IGF2: an endocrine hormone to improve islet transplant survival

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Abstract

In the week following pancreatic islet transplantation, up to 50% of transplanted islets are lost due to apoptotic cell death triggered by hypoxic and pro-inflammatory cytokine-mediated cell stress. Thus, therapeutic approaches designed to protect islet cells from apoptosis could significantly improve islet transplant success. IGF2 is an anti-apoptotic endocrine protein that inhibits apoptotic cell death through the mitochondrial (intrinsic pathway) or via antagonising activation of pro-inflammatory cytokine signalling (extrinsic pathway), in doing so IGF2 has emerged as a promising therapeutic molecule to improve islet survival in the immediate post-transplant period. The development of novel biomaterials coated with IGF2 is a promising strategy to achieve this. This review examines the mechanisms mediating islet cell apoptosis in the peri- and post-transplant period and aims to identify the utility of IGF2 to promote islet survival and enhance long-term insulin independence rates within the setting of clinical islet transplantation.

Key Words
- insulin-like growth factor
- apoptosis
- islets
- cell survival
- islet transplantation

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Introduction

Islet transplantation is an emerging therapy for highly selected patients with type 1 diabetes mellitus (T1D) and is now a funded treatment in the United Kingdom, France, Switzerland and recently in Australia (McCall & Shapiro 2012, O'Connell *et al*. 2013). Islet allotransplantation into a recipient with T1D exposes the transplanted islets to a number of apoptotic stresses, including the instant blood-mediated inflammatory reaction (IBMIR), hypoxia, inflammation, hyperglycemia, enzymatic and mechanical injury and immune-mediated rejection that contribute to islet allograft failure in the early post-transplant period (Robertson 2004, Tjernberg *et al*. 2008, Walters *et al*. 2013). Thus, inhibition of islet apoptosis is an attractive and potentially effective therapeutic strategy to prevent the loss of functional islet mass post transplantation and improve clinical islet transplant outcomes.

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The insulin-like growth factor (IGF) family consists of two IGF peptides (IGF1 and IGF2), the actions of which are regulated by six binding proteins (IGFBP1–6). IGF2 is more highly expressed than IGF1 during development in rodents, ruminants and humans (Delhanty & Han 1993, Hill & Pell 1998), suggesting that it may be the more important IGF during development (Sang *et al*. 2008). Recently, Hills *et al*. (2012) have described *IGF2* as a more effective anti-apoptotic survival factor compared with *IGF1* in human placental villous cytotrophoblasts. While others (Giannoukakis *et al*. 2000) have shown that adenoviral-mediated *IGF1* overexpression was unable to protect isolated human islets from pro-inflammatory cytokine-induced apoptosis *in vitro*. In comparison, *IGF2* exerts a robust anti-apoptotic effect on many cell and tissue types including neurons, ovarian pre-ovulatory cells and pancreatic islets (Jung *et al*. 1996, Stewart & Rotwein 1996, Petrik *et al*. 1998). Jourdan *et al*. (2011) have shown the usefulness of IGF2 as an islet survival factor in a rodent model of islet engraftment, and transgenic overexpression of IGF2 protects against pro-inflammatory cytokine-induced apoptosis *in vitro* and improves islet transplant outcomes *in vivo* (Hughes *et al*. 2013), a process mediated via the interaction of IGF2 with the IGF1 receptor (IGF1R) on the islet cell surface. For these reasons, we believe that IGF2 represents the more promising endocrine growth factor to improve islet transplant survival. This review provides an overview of the current mechanisms of islet cell apoptosis in the peri- and post-transplant period. In addition, this review focuses on the actions of IGF2 within native, isolated and transplanted islets and discusses the therapeutic potential of IGF2 to promote islet cell survival and function post transplantation.

