

# IGF2: an endocrine hormone to improve islet transplant survival

Amy Hughes<sup>1,2</sup>, Darling Rojas-Canales<sup>2,5</sup>, Chris Drogemuller<sup>2,3,4,5</sup>,  
Nicolas H Voelcker<sup>5,6</sup>, Shane T Grey<sup>7,\*</sup> and P T H Coates<sup>2,3,4,5,8,\*†</sup>

<sup>1</sup>Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, Illinois 60612, USA

<sup>2</sup>The Centre for Clinical and Experimental Transplantation (CCET) and <sup>3</sup>Australian Islet Transplant Consortium, The Royal Adelaide Hospital, Adelaide, South Australia 5000, Australia

<sup>4</sup>School of Medicine, University of Adelaide, Adelaide, South Australia 5000, Australia

<sup>5</sup>Cooperative Research Centre for Cell Therapy Manufacturing (CRC-CTM), Mawson Lakes, South Australia 5095, Australia

<sup>6</sup>Mawson Institute, University of South Australia, Mawson Lakes, South Australia 5095, Australia

<sup>7</sup>Gene Therapy and Autoimmunity Group, Immunology Program, Garvan Institute of Medical Research, Darlinghurst, New South Wales 2010, Australia

<sup>8</sup>Centre for Stem Cell Research, University of Adelaide, Adelaide, South Australia 5000, Australia

\*S T Grey and P T H Coates contributed equally to this work

†P T H Coates is now at Department of Nephrology and Transplantation Services, The Royal Adelaide Hospital, Frome Road, Adelaide, South Australia 5000, Australia

Correspondence should be addressed to S T Grey or P T H Coates  
**Email**  
s.grey@garvan.org.au or  
toby.coates@health.sa.gov.au

## 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

*Journal of Endocrinology*  
(2014) 221, R41–R48

## 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.

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-1 $\beta$  (IL1 $\beta$ ) and interferon- $\gamma$  (IFN $\gamma$ ) (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).

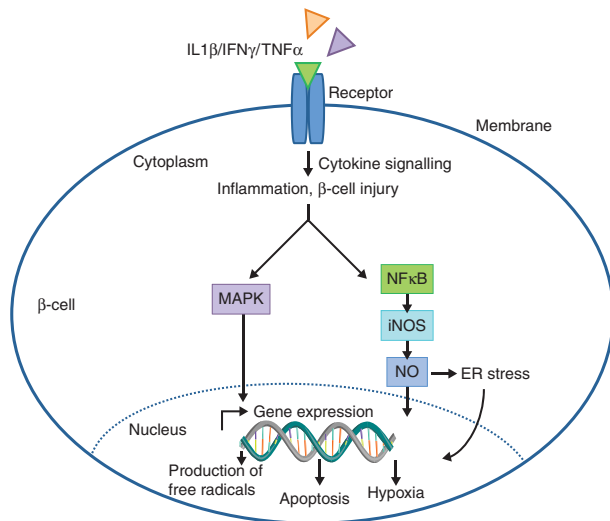
## Apoptosis in islet transplantation: the role for pro-inflammatory cytokines

In the early post-transplant period, multiple mechanisms are at play that negatively impact  $\beta$ -cell function and lead to islet cell apoptosis. Of these mechanisms, pro-inflammatory cytokines such as IL1 $\beta$ , IL6, IFN $\gamma$ , tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) 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.* 2007a,b). Exposure of rat islets to IL1 $\beta$  *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  $\kappa$ B (NF $\kappa$ B) 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, coincidentally; during this period, there is also substantial growth and structuring of the endocrine

**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  $TNF\alpha$ ,  $IL1\beta$  and  $IFN\gamma$  (coloured triangles). The release of these cytokines is associated with inflammation and their presence triggers activation of the  $NF\kappa B$  (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.

