We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK
CITATION
INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Present Accomplishments and Future Prospects of Cell-Based Therapies for Type 1 Diabetes Mellitus

Preeti Chhabra[1], Clark D. Kensinger[1,2],
Daniel J. Moore[3] and Kenneth L. Brayman[1,2]
[1]Department of Surgery, University of Virginia,
[2]Division of Transplantation, Department of Surgery, University of Virginia,
[3]Department of Pediatrics, Division of Endocrinology and Diabetes,
Vanderbilt Children's Hospital,
[1,2]Charlottesville,
[3]Nashville,
USA

1. Introduction

Type 1 diabetes mellitus is a classical autoimmune disease that results from immune-mediated destruction of pancreatic β-cells, primarily by T cells specific for β-cell antigens, leading to an absolute loss of insulin production (Gillard and Mathieu et al., 2011; van Belle et al. 2011). While the disease can become clinically apparent at any age, it commonly starts during childhood, and may appear later in adulthood in approximately 30-40% of affected individuals (Fändrich and Ungefroren, 2010; Knip, 1997). A combination of a genetic predisposition and autoimmune processes contribute to its development resulting in the gradual destruction of the insulin-producing β-cells. Daily insulin delivery by injection or pump to manage hyperglycemia by no means represents a cure, often resulting in hypoglycemic episodes (Leitão et al.,2008; Noguchi, 2009). Current strategies to prevent or reverse diabetes are broadly based on the concepts of β-cell regeneration, replacement or protection from T-cell-mediated autoimmune destruction. While transplantation of both, whole pancreas as well as islets of Langerhans, is able to restore endocrine function and glucose homeostasis, islet transplantation offers added advantage in terms of being minimally invasive, preventing the incidence of severe hypoglycemic episodes and significantly lowering hypoglycemic unawareness, thereby improving the quality of life of the transplant recipients (Bretzel et al, 2007; Langer, 2010; Noguchi et al, 2009). Furthermore, manipulation of islets *in vitro*, prior to transplantation, provides an opportunity for the development of various therapeutic manipulations aimed at achieving better transplant outcomes. However, despite islet transplantation being an excellent "proof of principle" for β-cell replacement therapy, its wide-spread applicability is limited by the scarcity of donor organs resulting in inadequate number of islets available for transplant as well as the harmful side-effects of immunosuppressive therapy (Huang et al., 2008). To overcome these limitations, generating a large quantity of β-cells that would allow transplantation of

sufficient β-cell mass to achieve normoglycemia, along with restoration of immunologic tolerance, represents a highly attractive alternative. To this end, proposed mechanisms for islet regeneration consist of replication of pre-existing β-cells, neogenesis from ductal and non β-cell progenitors, transdifferentiation of fully differentiated acinar cells and directed-differentiation of stem cells (putative β-cell progenitors/pancreatic stem cells; embryonic, mesenchymal, hematopoietic and umbilical cord blood-derived stem cells etc). While several studies demonstrate these mechanisms in mice, proving the occurrence of these phenomena in humans is hard to achieve. In this review, we will focus on various approaches to obtain an expandable mass of functional, insulin-secreting β-cells, emphasizing the major cell candidates for β-cell regeneration, the specific factors and stimuli involved in β-cell differentiation and expansion and alternate strategies that may enhance the effective β-cell mass and function. The basic science driving these discoveries and the obstacles that hinder clinical translation of these avenues will be highlighted from the perspective of islet transplantation.

2. Endocrine pancreas plasticity in physiological conditions

Although the formation of new islets in adults has primarily been demonstrated in response to pancreatic injury (eg. pancreatic duct ligation (PDL), β-cell ablation, partial pancreactomy etc.) and metabolic stress, there is ample evidence that β-cell replication from existing cells occurs throughout adulthood (Brennand et al, 2007; Dor et al., 2004; Levetan, 2010; Nir et al., 2007; Teta et al., 2007). This has been observed in several physiologic situations including amongst others, late pregnancy and obesity (Bernard-Kargar and Ktorza, 2001). In both these cases, increases in β-cell mass have been observed in response to insulin resistance and contribute to insulin oversecretion. Several studies have demonstrated a doubling of β-cell mass by the end of pregnancy that decreased progressively after parturition, a good illustration of the plasticity of the endocrine pancreas (Karnik et al., 2007; Rieck & Kaestner, 2010; Scaglia et al., 1995). As in pregnancy, euglycemia is maintained in obesity by increased insulin secretion, due not only to enhanced individual β-cell activity but also to β-cell growth. This increase in β-cell mass approximates 50% in obese, non-diabetic humans and seems consigned to β-cells, with α, δ and PP cell mass remaining unchanged (Bernard-Kargar & Ktorza, 2001). In addition to β-cell expansion based on self-duplication, there is evidence indicating the contribution of stem cell differentiation towards pancreatic β-cell maintenance (Bonner-Weir et al.,2004, 2006,2010). The mechanism of islet regeneration remains controversial, making identification of β-cell progenitors and the in-depth understanding of the underlying mechanisms that trigger β-cell regeneration and expansion absolutely critical in order to apply this strategy in a clinical scenario.