Apoptosis in islet transplantation

Apoptosis, also called programmed cell death, refers to a set of events within multicellular organisms, which lead to the breakdown of chromosomal DNA and the cessation of metabolic activity (Sia & Hanninen 2006). The key enzymes mediating the progression of apoptosis within a cell are the cysteine aspartate protease family of enzymes called caspases. Caspases reside within a cell as inactive procaspases (zymogens) until they are activated in response to pro-apoptotic stimuli. Once activated, caspases proceed to activate other caspases in a hierarchical manner, leading to the amplification of the apoptotic signalling cascade and cell death. Immediately following transplantation, islets experience the IBMIR that involves activation of coagulation pathways and complement and infiltration of pro-inflammatory cytokines, such as interleukin-1β (IL1β) and interferon-γ (IFNγ) (Moberg *et al*. 2002, Johansson *et al*. 2005). Allogenic rejection of islet grafts involves the activation of the adaptive immune system, in addition perforin and granzyme are primary mediators of islet cell death following transplantation (Sutton *et al*. 2006). This topic area has been extensively reviewed in the context of islet transplantation (Emamaullee & Shapiro 2006) and more broadly by references (Elmore 2007, Taylor *et al*. 2008).

Apoptosis in islet transplantation: the role for pro-inflammatory cytokines

In the early post-transplant period, multiple mechanisms are at play that negatively impact β-cell function and lead to islet cell apoptosis. Of these mechanisms, pro-inflammatory cytokines such as IL1β, IL6, IFNγ, tumour necrosis factor-α (TNF-α) and cyclooxygenase-2 mount a fierce inflammatory attack against the newly transplanted islet graft, triggering islet cell death (Cowley *et al*. 2012). The extent of cytokine release by islets in the early post-transplant period is directly related to islet transplant outcome in the recipient (Schroppel *et al*. 2005).

Experimentally, the release of pro-inflammatory cytokines by resident islet macrophages has been observed in rats following islet transplantation *in vivo* (Bottino *et al*. 1998, Montolio *et al*. 2007*a*,*b*). Exposure of rat islets to IL1β *in vitro* decreases islet insulin content, suppresses glucose-stimulated insulin secretion, induces DNA damage and leads to islet destruction (Bendtzen *et al*. 1986, Comens *et al*. 1987, Sandler *et al*. 1987, Wachlin *et al*. 2003). Recently, Yeung *et al*. (2012) have described morphological alterations in human islets following pro-inflammatory cytokine exposure *in vitro* and these changes are consistent with the cells undergoing apoptosis (i.e. cell shrinkage and chromosomal condensation). Mechanistically, pro-inflammatory cytokines mediate their inflammatory effects largely under the control of the nuclear factor κB (NFκB) and MAPK cell signalling pathways (Fig. 1), activation of which renders islets nonfunctional following the formation of cytotoxic nitric oxide (NO).

The role of IGF2 in the native, isolated and transplanted islet

IGF2 is highly expressed in the islet cells during embryonic development, coincidently; during this period, there is also substantial growth and structuring of the endocrine

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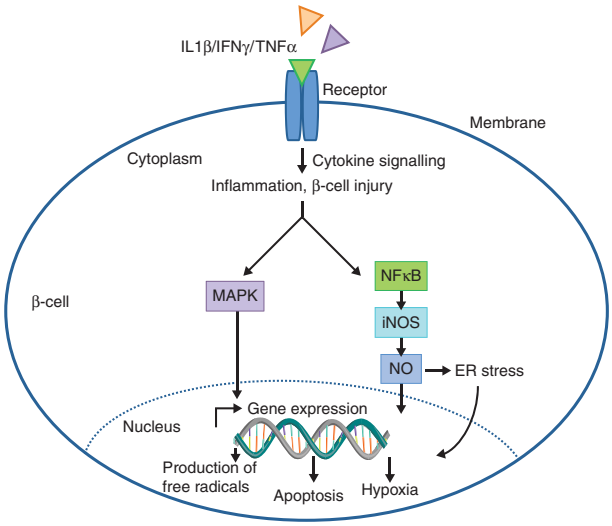

Figure 1
Cell signalling pathways activated in the pancreatic islets following pro-inflammatory cytokine exposure. Brain death is associated with the endogenous production of pro-inflammatory cytokines such as TNFα, IL1β and IFNγ (coloured triangles). The release of these cytokines is associated with inflammation and their presence triggers activation of the NFκB (green text box) and MAPK stress response pathways (purple text box) and renders islets non-functional, following induction of inducible nitric oxide synthase (iNOS; light blue box), the formation of NO (blue text box) and the induction of endoplasmic reticulum (ER) stress. As a result, islets experience subsequent stresses including the production of free radicals, apoptosis and hypoxia, among others.

