PKA	[31]
	Immunosuppressive drugs	GLP-1-transfected MIN6 cells	↓β-cell death	↑Bcl-2; ↓PARP, Smac/ Diablo	[53]
	*In vivo***:**				
	MLDS/48 hrs	GLP-1R$^{-/-}$ mice	↑β-cell death		[48]
	MLDS	C57Bl6 male mice exendin-4	↓β-cell death		[48]
	NOD female mice	GLP-1/4-8 weeks	↓β-cell death; delayed onset of diabetes		[61]
	db/db BKS female mice	Exendin-4/2 weeks	↓β-cell death; no hyperglycemia		[62]
	Male Zucker diabetic fatty rats	GLP-1/2 days	↓β-cell death	↓caspase-3	[63]
	Px rats ± STZ	Exendin-4/4 weeks	↓β-cell death	↓*XBP-1, ATF4, ATF6, CHOP*	[64]
	Pdx-1$^{-/-}$ mice	Exendin-4/3 days	↑β-cell death		[65]
	Syngeneic mouse islet transplants	Exendin-4/1,3,10 days	↔β-cell death		[66]
PTHrP					
	*In vitro***:**				
	Glucose/Serum deprivation	RIP-PTHrP Tg mouse islet cell culture	↓β-cell death	↑*Bcl-2*	[81]
		Mouse islet cells/PTHrP peptides	↓β-cell death	Mediated by amino-terminus PTHrP	[81]
	STZ	RIP-PTHrP Tg mouse islet cell culture	↓β-cell death		[81]
	*In vivo***:**				
	STZ	Adult RIP-PTHrP Tg mice	↓hyperglycemia; ↓β-cell death		[82,83] [81,83]

(Table 1) contd.....

Growth Factor	Cell Death Inducers	Approach	Effect	Intracellular Effectors	Ref.
		Neonatal RIP-PTHrP Tg mice	↓hyperglycemia; ↓β-cell death		[81]
	Neonatal apoptosis	Neonatal RIP-PTHrP Tg mice:11,13,15, 17 days	↔β-cell death		[Vasavada *et al.* unpublished results]
Lactogens					
	In vitro:				
	DEX	Rat islets/PRL INS-1cells/oPRL	↓β-cell death ↓β-cell death	↓caspase-3; ↑Jak2/Stat5; ↑Bcl-xL	[94] [32]
		Mouse islets/oPRL RIP-mPL1 Tg islets	↓β-cell death	↑Jak2/Stat5; ↑Bcl-xL	[32]
	STZ	INS-1cells/oPRL	↓β-cell death	↓caspase-3	[32]
		RIP-mPL1 Tg islets	↓β-cell death		[32]
	Culture	Human islets/PRL	↑viable β-cells		[95]
	Cytokines	INS-1 cells/bovine GH		↓iNOS; ↓phStat1; ↑SOCS-1,3	[97]
		INS-1 cells/human GH	↓β-cell death	↓iNOS; ↑phStat5; ↑SOCS-3; ↑Bcl-xL	[98]
	In vivo:				
	STZ	Adult RIP-mPL1 Tg mice	↓hyperglycemia; ↓β-cell death		[76,90] [76]
	NOD mice	PRL/13 weeks	delayed diabetes onset		[100]
HGF					
	In vitro:				
	Culture	Porcine NPCC/EBV-HGF	↑β-cell survival		[110]
		NHP islets/Ad-mouse HGF	↓β-cell death		[111]
	STZ	INS-1cells/HGF	↓β-cell death	↑PI3K	[112]
	Lipotoxicity				
	Palmitate+Oleate	RINm5F cells/HGF	↓β-cell death	↑PI3K; ↑Bcl-2	[113]
	Palmitate	Mouse islets/HGF	↑β-cell death		[114]
	Palmitate	RIP-HGF Tg islets	↑β-cell death	↓phAMPK, phACC, FAO, ↑SPT, Ceramide	[114]
	Palmitate	Human islets/Ad-HGF	↑β-cell death	↑Ceramide	[114]
	In vivo:				
	STZ	Adult RIP-HGF Tg mice	↓hyperglycemia; ↓β-cell death		[108] [112]
		Male CD1 mice/naked HGF DNA	↓hyperglycemia; ↓β-cell death		[115]
	MLDS	BalbC male mice/Ad-HGF	↓hyperglycemia		[116]
	Islet transplants	Diabetic SCIDs/RIP-HGF,	↓hyperglycemia; ↓β-cell death		[109] [112]
		Diabetic SCIDs/Ad-HGF NHP islets	↓hyperglycemia; ↓β-cell death		[111]
		Allogeneic rats/Ad-HGF rat islets intraportally	↓hyperglycemia		[117]
	High fat diet/15 weeks	Adult RIP-HGF Tg mice	↑β-cell death		[114]
IGFs/Insulin					
	In vitro:				
	Culture	Hamster islets/IGF-II	↓β-cell death		[126]
		Rat islets/IGF-II	↓β-cell death		[127]

(Table 1) contd…..