2.1 β-cell progenitors within the pancreas

The exocrine tissue of the pancreas consists of acinar cells that secrete digestive enzymes into a branched ductal network that drains into the gastrointestinal tract. The endocrine cells consist of α, β, δ, ε, and PP cells that are grouped into islets of Langerhans and secrete insulin and other polypeptide hormones into the bloodstream. Numerous studies propose that in addition to replication of pre-existing β-cells, new β-cells can be produced from differentiated adult cells by interconversions amongst different pancreatic cell compartments as well as neogenesis from ductal and circulating progenitors and putative pancreatic stem cells (Bonner-Weir et al., 2004,2006; Gao et al., 2003, 2005; Granger and

Kushner, 2009; Juhl et al., 2010; Kikugawa et al.,2009; Pittenger et al., 2009). However, while these strategies demonstrate the generation of insulin-producing cells, the major challenge encountered is the inability to generate sufficient amounts of 'glucose-responsive' β-cells to normalize hyperglycemia. True mature β-cells are defined by the ability to store large amounts of insulin and secrete it in a regulated manner in response to glucose challenge. This inability to mature into 'glucose responsive' β-cells may indicate an underlying inability to dedifferentiate completely to a progenitor state or to efficiently redifferentiate/transdifferentiate into β-cells. Therefore, while these studies outline the therapeutic potential of various regenerative strategies for β-cell expansion, they also highlight the importance of elucidating the underlying mechanisms required to form islets that perform similar to primary islets used in clinical applications.

2.1.1 Can β-cells be derived from the exocrine pancreas - the plasticity of the pancreatic acinar cell.

The exocrine tissue consisting of acinar and ductal cells, comprises approximately 95% of the adult pancreas and shares a common progenitor with endocrine cells, namely, pancreatic and duodenal homeobox 1(Pdx1)-expressing cells (Gu et al, 2002). Several studies indicate the capability of acinar cells to transdifferentiate into insulin positive cells (IPCs) either directly (Minami et al., 2005; Minami and Seino S, 2008) or via a ductal intermediate (Means et al., 2005), usually accompanied by a corresponding increase in functional β-cell mass. However, other studies using cultured, genetically marked, murine acinar cells indicate that these cells are only able to rapidly transdifferentiate into a amylase-negative, keratin 19- and mucin-antigens positive ductal phenotype and not into functional β-cells (Blaine et al., 2010). Using a mouse model that develops hyperplastic ducts containing IPCs in response to the transforming growth factor (TGF-α), Blaine et al. performed genetic lineage tracing experiments and demonstrated that hyperplastic ductal cells arose largely from acinar cells that transdifferentiated into ductal cells, while IPCs adjacent to acinar-derived ductal cells arose from pre-existing IPCs, suggesting that islet endocrine cells can intercalate into hyperplastic ducts as they develop. Thus, the apparent pancreatic plasticity resulted from both, the ability of acinar cells to transdifferentiate and of endocrine cells to reorganize in association with duct structures. Enthusiasm was further curbed by a Cre/loxP-based lineage tracing study suggesting that transdifferentiation of acinar to β-cells was unlikely a part of the normal β-cell turnover, even after injury such as pancreatectomy, ductal ligation, or pancreatitis (Desai et al., 2007). A recent time-specific lineage tracing study indicated that in mice containing genetically marked ductal and acinar cells carrying the mucin gene Muc1, the Muc1 positive cells only gave rise to β-cells and other islet cells *in utero* (Kopinke & Murtaugh, 2010). From birth onwards, Muc1 lineage-labeled cells were confined to the exocrine compartment with no detectable contribution to islet cells. In contrast, other studies showed that differentiated exocrine cells were capable of reverting to a partially dedifferentiated state with the capacity to transdifferentiate into different phenotypes, including ductal cells (Rooman et al., 2000), hepatocyte-like cells (Lardon et al., 2004) and IPCs (Baeyens et al., 2005, 2008). Treatment of rat exocrine pancreatic cells *in vitro* with epidermal growth factor (EGF) and leukaemia inhibitory factor (LIF) resulted in an 11-fold increase of the β-cell mass in a 3 day culture period with the newly-formed cells secreting insulin in response to glucose, containing insulin immunoreactive secretory granules, immunoreactive for C-peptide-I, Pdx-1 and Glucose transporter-2 (Glut-2) and able to