$\beta$ -cell component in the pancreatic islets (Hill *et al.* 1999a). 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  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -cell line (Milo-Landesman & Efrat 2002). IGF2 protects pre-diabetic NOD mouse islets from the cytotoxic actions of  $IL1\beta$  by the mechanisms that include a reduction in apoptosis (Hill *et al.* 1999b). Similarly, adenoviral-mediated overexpression of IGF2 in isolated rat islets conferred significant protection against  $IL1\beta$ -induced apoptosis (Estil les *et al.* 2009) and increased  $\beta$ -cell replication and  $\beta$ -cell mass regeneration in transplanted islets, effectively reducing the  $\beta$ -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-1 $\alpha$  (HIF1 $\alpha$ ), a master regulator of cellular and systemic homeostatic response to hypoxia. Interestingly, HIF1 $\alpha$  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 HIF1 $\alpha$  exists in the context of islet transplant survival;

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 <i>et al.</i> 1999) Effective as a pre-transplant method to ensure viable islet mass for application in downstream strategies, such as islet encapsulation	Effect limited by biological half-life of growth factors (McGeachie & Tennant 1997) Residual IGF2 expression unlikely to have additional anti apoptotic benefit <i>in vivo</i>
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 <i>et al.</i> 2012)
Tissue engineering scaffolds	Can reduce or prevent chronic administration of immunosuppressants and their associated side effects (i.e. islet toxicity and malignancy) Provide a supportive microenvironment for transplanted cells Can be designed with varying biocompatible and biodegradable materials, allowing them to be tailored for specific applications (Salvay & Shea 2006, Hutmacher & Cool 2007)	Unable to revascularise after transplantation, exacerbating islet hypoxia and subsequent islet cell death (Narang & Mahato 2006) Cell seeding to scaffolds can be time consuming and inefficient due to the limited penetration ability of cells into the scaffolds (Chan & Leong 2008)
Gene therapy to overexpress IGF2 (viral/non-viral)	<i>Ex vivo</i> 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 <i>et al.</i> 2013) Option for transient (adenoviral) or stable (adeno associated viral and lenti-viral) transgene expression to suit required application	Non-viral vectors are associated with poor transduction efficiency compared with their viral vector counterparts (Mahato <i>et al.</i> 2003) Stimulation of the immune system (viral vectors) (Muruve <i>et al.</i> 1999)
Cell line overexpressing IGF2	Can be combined with other anti-apoptotic strategies, such as islet encapsulation or co-culture transplantation Provides the option to overexpress genes alone or in combination	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 <i>et al.</i> 1996)

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 multi-layer 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  $\beta$ -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  $\beta$ -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 IL1 $\beta$ - and IFN $\gamma$ -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

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.

#### Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

#### References

- Asfari M, De W, Noel M, Holthuisen PE & Czernichow P 1995 Insulin-like growth factor-II gene expression in a rat insulin-producing  $\beta$ -cell line (INS-1) is regulated by glucose. *Diabetologia* **38** 927–935. (doi:10.1007/BF00400581)
- Bendtzen K, Mandrup-Poulsen T, Nerup J, Nielsen JH, Dinarello CA & Svenson M 1986 Cytotoxicity of human  $\text{p}17$  interleukin-1 for pancreatic islets of Langerhans. *Science* **232** 1545–1547. (doi:10.1126/science.3086977)
- Bergmann U, Funatomi H, Kormmann M, Ishiwata T, Beger H & Korc M 1996 Insulin-like growth factor II activates mitogenic signaling in pancreatic cancer cells via IRS-1. *International Journal of Oncology* **9** 487–492. (doi:10.3892/ijo.9.3.487)
- Bottino R, Fernandez LA, Ricordi C, Lehmann R, Tsan MF, Oliver R & Inverardi L 1998 Transplantation of allogeneic islets of Langerhans in the rat liver: effects of macrophage depletion on graft survival and microenvironment activation. *Diabetes* **47** 316–323. (doi:10.2337/diabetes.47.3.316)
- Braulke T 1999 Type-2 IGF receptor: a multi-ligand binding protein. *Hormone and Metabolic Research* **31** 242–246. (doi:10.1055/s-2007-978725)
- Bryson JM, Tuch BE & Baxter RC 1989 Production of insulin-like growth factor-II by human fetal pancreas in culture. *Journal of Endocrinology* **121** 367–373. (doi:10.1677/joe.0.1210367)
- Chan BP & Leong KW 2008 Scaffolding in tissue engineering: general approaches and tissue-specific considerations. *European Spine Journal* **17**(Suppl 4) 467–479. (doi:10.1007/s00586-008-0745-3)
- Chen G, Hohmeier HE, Gasa R, Tran VV & Newgard CB 2000 Selection of insulinoma cell lines with resistance to interleukin- $\beta$ - and  $\gamma$ -interferon-induced cytotoxicity. *Diabetes* **49** 562–570. (doi:10.2337/diabetes.49.4.562)
- Chen FM, Chen R, Wang XJ, Sun HH & Wu ZF 2009 *In vitro* cellular responses to scaffolds containing two microencapsulated growth factors. *Biomaterials* **30** 5215–5224. (doi:10.1016/j.biomaterials.2009.06.009)
- Comens PG, Wolf BA, Unanue ER, Lacy PE & McDaniel ML 1987 Interleukin 1 is potent modulator of insulin secretion from isolated rat islets of Langerhans. *Diabetes* **36** 963–970. (doi:10.2337/diab.36.8.963)
- Cowley MJ, Weinberg A, Zammit NW, Walters SN, Hawthorne WJ, Loudovaris T, Thomas H, Kay T, Gunton JE, Alexander SI *et al.* 2012 Human islets express a marked proinflammatory molecular signature prior to transplantation. *Cell Transplantation* **21** 2063–2078. (doi:10.3727/096368911X627372)
- Czibik G, Sagave J, Martinov V, Ishaq B, Sohl M, Sefland I, Carlsen H, Farnebo F, Blomhoff R & Valen G 2009 Cardioprotection by hypoxia-inducible factor 1  $\alpha$  transfection in skeletal muscle is dependent on haem oxygenase activity in mice. *Cardiovascular Research* **82** 107–114. (doi:10.1093/cvr/cvp035)
- Damien E, Hing K, Saeed S & Revell PA 2003 A preliminary study on the enhancement of the osteointegration of a novel synthetic hydroxyapatite scaffold *in vivo*. *Journal of Biomedical Materials Research. Part A* **66** 241–246. (doi:10.1002/jbm.a.10564)
- Delhanty PJ & Han VK 1993 The expression of insulin-like growth factor (IGF)-binding protein-2 and IGF-II genes in the tissues of the developing ovine fetus. *Endocrinology* **132** 41–52.
- Elmore S 2007 Apoptosis: a review of programmed cell death. *Toxicologic Pathology* **35** 495–516. (doi:10.1080/01926230701320337)
- Emamaullee JA & Shapiro AM 2006 Interventional strategies to prevent  $\beta$ -cell apoptosis in islet transplantation. *Diabetes* **55** 1907–1914. (doi:10.2337/db05-1254)