Growth Factor	Cell Death Inducers	Approach	Effect	Intracellular Effectors	Ref.
		Rat islets/Insulin antibody	↑β-cell death		[128]
	Glucose/Serum	Rat islets/Insulin	↓β-cell death		[128]
	Deprivation	Mouse, human islets/Insulin	↓β-cell death	↑Pdx-1 nuclear localization; ↑Bridge-1	[130]
		Pdx-1^{+/-} islets	↑β-cell death		[130]
		RINm5F cells; rat, human Islets/Insulin; IGF-I	↓β-cell death	↑PI3K, c-src; ↑NOS3, NO; ↑phIRS-1, phIRS-2	[131]
		β-cell specific IRKO cells	↑β-cell death	↓phAkt; ↑caspase3, phFoxo1	[132]
		Mouse beta cells/Insulin;	↓β-cell death		[132]
		IR antibody	↑β-cell death		
		MIN6 cells/cisplatin/Insulin	↓β-cell death		[129]
		Rat islets/Insulin	↓β-cell death		[128]
	STZ	RINm5F cells/Ad-IGF-I	↓β-cell death	↓NO	[133]
	DEX	INS-1 cells/IGF-I	↓β-cell death	↑phERK	[134]
	ER-stress	MIN6 cells/IGF-I	↓β-cell death	↑phAkt; ↓PARP cleavage	[135]
		MIN6 cells/IR siRNAs	↑β-cell death		[135]
	Cytokines	Rat islets/IGF-I	↓β-cell death	↓NOS	[136]
		Neonatal rat islets/IGF-I, insulin	↓β-cell death		[137]
		~22 day rat islets/IGF-I, IGF-II	↓β-cell death		[138]
		~5 day rat islets/IGF-II antibody	↑β-cell death		[138]
		Pre-diabetic female NOD mice islets/IGF-I,IGF-II	↓β-cell death		[139]
		Human islets/Ad-IGF-I	↓β-cell death	↓NO	[140]
		INS-1 cells/IGF-I	↓β-cell death	↑PI3K; ↓IKK activity; ↓NOS2	[141]
		NIT-1 cells, NOD mouse islets/IGF-I	↓β-cell death	↑PI3K, phAkt; ↓caspase activity; ↑Bcl-xL, Bcl-2	[142]
***In vivo*:**					
	MLDS	Metallothionein-I promoter IGF-I Tg mice	↓hyperglycemia ↓β-cell death		[143]
		RIP-IGF-I Tg mice on CD1, C57BL/6-SJL strains	↓hyperglycemia ↓β-cell death		[125]
		Pdx-Cre x IGF-I/loxP KO mice	↓β-cell death		[148]
	RIP-IFNγ mice/STZ	RIP-IFNγ/RIP-IGF-I double Tg mice	↓β-cell death	↓2-microglobulin, Fas	[144]
	NOD mice	IGF-I peptides	↓hyperglycemia		[145,146]
		IGF-I/IGFBP-3 complex	↓β-cell death	↓caspase-3	[142]
	Neonatal apoptosis	Keratin promoter IGF-IITg mice	↓neonatal β-cell death		[147]
	Islet transplants	Diabetic SCIDs/Rat islets pre-treated with IGF-II	↓hyperglycemia;		[127]
	Ins1^{-/-}/Ins2^{-/-} double KO mice	e18.5, newborn	↓β-cell death		[149]
	β-cell specific	2 weeks	↑β-cell death		[150]
	IR^{-/-}/IGF-IR^{-/-} double KO mice		↔glycemia		

Note: Transgenic mouse (Tg); No changes (↔); Knockout (KO); refer to other abbreviations in the text.

the factors described above not only augment beta cell survival but are also known to potentiate beta cell growth and function, thereby enhancing their potential as future therapeutic agents for beta cell regeneration. As each of these peptides is secreted, and can exogenously manifest its action through receptors, it has the potential to be acutely

administered for treatment. A drawback to this method would be the non-specific effects of these factors on other organs where receptors are present. However, several of these factors are already being administered as drugs or are in clinical trial, underscoring the feasibility of this approach. Finally, understanding the downstream mechanisms that mediate the pro-survival effects of these factors could identify common pathways that can be targeted in the future for development of drugs to improve beta cell survival.

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