restore normoglycemia upon transplantation into alloxan-diabetic mice. Interestingly, the cells were immunoreactive for amylase and cytokeratin-20, characteristics of exocrine cells. Similar results were obtained using adult mouse pancreatic acinar cells cultured in suspension in the presence of EGF and nicotinamide. Analysis using a Cre/loxP-based direct cell lineage tracing system indicated that newly made cells originated from amylase/elastase-expressing pancreatic acinar cells and had insulin-containing secretory granules (Minami et al., 2005). Insulin secretion was stimulated by glucose, sulfonylurea, and carbachol; potentiation by glucagon-like peptide-1 (GLP-1) was also observed. In addition, enzymatic dissociation of pancreatic acini itself lead to activation of EGF signaling and inhibition of EGF receptor kinase blocked transdifferentiation, suggesting that activation of EGF signaling was required for transdifferentiation of pancreatic acinar cells into insulin-secreting cells with secretory properties similar to native pancreatic β-cells (Minami & Seino, 2008). Using a different approach, Melton and coworkers demonstrated that a specific combination of transcription factors Neurogenin3 (Ngn3), Pdx1 and MafA could reprogram differentiated, pancreatic exocrine cells in adult mice into cells that were indistinguishable from endogenous islet β-cells in size, shape and ultrastructure and expressed genes essential for β-cell function (Zhou et al., 2008). These cells increased by 20% after the triple adenoviral transduction and were capable of ameliorating hyperglycemia by remodeling local vasculature and secreting insulin, providing proof that 1) exocrine cells can assume an insulin-secreting phenotype and 2) that cellular reprogramming of exocrine pancreatic tissue using defined factors in an adult organ was possible without reverting to a pluripotent stem cell state or involving activation of the cell cycle. The drawback of this study however, was that these cells did not form islets nor became incorporated into them. Also, the use of viruses raises concerns about insertional mutagenesis and tumor initiation. A more clinically suitable study through induction of acinar-to-islet transdifferentiation was the expansion of adult, human islet tissue via a duct epithelial-like intermediate using a combination of gastrin, hepatocyte growth factor (HGF), and the Reg family member INGAP, islet neogenesis gene associated protein (Lipsett et al., 2007). A 6-fold increase in total dithizone (DTZ) positivity and approximately 15-fold increase in neoislets compared with untreated control tissue cultures was observed. However, these cells were not as efficient as native islets in the production of insulin and the success of this process depended heavily on the reorganization of the extracellular matrix begging definition of the role of extracellular scaffolding in increasing the output of islet-like cells or their insulin-secreting function(Okuno et al., 2007). Thus, transdifferentiation into insulin-secreting cells with low insulin expression levels and secretory response, absence of transcription factors essential for β-cell development and the inability to fully mature remain serious concerns.

2.1.2 The feasibility of β-cell regeneration through neogenesis from pancreatic ductal cells

Morphological observations of islet-ductal complexes comprising IPCs within or near adult pancreatic ducts in instances such as pregnancy or obesity, damage or disease, supported by various lineage-tracing studies in both human and rodent pancreata, suggest that differentiated pancreatic ductal cells act as *in vivo* progenitors for pancreatic β-cells (Bonner-Weir et al., 2008; Inada et al., 2008; Suarez-Pinzon et al.,2005; Xu et al., 2008, Xia et al. 2009). This is particularly impressive in the pancreata of individuals with a clinical diagnosis of chronic pancreatitis or with asymptomatic pancreatic fibrosis wherein immunophenotypical