- Estil les E, Tellez N, Soler J & Montanya E 2009 High sensitivity of  $\beta$ -cell replication to the inhibitory effects of interleukin- $\beta$ : modulation by adenoviral overexpression of IGF2 in rat islets. *Journal of Endocrinology* **203** 55–63. (doi:10.1677/JOE-09-0047)
- Estil les E, Tellez N, Escoriza J & Montanya E 2012 Increased  $\beta$ -cell replication and  $\beta$ -cell mass regeneration in syngeneically transplanted rat islets overexpressing insulin-like growth factor II. *Cell Transplantation* **21** 2119–2129. (doi:10.3727/096368912X638955)
- Fehmann HC, Jehle P, Markus U & Goke B 1996 Functional active receptors for insulin-like growth factors-I (IGF-I) and IGF-II on insulin-, glucagon-, and somatostatin-producing cells. *Metabolism: Clinical and Experimental* **45** 759–766. (doi:10.1016/S0026-0495(96)90143-2)
- Feldser D, Agani F, Iyer NV, Pak B, Ferreira G & Semenza GL 1999 Reciprocal positive regulation of hypoxia-inducible factor 1 $\alpha$  and insulin-like growth factor 2. *Cancer Research* **59** 3915–3918.
- Foulstone E, Prince S, Zaccheo O, Burns JL, Harper J, Jacobs C, Church D & Hassan AB 2005 Insulin-like growth factor ligands, receptors, and binding proteins in cancer. *Journal of Pathology* **205** 145–153. (doi:10.1002/path.1712)
- Franke K, Kalucka J, Mamlouk S, Singh RP, Muschter A, Weidemann A, Iyengar V, Jahn S, Wiczorek K, Geiger K *et al.* 2013 HIF-1 $\alpha$  is a protective factor in conditional PHD2-deficient mice suffering from severe HIF-2 $\alpha$ -induced excessive erythropoiesis. *Blood* **121** 1436–1445. (doi:10.1182/blood-2012-08-449181)
- Giannoukakis N, Mi Z, Rudert WA, Gambotto A, Trucco M & Robbins P 2000 Prevention of  $\beta$  cell dysfunction and apoptosis activation in human islets by adenoviral gene transfer of the insulin-like growth factor I. *Gene Therapy* **7** 2015–2022. (doi:10.1038/sj.gt.3301333)
- van Haeften TW & Twickler TB 2004 Insulin-like growth factors and pancreas  $\beta$  cells. *European Journal of Clinical Investigation* **34** 249–255. (doi:10.1111/j.1365-2362.2004.01337.x)
- Han VK, Hill DJ, Strain AJ, Towle AC, Lauder JM, Underwood LE & D'Ercole AJ 1987 Identification of somatomedin/insulin-like growth factor immunoreactive cells in the human fetus. *Pediatric Research* **22** 245–249. (doi:10.1203/00006450-198709000-00001)
- Han VK, Lund PK, Lee DC & D'Ercole AJ 1988 Expression of somatomedin/insulin-like growth factor messenger ribonucleic acids in the human fetus: identification, characterization, and tissue distribution. *Journal of Clinical Endocrinology and Metabolism* **66** 422–429. (doi:10.1210/jcem-66-2-422)
- Hill DJ 1990 Relative abundance and molecular size of immunoreactive insulin-like growth factors I and II in human fetal tissues. *Early Human Development* **21** 49–58. (doi:10.1016/0378-3782(90)90110-5)
- Hill RA & Pell JM 1998 Regulation of insulin-like growth factor I (IGF-I) bioactivity *in vivo*: further characterization of an IGF-I-enhancing antibody. *Endocrinology* **139** 1278–1287. (doi:10.1210/endo.139.3.5804)
- Hill DJ, Hogg J, Petrik J, Arany E & Han VK 1999a Cellular distribution and ontogeny of insulin-like growth factors (IGFs) and IGF binding protein messenger RNAs and peptides in developing rat pancreas. *Journal of Endocrinology* **160** 305–317. (doi:10.1677/joe.0.1600305)
- Hill DJ, Petrik J, Arany E, McDonald TJ & Delovitch TL 1999b Insulin-like growth factors prevent cytokine-mediated cell death in isolated islets of