β-cell component in the pancreatic islets (Hill *et al.* 1999*a*). In humans, immunofluorescence staining has revealed co-localisation of IGF2 and insulin cells in both fetal and adult pancreas; however, expression is more pronounced in the fetal setting (Portela-Gomes & Hoog 2000). Interestingly, IGF2 expression declines in mice and rats postnatally and this correlates with increased β-cell apoptosis *in vivo* (Petrik *et al.* 1998, 1999), suggesting that endogenous IGF2 exerts an early anti-apoptotic effect within native pancreatic islets. In contrast to this, in humans, IGF2 remains the most abundant IGF in circulation throughout life, the expression of which is detected in many tissues including the CNS, adrenal medulla, pancreas and purified β-cells (Han *et al.* 1987, Bryson *et al.* 1989, Hill 1990, Hogg *et al.* 1993, Miettinen *et al.* 1993, Asfari *et al.* 1995, Bergmann *et al.* 1996, Katz *et al.* 1997). The post-natal decline in IGF2 expression may be a result of differences in the *IGF2* gene promoter structure between humans and rodents (Foulstone *et al.* 2005).

The major role of IGF2 during embryonic development is the regulation of islet growth and differentiation, a role that IGF2 performs in part through its mitogenic growth-promoting effects (Han *et al.* 1988, Hogg *et al.* 1993, Miettinen *et al.* 1993). IGF2 has been shown to promote pancreatic β-cell survival against apoptotic stimuli *in vitro* and *in vivo* (Rabinovitch *et al.* 1982, Swenne *et al.* 1987, Hogg *et al.* 1993, Ilieva *et al.* 1999, Robitaille *et al.* 2003, Jourdan *et al.* 2011) and induce proliferation in a growth-arrested mouse β-cell line (Milo-Landesman & Efrat 2002). IGF2 protects pre-diabetic NOD mouse islets from the cytotoxic actions of IL1β by the mechanisms that include a reduction in apoptosis (Hill *et al.* 1999*b*). Similarly, adenoviral-mediated overexpression of IGF2 in isolated rat islets conferred significant protection against IL1β-induced apoptosis (Estil les *et al.* 2009) and increased β-cell replication and β-cell mass regeneration in transplanted islets, effectively reducing the β-cell mass required to achieve normoglycemia in diabetic rats (Estil les *et al.* 2012). Recently, data from our laboratory has shown the ability of adenoviral-mediated IGF2 overexpression to protect islets from pro-inflammatory cytokine-induced apoptosis *in vitro* and improve transplant outcomes in a minimal mass islet transplant model *in vivo* (Hughes *et al.* 2013). Most of the biological actions of IGF2, including its anti-apoptotic effects are mediated via the IGF1R (O'Dell & Day 1998, Braulke 1999). All endocrine pancreas cell types express the IGF1R (Van Schravendijk *et al.* 1987, Fehmann *et al.* 1996), providing support for an IGF2-mediated anti-apoptotic strategy to promote islet survival post transplantation. In the context of IGF2 function, binding of IGF2 to the IGF1R leads to phosphorylation of insulin receptor substrate-2, which activates the phosphoinositide 3-kinase/Akt cell signalling pathway to mediate cell survival (van Haeften & Twickler 2004).

In addition to its anti-apoptotic properties, IGF2 upregulates the expression of vascular endothelial growth factor (VEGF), an endothelial cell-specific mitogen involved in vasculogenesis and angiogenesis (Kwon *et al.* 2004). The production of VEGF in this manner is particularly advantageous when considering the reduction in islet vascular density and function that occurs following the islet isolation procedure. Mechanistically, IGF2 upregulates VEGF by increasing the expression of hypoxia-inducible factor-1α (HIF1α), a master regulator of cellular and systemic homeostatic response to hypoxia. Interestingly, HIF1α itself has been shown to play a protective role in various experimental settings of apoptosis, including islet transplantation (Kim & Kim 2005, Czibik *et al.* 2009, Franke *et al.* 2013, Stokes *et al.* 2013). To our knowledge, there have been no studies aiming to identify whether a synergistic role of IGF2 with HIF1α exists in the context of islet transplant survival;

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however, when considering the significant crosstalk that occurs between both systems (Feldser *et al*. 1999), it would be an interesting hypothesis to explore.