characterization of duct-associated islet-like cells revealed positivity for both ductal and endocrine markers (Gianani et al., 2006, 2011). The presence of insulin- and cytokeratin-positive cells has also been reported in transplanted pancreata of individuals with recurrence of β-cell autoimmunity (Martin-Pagola et al., 2008). Transient expression of the Ngn3 marks progenitor cells in the pancreas as they differentiate into islet cells. Using lineage-tracing and pancreatic ductal ligation in adult mice, Xu et al. revealed the appearance of facultative Ngn3+ Cytokeratin19+ progenitors in the ductal lining of the regenerating portion but not in the non-injured pancreas during the robust β-cell expansion that followed pancreatic duct ligation (PDL), suggesting that endogeneous duct cells represented an obvious target for therapeutic regeneration of β-cells in diabetes (Xu et al., 2008). Concerns raised about using Ngn3 expression as a marker of endocrine progenitors and neogenesis were allayed in a very recent study that developed a transgenic mouse line that could monitor Ngn-3 expression, and thus islet cell genesis, by surrogate markers that secreted alkaline phosphatase (SeAP) and enhanced green florescent protein (EGFP) (Shimajiri et al., 2011). They demonstrated that in transgenic embryos, cells expressing EGFP lined the pancreatic ducts while SeAP was readily detectable in embryos, in the media of cultured embryonic pancreases and in the serum of adult animals. Duct ligation in adult mice caused an increase in circulating SeAP levels. A genetic lineage tracing study using a human carbonic anhydrase II (CA2) promoter fragment to express Cre and CreER in differentiated duct cells showed that a significant proportion of adult pancreatic ductal cells retain the potential to differentiate into other pancreatic cell types including β-cells. Impressively, two weeks after duct ligation, 42% of islets and 24% of β-cells expressed the lineage marker (Inada et al., 2008). In fact, dedifferentiation of ductal epithelial cells is often associated with the epithelial-mesenchymal transition (EMT)(Fanjul et al., 2010). An interesting study by Seaberg et al reported the identification of multipotent precursor cells from the adult mouse pancreatic islet and ductal populations (Seaberg et al., 2004) that proliferated *in vitro* to form clonal colonies coexpressing neural and pancreatic precursor markers. Upon differentiation, individual clonal colonies produced distinct populations of neurons and glial cells, pancreatic endocrine β-, α-and δ-cells and pancreatic exocrine and stellate cells. The newly generated β-like cells demonstrated glucose-dependent Ca(2+) responsiveness and insulin release, representing a previously unidentified, intrinsic, pancreatic precursor population that is a promising candidate for cell-based therapeutic strategies. Taken together, these data show that the ductal epithelium in human as well as rodent pancreata is capable of producing IPCs, however whether these cells are mature β-cells with the full complement of molecules necessary for maintaining optimal glucose homeostasis remains to be seen. Despite these advances, the ductal origin hypothesis of pancreatic regeneration is under attack (Kushner et al., 2010). While some reports suggest that β-cell replication rather than neogenesis is the main source of regenerating islets in the murine pancreas (Dor et al., 2004), others using genetic labeling to fate map embryonic and adult pancreatic duct cells have demonstrated that pancreatic ductal epithelial cells do not contribute to endocrine or acinar cells postnatally (Solar et al., 2009). The latter study demonstrated that as the embryonic Hnf1β+ epithelium gradually acquires the anatomical configuration of the pancreatic ductal network, it sequentially sets aside cells for the acinar and endocrine lineages and finally takes on a differentiated pancreatic duct phenotype. The embryonic ducts give rise to both differentiated endocrine and ductal cells but once the pancreatic duct epithelium acquires a differentiated phenotype, it does not contribute

significantly to new β-cells that are formed in diverse physiological or regenerative settings. However, certain caveats have not been addressed in this study; amongst them being low labeling efficiency during embryonic development and variable expression of Hnf1β in ductal progenitors with the cells that do become marked, representing a population of duct cells with a higher activity of Hnf1β transcription or greater accessibility to Cre recombinase. Thus, taking into account possible low levels of leakiness of the Cre-lox system over time with the strong insulin promoter, the small proportion of the islets counted, limited (only 30%) labeling of the β-cells and the lack of examination of the new lobes after pancreatectomy, neogenesis in the adult cannot be ruled out by negative data of a lack of dilution of labeled β-cells (Kushner et al., 2010). The observed discrepancies warrant rigorous investigation with definitive experiments, using lineage tracing studies with additional markers that label either ducts or other candidate precursor cells to tease out the underlying mechanisms. Until then, however, the identification of differentiated pancreatic duct epithelium represents an expandable source of facultative progenitor cells for generating β-cells with major implications for replenishment therapy for diabetes.