- Langerhans from pre-diabetic non-obese diabetic mice. *Journal of Endocrinology* **161** 153–165. (doi:10.1677/joe.0.1610153)
- Hills FA, Mehmet H & Sullivan MH 2012 Insulin-like growth factor-II and heparin are anti-apoptotic survival factors in human villous cytotrophoblast. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* **163** 11–16. (doi:10.1016/j.ejogrb.2012.03.013)
- Hogg J, Han VK, Clemmons DR & Hill DJ 1993 Interactions of nutrients, insulin-like growth factors (IGFs) and IGF-binding proteins in the regulation of DNA synthesis by isolated fetal rat islets of Langerhans. *Journal of Endocrinology* **138** 401–412. (doi:10.1677/joe.0.1380401)
- Hughes A, Mohanasundaram D, Kireta S, Jessup CF, Drogemuller CJ & Coates PT 2013 Insulin-Like growth factor-II (IGF-II) prevents proinflammatory cytokine-induced apoptosis and significantly improves islet survival after transplantation. *Transplantation* **95** 671–678. (doi:10.1097/TP.0b013e31827fa453)
- Hutmacher DW & Cool S 2007 Concepts of scaffold-based tissue engineering – the rationale to use solid free-form fabrication techniques. *Journal of Cellular and Molecular Medicine* **11** 654–669. (doi:10.1111/j.1582-4934.2007.00078.x)
- Ichii H, Sakuma Y, Pileggi A, Fraker C, Alvarez A, Montelongo J, Szust J, Khan A, Inverardi L, Naziruddin B *et al.* 2007 Shipment of human islets for transplantation. *American Journal of Transplantation* **7** 1010–1020. (doi:10.1111/j.1600-6143.2006.01687.x)
- Ilieva A, Yuan S, Wang RN, Agapitos D, Hill DJ & Rosenberg L 1999 Pancreatic islet cell survival following islet isolation: the role of cellular interactions in the pancreas. *Journal of Endocrinology* **161** 357–364. (doi:10.1677/joe.0.1610357)
- Jaklenc A, Hinckfuss A, Bilgen B, Ciombor DM, Aaron R & Mathiowitz E 2008 Sequential release of bioactive IGF-I and TGF- $\beta$  1 from PLGA microsphere-based scaffolds. *Biomaterials* **29** 1518–1525. (doi:10.1016/j.biomaterials.2007.12.004)
- Johansson H, Lukinius A, Moberg L, Lundgren T, Berne C, Foss A, Felldin M, Kallen R, Salmela K, Tibell A *et al.* 2005 Tissue factor produced by the endocrine cells of the islets of Langerhans is associated with a negative outcome of clinical islet transplantation. *Diabetes* **54** 1755–1762. (doi:10.2337/diabetes.54.6.1755)
- Jourdan G, Dusseault J, Benhamou PY, Rosenberg L & Halle JP 2011 Co-encapsulation of bioengineered IGF-II-producing cells and pancreatic islets: effect on  $\beta$ -cell survival. *Gene Therapy* **18** 539–545. (doi:10.1038/gt.2010.166)
- Jung Y, Miura M & Yuan J 1996 Suppression of interleukin-1  $\beta$ -converting enzyme-mediated cell death by insulin-like growth factor. *Journal of Biological Chemistry* **271** 5112–5117. (doi:10.1074/jbc.271.9.5112)
- Katz LE, Bhala A, Camron E, Nunn SE, Hintz RL & Cohen P 1997 IGF-II, IGF-binding proteins and IGF receptors in pancreatic  $\beta$ -cell lines. *Journal of Endocrinology* **152** 455–464. (doi:10.1677/joe.0.1520455)
- Kim HJ & Kim TY 2005 Regulation of vascular endothelial growth factor expression by insulin-like growth factor-II in human keratinocytes, differential involvement of mitogen-activated protein kinases and feedback inhibition of protein kinase C. *British Journal of Dermatology* **152** 418–425. (doi:10.1111/j.1365-2133.2004.06397.x)
- Kim S, Kang Y, Krueger CA, Sen M, Holcomb JB, Chen D, Wenke JC & Yang Y 2012 Sequential delivery of BMP-2 and IGF-1 using a chitosan gel with gelatin microspheres enhances early osteoblastic differentiation. *Acta Biomaterialia* **8** 1768–1777. (doi:10.1016/j.actbio.2012.01.009)
- Kodama S, Kojima K, Furuta S, Chambers M, Paz AC & Vacanti CA 2009 Engineering functional islets from cultured cells. *Tissue Engineering* **15A** 3321–3329. (doi:10.1089/ten.tea.2008.0459)
- Kwon YW, Kwon KS, Moon HE, Park JA, Choi KS, Kim YS, Jang HS, Oh CK, Lee YM, Kwon YG *et al.* 2004 Insulin-like growth factor-II regulates the expression of vascular endothelial growth factor by the human keratinocyte cell line HaCaT. *Journal of Investigative Dermatology* **123** 152–158. (doi:10.1111/j.0022-202X.2004.22735.x)