The optimal delivery method(s) for IGF2 expression in pancreatic islet cells

Protecting islets from early transplant stresses such as apoptosis is crucial to prevent islet allograft failure and ensure long-term insulin independence in diabetic patients. IGF2 is a promising candidate molecule to fulfill this need; however, the question remains regarding the optimal delivery method that should be paired with IGF2 to provide adequate protection against apoptosis. Theoretically, IGF2 could be delivered in a variety of ways to protect against islet cell death, including viral or non-viral gene therapy methods, islet encapsulation methods or within polymeric scaffolds, via supplementation of culture medium with IGF2 before transplantation or under islet coculture conditions, each with their own advantages and disadvantages (Table 1).

The culture of isolated islets before transplantation allows the islets to recover following the aggressive islet isolation procedure that leads to destruction of the islet microenvironment and contributes significantly to islet cell apoptosis in the early post-transplant period. Thus, the islet culture before transplantation provides an excellent window of opportunity to supplement the islet culture medium with anti-apoptotic molecules such as IGF2. This approach has been confirmed by Ilieva *et al*. (1999) who demonstrate that the presence of IGF2 during culture protects islets from isolation-induced apoptosis and necrosis. A possible disadvantage with pre-culture incubation of islets is the risk of islet fusion, which may lead to hypoxia, islet necrosis and significant loss of islet viability (Ichii *et al*. 2007). In addition, when considering the short half-life of growth factors (McGeachie & Tennant 1997), it is likely that the *in vitro* benefit would be short lived *in vivo*.

Table 1 Potential delivery method(s) for IGF2 expression in pancreatic islet cells

Approach	Advantages	Disadvantages
Supplementation of culture medium with exogenous IGF2	Minimises islet cell loss during pre-transplant culture, leading to increased islet survival (Ilieva *et al*. 1999)	Effect limited by biological half-life of growth factors (McGeachie & Tennant 1997)
	Effective as a pre-transplant method to ensure viable islet mass for application in down-stream strategies, such as islet encapsulation	Residual IGF2 expression unlikely to have additional anti apoptotic benefit *in vivo*
Islet encapsulation	Immunoprotection (cellular and humoral; McGeachie & Tennant 1997)	Bioincompatibility, characterised by fibrotic overgrowth upon the microcapsule surface and reduced diffusion of oxygen and nutrients (Sakata *et al*. 2012)
	Can reduce or prevent chronic administration of immunosupressants and their associated side effects (i.e. islet toxicity and malignancy)	Unable to revascularise after transplantation, exacerbating islet hypoxia and subsequent islet cell death (Narang & Mahato 2006)
Tissue engineering scaffolds	Provide a supportive microenvironment for transplanted cells	Cell seeding to scaffolds can be time consuming and inefficient due to the limited penetration ability of cells into the scaffolds (Chan & Leong 2008)
	Can be designed with varying biocompatible and biodegradable materials, allowing them to be tailored for specific applications (Salvay & Shea 2006, Hutmacher & Cool 2007)	
Gene therapy to overexpress IGF2 (viral/non-viral)	*Ex vivo* gene therapy ensures that the expression of IGF2 would be localised to the islets, providing cell protection while leaving the immune system and other organs unaffected (Hughes *et al*. 2013)	Non-viral vectors are associated with poor transduction efficiency compared with their viral vector counterparts (Mahato *et al*. 2003)
	Option for transient (adenoviral) or stable (adeno associated viral and lenti-viral) transgene expression to suit required application	Stimulation of the immune system (viral vectors) (Muruve *et al*. 1999)
Cell line overexpressing IGF2	Can be combined with other anti-apoptotic strategies, such as islet encapsulation or co-culture transplantation	Results in permanent transgene expression by the transduced cells which raises potential concern for malignancy as the cells permanently overexpress an anti-apoptotic factor (Bergmann *et al*. 1996)
	Provides the option to overexpress genes alone or in combination	

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Another interesting approach involves the use of biomaterials, designed to entrap or encapsulate growth factors into, or adsorb them onto biological scaffolds (Hutmacher & Cool 2007, Chan & Leong 2008). Significant work in this area has been undertaken using the sister compound IGF1 (Jaklenec *et al*. 2008, Chen *et al*. 2009, Sun *et al*. 2011, Kim *et al*. 2012). In one example, Nelson *et al*. (2011) used porous poly(ester urethane)urea scaffolds to demonstrate the long-term delivery of bioactive IGF1 *in vitro*. Likewise, the Meinel group showed that IGF1 releasing silk fibroin scaffolds initiated chondrogenesis from human mesenchymal stem cells *in vitro* (Uebersax *et al*. 2008). Adsorbing IGF1 onto porous hydroxyapatite, or chitosan scaffolds, enhanced osseointegration *in vivo* due to enhanced osteoblastic proliferation and vascularisation (Damien *et al*. 2003, Nandi *et al*. 2013). Kodama *et al*. (2009) engineered functional islets from single-cell suspensions using a protocol that involved seeding islets onto a polyglycolic acid scaffold and supplementing the islet culture medium with growth factors including IGF2. The resulting islets restored normoglycemia in diabetic mice.