2.1.3 Replication of pre-existing β-cells: contribution to β-cell growth and regeneration

Following stimuli such as pregnancy, obesity, glucose infusion, manipulating growth-hormone expression, toxigene-mediated β-cell ablation and partial pancreatectomy, self-renewal by β-cell replication has emerged as a dominant mechanism for homeostatic maintenance of β-cell mass postnatally. This was elegantly demonstrated in a study by Melton and coworkers wherein, using lineage-tracing highly specific for β-cells (double transgenic mice bearing a tamoxifen-dependent Cre-recombinase construct under the control of a rat insulin promoter together with a reporter Z/AP gene) they showed that pre-existing terminally differentiated β-cells, rather than pluripotent stem cells, were the major source of new β-cells retaining significant proliferative capacity *in vivo* during adult life, even after pancreatectomy (Dor et al., 2004). This conclusion was supported by the observation that forced cell-cycle arrest of β-cells by transgenic expression of the cyclin-dependent kinase inhibitor p27 blocked β-cell proliferation, severely restricting postnatal but not embryonic β-cell mass, indicating that non β-cells were unable to compensate (Nir & Dor, 2005). Deletion of cyclin-dependent kinase 4 (Cdk4) showed similar results that could be rescued by β-cell-specific expression of *Cdk4*, suggesting that the underlying proliferative defect in *Cdk4*-null mice lay in β-cells (Martin et al., 2003; Rane et al., 1999, Uchida et al., 2005). A recent *in vivo* pulse-chase labeling assay investigating the replication dynamics of adult mouse β-cells indicated that replicated β-cells were able to re-enter the cell division cycle shortly after mitosis and regained their normal proliferative potential after a short quiescence period of several days (Salpeter et al., 2010). Metabolic demand was a key determinant of cell cycle re-entry as quiescence period was lengthened with advanced age, but shortened during injury-driven β-cell regeneration and following treatment with a pharmacological activator of glucokinase. Their results implicated glucose control of cyclin D2 expression in regulating the capacity of β-cells to re-enter the cell cycle post-mitosis. A novel DNA analog-based lineage-tracing technique to detect multiple rounds of cell division *in vivo* indicated that β-cells rather than specialized progenitors contributed to adult β-cells, slowed by a replication refractory period that prevented β-cells from immediately redividing (Teta et al., 2007). Also, label-retaining experiments, clonal analysis and sequential thymidine analogue labeling indicated that all β-cells replicated homogeneously,

contributing equally to islet growth and maintenance in healthy adult mice as well as during periods of increased replication such as pregnancy, neonatal growth and following pancreatectomy (Brennand et al., 2007, Teta et al., 2007). By counting accumulated mitotic figures following colchicine treatment (Bonner-weir et al., 1989), 5-bromo-2'-deoxyuridine (BrdU) incorporation (Montana et al., 1994), tritiated thymidine incorporation (Kaung, 1994) or expression of the cell cycle marker Ki67 (Meier et al., 2008), it has been shown that β-cell replication rates in rodents and human beings *in vivo* are a function of age, highest during late embryonic development and the neonatal period and declining significantly throughout adulthood (Brennand & Melton, 2009). However, hyperglycemia and hyperinsulinemia can induce a robust increase in β-cell replication even in older mice. Unfortunately human β-cell replication is difficult to demonstrate compared to murine pancreas (Butler et al., 2003, 2010; Menge et al., 2008). Although there have been reports of human β-cell proliferation in the presence of HGF (Kayali et al., 2007) or by overexpression of Cdk6 (Fiaschi-Taesch et al., 2010), the results were questionable, since insulin expression was lost after 5 doublings in the former study and the investigators in the latter study did not measure β-cell mass or number thereby raising doubts whether their approach did induce proliferation of β-cells. While β-cell replication offers an attractive mechanism for postnatal homeostatic maintenance of β-cells, the complete absence of β-cells in patients with type 1 diabetes and a declining ability to replicate with age are questions that need to be addressed.