- Mahato RI, Henry J, Narang AS, Sabek O, Fraga D, Kotb M & Gaber AO 2003 Cationic lipid and polymer-based gene delivery to human pancreatic islets. *Molecular Therapy* **7** 89–100. (doi:10.1016/s1525-0016(02)00031-x)
- McCall M & Shapiro AM 2012 Update on islet transplantation. *Cold Spring Harbor Perspectives in Medicine* **2** a007823. (doi:10.1101/cshperspect.a007823)
- McGeachie J & Tennant M 1997 Growth factors and their implications for clinicians: a brief review. *Australian Dental Journal* **42** 375–380. (doi:10.1111/j.1834-7819.1997.tb06081.x)
- Miettinen PJ, Otonkoski T & Voutilainen R 1993 Insulin-like growth factor-II and transforming growth factor- $\alpha$  in developing human fetal pancreatic islets. *Journal of Endocrinology* **138** 127–136. (doi:10.1677/joe.0.1380127)
- Milo-Landesman D & Efrat S 2002 Growth factor-dependent proliferation of the pancreatic  $\beta$ -cell line  $\beta$ TC-tet: an assay for  $\beta$ -cell mitogenic factors. *International Journal of Experimental Diabetes Research* **3** 69–74. (doi:10.1080/15604280212526)
- Moberg L, Johansson H, Lukinius A, Berne C, Foss A, Kallen R, Ostraat O, Salmela K, Tibell A, Tufveson G *et al.* 2002 Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. *Lancet* **360** 2039–2045. (doi:10.1016/S0140-6736(02)12020-4)
- Montolio M, Biarnes M, Tellez N, Escoriza J, Soler J & Montanya E 2007a Interleukin-1 $\beta$  and inducible form of nitric oxide synthase expression in early syngeneic islet transplantation. *Journal of Endocrinology* **192** 169–177. (doi:10.1677/joe.1.06968)
- Montolio M, Tellez N, Soler J & Montanya E 2007b Role of blood glucose in cytokine gene expression in early syngeneic islet transplantation. *Cell Transplantation* **16** 517–525. (doi:10.3727/000000007783464920)
- Muruve DA, Barnes MJ, Stillman IE & Libermann TA 1999 Adenoviral gene therapy leads to rapid induction of multiple chemokines and acute neutrophil-dependent hepatic injury *in vivo*. *Human Gene Therapy* **10** 965–976. (doi:10.1089/10430349950018364)
- Nandi SK, Kundu B & Basu D 2013 Protein growth factors loaded highly porous chitosan scaffold: a comparison of bone healing properties. *Materials Science & Engineering. C, Materials for Biological Applications* **33** 1267–1275. (doi:10.1016/j.msec.2012.12.025)
- Narang AS & Mahato RI 2006 Biological and biomaterial approaches for improved islet transplantation. *Pharmacological Reviews* **58** 194–243. (doi:10.1124/pr.58.2.6)
- Nelson DM, Baraniak PR, Ma Z, Guan J, Mason NS & Wagner WR 2011 Controlled release of IGF-1 and HGF from a biodegradable polyurethane scaffold. *Pharmaceutical Research* **28** 1282–1293. (doi:10.1007/s11095-011-0391-z)
- O'Connell PJ, Holmes-Walker DJ, Goodman D, Hawthorne WJ, Loudovaris T, Gunton JE, Thomas HE, Grey ST, Drogemuller CJ, Ward GM *et al.* 2013 Multicenter Australian trial of islet transplantation: improving accessibility and outcomes. *American Journal of Transplantation* **13** 1850–1858. (doi:10.1111/ajt.12250)
- O'Dell SD & Day IN 1998 Insulin-like growth factor II (IGF-II). *International Journal of Biochemistry & Cell Biology* **30** 767–771. (doi:10.1016/S1357-2725(98)00048-X)
- Petrik J, Arany E, McDonald TJ & Hill DJ 1998 Apoptosis in the pancreatic islet cells of the neonatal rat is associated with a reduced expression of insulin-like growth factor II that may act as a survival factor. *Endocrinology* **139** 2994–3004. (doi:10.1210/endo.139.6.6042)
- Petrik J, Reusens B, Arany E, Remacle C, Coelho C, Hoet JJ & Hill DJ 1999 A low protein diet alters the balance of islet cell replication and apoptosis in the fetal and neonatal rat and is associated with a reduced pancreatic expression of insulin-like growth factor-II. *Endocrinology* **140** 4861–4873. (doi:10.1210/endo.140.10.7042)
- Portela-Gomes GM & Hoog A 2000 Insulin-like growth factor II in human fetal pancreas and its co-localization with the major islet hormones: comparison with adult pancreas. *Journal of Endocrinology* **165** 245–251. (doi:10.1677/joe.0.1650245)