Alginate microcapsules represent an alternative yet versatile approach to biological scaffolds, designed to encapsulate one or a few islets within a semi permeable membrane. In one strategy, bioengineered TM4 cells stably overexpressing IGF2 were coencapsulated with islet cells into alginate microcapsules, leading to significantly improved islet survival *in vitro* and *in vivo*, and improved normoglycemia maintenance (Jourdan *et al*. 2011). Since their introduction over 30 years ago, significant advances have been made in the engineering of alginate microcapsules including refinement of their sophisticated multilayer architectures leading to enhanced biocompatibility and biodegradability *in vivo* (Schneider *et al*. 2001). However, the current major limitation of encapsulated islets is the fact that they are unable to revascularise after transplantation, exacerbating islet hypoxia and subsequent β-cell death (Sakata *et al*. 2012).

Viral and non-viral-mediated transduction of isolated islets to overexpress IGF2 represents another interesting option to enhance islet survival. Non-viral vectors offer the major advantage of high clinical safety and lack of vector-mediated immunogenicity, but they are significantly disadvantaged by their low-efficiency transduction (10–20%) in pancreatic islets (Narang & Mahato 2006). On the other hand, viral vectors offer the advantage of high-efficiency transduction but are limited by their unstable expression, need for repeated administration and stimulation of the immune system (Muruve *et al*. 1999). The major difficulty of any gene therapy strategy lies in the requirement for all or most cells to express the gene in order to gain protection. However, in this regard, IGF2 proves optimal as it mediates its anti-apoptotic effect via autocrine and paracrine mechanisms which negates the need for every islet cell to express the therapeutic gene. In the context of islet transplantation, the isolated islets are transduced *ex vivo* outside the body and any remaining viral particles are 'washed off' or removed before transplantation. This considerably limits the likelihood of any viral vector-mediated systemic response, which can be potentially life threatening *in vivo* (Raper *et al*. 2003). Moreover, considering that up to 70% of islets can die due to apoptosis and necrosis in the immediate post-transplant period, a potential major advantage of a gene therapy approach is that islets would be exposed to a constitutively produced supply of anti-apoptotic IGF2 before transplantation and during the immediate post-transplant period (Hughes *et al*. 2013).

Engineering of β-cell lines that can protect against pro-inflammatory cytokine-mediated damage represents an interesting alternative to isolated islets for transplantation. Chen *et al*. (2000) developed a cytokine resistant rat insulinoma INS-1 cell line capable of protecting against IL1β- and IFNγ-mediated apoptosis more efficiently than cells stably overexpressing the anti-apoptotic gene *Bcl2*. There was an enhanced anti-apoptotic effect when the cytokine selection strategy was applied to the *Bcl2* overexpressing cells. Importantly, the cells displayed no loss of glucose responsiveness, a critical function that ordinarily disappears very early during apoptosis. Combining this cytokine-resistant selection strategy with a cell line overexpressing IGF2, such as that used by Jourdan *et al*. (2011), could provide a novel approach for improving islet cell survival in the early post-transplant period.

Conclusions and future perspectives

The primary goal of islet transplantation is to achieve stable and long-term normoglycaemia in diabetic patients without the risks of hypoglycaemia. The first barrier that must be overcome to accomplish this is to improve the survival of islets in the immediate transplant period. The endocrine growth factor IGF2 represents a promising candidate molecule to promote islet survival post transplantation, but additional investigation is required to identify the optimal delivery approach to ensure sufficient expression of IGF2 within the islet microenvironment. As such, the full therapeutic potential of IGF2 to promote

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islet survival and function post transplantation may not be realised until improvements are made in the efficacy, biocompatibility and safety of the gene delivery technologies currently under investigation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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