2.1.4 Intra-Islet cells: role of α and δ cells in β-cell regeneration

Several studies using the streptozotocin (STZ)-induced diabetic model have indicated the presence of intra-islet precursor cells with the potential to differentiate into neo islets/pancreatic β-cells upon appropriate stimulation (Banerjee & Bhonde, 2003; Guz et al., 2001; Kodama et al., 2005). While some studies indicate that administration of betacellulin improved STZ-induced hyperglycemia by promoting neoformation of β-cells mainly from somatostatin-positive islet cells (Li et al., 2003), others suggested that differentiation of multipotent nestin-positive stem cells isolated from adult pancreatic islets resulted in pancreatic endocrine, exocrine, and hepatic phenotypes *ex vivo* (Zulewski et al., 2001). By conditional and ectopic expression of Pax4 using different cell-specific promoters, Collombat et al have demonstrated that Pax4 forces endocrine precursor cells as well as mature α-cells to adopt a β-cell destiny (Collombat et al., 2009). Upon Pax4 ectopic expression, the resulting glucagon deficiency provoked a compensatory and continuous glucagon-positive cell neogenesis requiring the re-expression of the proendocrine gene Ngn3, with the newly-formed α-cells acquiring a β-cell phenotype. Thus, ectopic expression of Pax4 in α-cells caused a cycle of neogenesis and redifferentiation capable of restoring a functional β-mass and restoring normoglycemia in animals that had been chemically depleted of β-cells. In an elegant experiment using alloxan damage to eliminate all pre-existing β-cells followed by PDL to stimulate β-cell neogenesis, Fred Levine and coworkers demonstrated β-cell neogenesis from α-cells (Chung et al., 2010a,2010b). They showed that virtually all β-cells that appeared after treatment were neogenic, mostly arising from adult α-cells within 14 days. At one week, 58% of IPCs coexpressed glucagon and β-cell-specific transcription factors such as Pdx1 and Nkx6.1. By two weeks, these MafB-expressing immature IPCs demonstrated a predominant mature phenotype defined by MafA expression and lack of glucagon. Furthermore, in this PDL plus alloxan model, in response to environmental cues, α-cells could a) replicate first, forming a potential reservoir of β-cell

progenitors; b) could directly differentiate into β-cells without intervening replication; or c) replicate first and then convert to β-cells. β-cell regeneration from α-cells was also demonstrated using a transgenic model of diphtheria-toxin-induced, acute, selective, near-total β-cell ablation (Thorel et al., 2010). Administration of insulin resulted in β-cell mass augmentation with time and lineage-tracing to label the glucagon-producing α-cells before β-cell ablation tracked large fractions of regenerated β-cells as derived from α-cells. Transdifferentiation of glucagon-expressing cells into insulin-expressing cells on menin inactivation, without the necessity of Pdx1, MafA, Pax4, and Ngn3 expression, has also been demonstrated albeit in a scenario relating to islet tumorigenesis (Lu et al., 2010). The lack of Ngn3, Pdx1, and MafA expression in these cells makes them more similar to the immature MafB+Ins+ cells that have been documented during development, however, further investigation will be required to clarify the similarities and the differences. Taken together, these results demonstrate that adult α-cells can serve as *in vivo* source for generating β-cells. There are only a few published records of this phenomenon in human pancreata. Gianani et al. reported the presence of insulin–glucagon double-positive cells in fibrotic pancreata and speculated that in conditions like chronic pancreatitis and pancreatic fibrosis, α-cells increased through neogenesis from ductal cells followed by transdifferentiation into insulin-producing cells (Gianani et al., 2006, 2011). This theory is supported by experiments demonstrating insulin–glucagon double-positive cells intermixed with a severe insulitic islet infiltrate in the pancreata of a subset of NOD mice that remain non-diabetic despite the total loss of insulin-producing cells. Since α-cells appear to be capable of resisting immune-mediated destruction in autoimmune diabetes, with residual α-cells persisting long after the onset of disease, this scenario is particularly attractive in terms of potential therapy. Other studies indicating the presence of intra-islet β-cell precursors include the development of a tissue culture platform wherein isolated adult human pancreatic islets formed proliferative duct-like structures expressing ductal and progenitor markers. Short-term treatment with INGAP induced these structures to reform islet-like structures that resembled freshly isolated islets with respect to the frequency and distribution of the four endocrine cell types, islet gene expression and hormone production, insulin content, and glucose-responsive insulin secretion (Hanley & Rosenberg, 2009). Similar results were obtained by dedifferentiation of fresh human islets into a duct cell phenotype with further redifferentiation into β-cells in appropriate conditions *in vivo* (Gao et al., 2005). While the plasticity of adult human islets has significant implications for islet regeneration, until the maximum extent of neogenesis achieved using intra-islet cells can be determined, significant investment in developing regenerative strategies based on these cells should be reconsidered.