- Rabinovitch A, Quigley C, Russell T, Patel Y & Mintz DH 1982 Insulin and multiplication stimulating activity (an insulin-like growth factor) stimulate islet  $\beta$ -cell replication in neonatal rat pancreatic monolayer cultures. *Diabetes* **31** 160–164. (doi:10.2337/diab.31.2.160)
- Raper SE, Chirmule N, Lee FS, Wivel NA, Bagg A, Gao GP, Wilson JM & Batshaw ML 2003 Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. *Molecular Genetics and Metabolism* **80** 148–158. (doi:10.1016/j.ymgme.2003.08.016)
- Robertson RP 2004 Islet transplantation as a treatment for diabetes – a work in progress. *New England Journal of Medicine* **350** 694–705. (doi:10.1056/NEJMra032425)
- Robitaille R, Dusseault J, Henley N, Rosenberg L & Halle JP 2003 Insulin-like growth factor II allows prolonged blood glucose normalization with a reduced islet cell mass transplantation. *Endocrinology* **144** 3037–3045. (doi:10.1210/en.2002-0185)
- Salvay DM & Shea LD 2006 Inductive tissue engineering with protein and DNA-releasing scaffolds. *Molecular Biosystems* **2** 36–48. (doi:10.1039/b514174p)
- Sakata N, Sumi S, Yoshimatsu G, Goto M, Egawa S & Unno M 2012 Encapsulated islets transplantation: past, present and future. *World Journal of Gastrointestinal Pathophysiology* **3** 19–26. (doi:10.4291/wjgp.v3.i1.19)
- Sandler S, Andersson A & Hellerstrom C 1987 Inhibitory effects of interleukin 1 on insulin secretion, insulin biosynthesis, and oxidative metabolism of isolated rat pancreatic islets. *Endocrinology* **121** 1424–1431. (doi:10.1210/endo-121-4-1424)
- Sang X, Curran MS & Wood AW 2008 Paracrine insulin-like growth factor signaling influences primordial germ cell migration: *in vivo* evidence from the zebrafish model. *Endocrinology* **149** 5035–5042. (doi:10.1210/en.2008-0534)
- Schneider S, Feilen PJ, Slotty V, Kampfner D, Preuss S, Berger S, Beyer J & Pommersheim R 2001 Multilayer capsules: a promising microencapsulation system for transplantation of pancreatic islets. *Biomaterials* **22** 1961–1970. (doi:10.1016/S0142-9612(00)00380-X)
- Schroppel B, Zhang N, Chen P, Chen D, Bromberg JS & Murphy B 2005 Role of donor-derived monocyte chemoattractant protein-1 in murine islet transplantation. *Journal of the American Society of Nephrology* **16** 444–451. (doi:10.1681/ASN.2004090743)
- Sia C & Hanninen A 2006 Apoptosis in autoimmune diabetes: the fate of  $\beta$ -cells in the cleft between life and death. *Review of Diabetic Studies* **3** 39–46. (doi:10.1900/RDS.2006.3.39)
- Stewart CE & Rotwein P 1996 Insulin-like growth factor-II is an autocrine survival factor for differentiating myoblasts. *Journal of Biological Chemistry* **271** 11330–11338. (doi:10.1074/jbc.271.19.11330)