3. Stem cells strategies for β-cell regeneration

Obtaining a large source of β-cells for cellular therapy is a major challenge in the treatment of diabetes. While efforts thus far are based on deriving maximal utilization of all the unwanted tissue from the donor organ, the insignificant yield of differentiated β-cells, diminished function and insignificant amounts of insulin secreted both *in vitro* and *in vivo*, make it necessary to seek alternative approaches. Regeneration of β-cells from a self renewing, expandable stock of stem/progenitor cells via processes that include dedifferentiation, proliferation, tissue-specific directed differentiation and genetic

reprogramming offer an attractive alternate source towards achieving insulin independency in diabetic patients. Based on their origins, stems cells from blastocyst-stage embryos can be classified as pluripotent embryonic stem cells (ESCs), whilst those obtained from niches of mature adult tissues and bone marrow as adult multipotent stem cells. The tissue-specific differentiation of ESCs, pancreas-derived multipotent progenitor/stem cells, extra-pancreatic adult stem cells (bone-marrow (BM) derived stem cells, neural progenitor cells, umbilical cord blood (UCB)-derived stem cells, etc.) or induced pluripotent stem cells (iPSCs) established from adult differentiated cells, into unprecedented quantities of cells with an insulin expressing phenotype *in vitro* has tremendous potential in β-cells replacement therapy(Aguayo-Mazzucato & Bonner-Weir, 2010; Furth etal., 2009; Guo et al., 2009; Santana et al., 2006; Sordi et al., 2008; Stanley et al., 2008; Tang 2004). Additionally, the immunosuppressive, anti-inflammatory properties of stem cells shared among ESCs and several types of non-haematopoietic stem cells (HSCs) such as BM-derived mesenchymal stem cells (BM-MSCs) and UCB-derived stem cells are an added advantage. Better yet is the ability of iPSCs to generate an unlimited supply of clinically compliant, functional β-cells derived from a patient's own somatic cells, and therefore not subject to allograft rejection, providing a framework for a solution to the cited limits of islet transplantation, tissue supply and chronic immunosuppression.

3.1 Potential stem/progenitor cell sources for regeneration of insulin-producing β-cells

3.1.1 Regeneration of β-cells from embryonic stem cells

ESCs are pluripotent with high self-renewal potential and a limitless capacity of proliferation. There are several elegant strategies to induce β-cell generation from ESCs, based on sequential exposure of human ESCs to epigenetic signals that mimic *in-vivo* pancreatic development, beginning with the formation of definite endoderm, followed by pancreatic endoderm, endocrine tissue and finally β-cell maturation (Aguayo-Mazzucato & Bonner-Weir, 2010). These differentiated β-cells display architecture consistent with mature islets, contain islet hormones, respond successfully to glucose challenge in glucose-tolerance tests (GTT) and reverse hyperglycemia in diabetic mice. While several growth factors, such as activin A, fibroblast growth factor 10 (FGF10) and retinoic acid (RA) drive differentiation of hESCs into cells expressing Pdx1, recent efforts have focused on the identification of efficient, less expensive, small molecule inducers capable of controlling differentiation by modulating signal transduction pathways, gene expression or metabolism (Borowiak et al., 2009). For eg. induce definitive endoderm (IDE) 1 and 2 have been shown to induce the formation of large amounts of definitive endoderm expressing multiple endodermal markers from mouse and human ESCs with a 70–80% efficiency, higher than Activin A or Nodal. Further development of these endodermal cells into pancreatic progenitors *in vitro* has been shown to occur in response to FGF10, RA, inhibitors of hedgehog signaling and more recently small molecule indolactam V (Aguayo-Mazzucato & Bonner-Weir, 2010; Chen et al., 2009). While these hESC-derived β-cells resemble human islets, in that they are capable of synthesizing insulin, glucagon, somatostatin, pancreatic polypeptide and ghrelin (D'Amour et al., 2006; Kroon et al., 2008) and prolong graft survival, the lack of glucose-stimulated insulin secretion (GSIS) is one of the main drawbacks. In an elegant study utilizing a five-stage hESC differentiation protocol, Kroon et al assessed the competence of hESC–derived pancreatic endoderm to produce functional endocrine cells *in vivo*. Direct

engraftment of hESC derived pancreatic endoderm from the penultimate fourth stage of the five-step differentiation protocol into immunodeficient mice generated 'glucose-responsive' endocrine cells with maximal insulin secretion which developed during a 3 month period post implant. These glucose-responsive cells had insulin secretory properties similar in kinetics and magnitude to ~ 3000 adult human islets similarly engrafted, expressed critical β-cell transcription factors, demonstrated appropriate processing of proinsulin and the presence of mature endocrine secretory granules. 92% of the mice receiving hESC–derived pancreatic endoderm implants achieved insulin levels sufficient to fully protect against STZ-induced hyperglycemia, providing definitive and compelling evidence that hESCs are competent to generate glucose-responsive, insulin-secreting cells and serve as a renewable source of mature functional islets for diabetes cell-replacement therapies. This study also demonstrates the contribution of *in vivo* factors for the final stages of maturation and the need to identify these factors for efficient *in vitro* differentiation in order to produce sufficient amounts of functional β-cells.