- Stokes RA, Cheng K, Deters N, Lau SM, Hawthorne WJ, O'Connell PJ, Stolp J, Grey S, Loudovaris T, Kay TW *et al.* 2013 Hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) potentiates  $\beta$ -cell survival after islet transplantation of human and mouse islets. *Cell Transplantation* **22** 253–266. (doi:10.3727/096368912X647180)
- Sun G, Shen YI, Kusuma S, Fox-Talbot K, Steenbergen CJ & Gerecht S 2011 Functional neovascularization of biodegradable dextran hydrogels with multiple angiogenic growth factors. *Biomaterials* **32** 95–106. (doi:10.1016/j.biomaterials.2010.08.091)
- Sutton VR, Estella E, Li C, Chen M, Thomas HE, Kay TW & Trapani JA 2006 A critical role for granzyme B, in addition to perforin and TNF $\alpha$ , in alloreactive CTL-induced mouse pancreatic  $\beta$  cell death. *Transplantation* **81** 146–154. (doi:10.1097/01.tp.0000191939.68451.d9)
- Swenne I, Hill DJ, Strain AJ & Milner RD 1987 Growth hormone regulation of somatomedin C/insulin-like growth factor I production and DNA replication in fetal rat islets in tissue culture. *Diabetes* **36** 288–294. (doi:10.2337/diab.36.3.288)
- Taylor RC, Cullen SP & Martin SJ 2008 Apoptosis: controlled demolition at the cellular level. *Nature Reviews. Molecular Cell Biology* **9** 231–241. (doi:10.1038/nrm2312)
- Tjernberg J, Ekdahl KN, Lambris JD, Korsgren O & Nilsson B 2008 Acute antibody-mediated complement activation mediates lysis of pancreatic islets cells and may cause tissue loss in clinical islet transplantation. *Transplantation* **85** 1193–1199. (doi:10.1097/TP.0b013e31816b22f3)
- Uebersax L, Merkle HP & Meinel L 2008 Insulin-like growth factor I releasing silk fibroin scaffolds induce chondrogenic differentiation of human mesenchymal stem cells. *Journal of Controlled Release* **127** 12–21. (doi:10.1016/j.jconrel.2007.11.006)
- Van Schravendijk CF, Foriers A, Van den Brande JL & Pipeleers DG 1987 Evidence for the presence of type I insulin-like growth factor receptors on rat pancreatic A and B cells. *Endocrinology* **121** 1784–1788. (doi:10.1210/endo-121-5-1784)
- Wachlin G, Augstein P, Schroder D, Kuttler B, Kloting I, Heinke P & Schmidt S 2003 IL-1 $\beta$ , IFN- $\gamma$  and TNF- $\alpha$  increase vulnerability of pancreatic  $\beta$  cells to autoimmune destruction. *Journal of Autoimmunity* **20** 303–312. (doi:10.1016/S0896-8411(03)00039-8)
- Walters SN, Luzuriaga J, Chan JY, Grey ST & Laybutt DR 2013 Influence of chronic hyperglycemia on the loss of the unfolded protein response in transplanted islets. *Journal of Molecular Endocrinology* **51** 225–232. (doi:10.1530/JME-13-0016)
- Yeung TY, Seeberger KL, Kin T, Adesida A, Jomha N, Shapiro AM & Korbitt GS 2012 Human mesenchymal stem cells protect human islets from pro-inflammatory cytokines. *PLoS ONE* **7** e38189. (doi:10.1371/journal.pone.0038189)

Received in final form 5 February 2014

Accepted 11 February 2014