3.1.2 Islet-derived stem/precursor cells as a source for insulin-producing β-cell regeneration

While ESCs have tremendous potential in tissue engineering, their use is hampered by ethical, legal and scientific considerations. On the contrary, non-embryonic adult stem cells are multipotent and can be derived from several sources including bone marrow, umbilical cord tissue, amniotic fluid, fat tissue, skin, retina and central nervous system (Chhabra et al., 2009). The existence of putative pancreatic stem cells that express low amounts of insulin mRNA *in vitro* with clonogenic potential and multipotency has been described previously(Bonner-Weir & Weir, 2005; Seaberg et al., 2004). Suzuki et al also demonstrated the existence of a possible pancreatic stem/progenitor cell candidate that expressed HGF c-Met receptor, did not express hematopoietic and vascular endothelial antigens such as CD45, TER119, c-Kit, and Flk-1, formed clonal colonies *in vitro,* differentiated into multiple pancreatic lineage cells and expanded with self-renewing cell divisions in culture (Suzuki et al., 2004). Following transplantation, these cells differentiated into pancreatic endocrine and acinar cells *in vivo*. A recent study reported the isolation, culture and functional characterization of islet-derived stem/precursor cells from purified human islet preparations (Carlotti et al.,2011). The cultured stem/precursor cells did not express the genes for endocrine hormones and when transferred to serum-free medium, aggregated to form clusters expressing insulin, glucagon, and somatostatin genes. They were not of hematopoietic, endothelial, or of ductal origin, expressed MSC markers CD105, CD90, CD73, CD44, CD29, and CD13 as well as nestin and vimentin and pericyte markers CD146, NG2, αSMA and PDGF-Rβ. Immuno-flowcytometry and confocal microscopic analyses revealed 2.0±0.8% of CD105/CD90 double-positive cells resided within the human islets, supporting the presence of a distinct MSC-like stem cell population in isolated human islets.

3.1.3 Mesenchymal stem cells: stopping immune destruction and promoting β-cell regeneration

Whole bone marrow contains a mixture of multiple types of stem cells, including BM-HSCs, BM-MSCs, endothelial progenitor cells (EPCs), multipotent adult progenitor cells and side population (SP) cells. BM-MSCs are plastic-adherent cells, typically expressing surface markers such as CD90, CD73, CD105, CD44 and CD29. They lack hematopoietic lineage

markers such as CD34, CD45, CD14 and HLA-DR and can differentiate into cells of connective tissue lineages, including bone, fat, cartilage and muscle (Volarevik et al., 2011). These multipotent cells can be isolated and expanded with high efficiency in culture, are non-immunogenic and display immunosuppressive properties, for eg. inhibiting the proliferation and function of major immune cell populations, including T cells, B cells and natural killer (NK) cells as well as modulating the activities of dendritic cells (DCs) and inducing regulatory T cells (Tregs) both *in vivo* and *in vitro* (Shi et al.,2011). They also express a number of growth factors (GF) such as vascular endothelial growth factor (VEGF), HGF and insulin-like growth factor-1 (IGF-1), secrete tolerogenic cytokines as well as display significant anti-inflammatory and anti-apoptotic features (Uccelli et al., 2011). Thus, non-immunogenic BM-MSCs can enhance repair and regeneration, not only by repopulating damaged tissue, but also by reducing inflammation. These cells also do not possess cell surface human leukocyte antigen (HLA) or major histocompatibility complex (MHC) class II molecules, thereby allowing transplantation across MHC barriers. The possible therapeutic effect of BM-MSCs in type 1 diabetes was suggested by their capacity to generate insulin-producing cells and to abrogate immune injury (Chen et al., 2004; Sun et al., 2007; Tang et al., 2004; Volarevic et al., 2011; Xie et al., 2009). These insulin producing cells expressed multiple genes related to the development or function of pancreatic β-cells including high expression of pdx1, insulin, and glucagon and were able to release insulin in a glucose-dependent manner that led to amelioration of diabetic conditions in STZ-treated nude mice. Bouzama et al. also showed that administration of *in vitro*-expanded syngeneic BM-MSCs in STZ-induced diabetic rats promoted Pdx1 and insulin expression in the islets, altered T cell cytokine pattern toward IL-10/IL-13 production, preserved CD4(+)/CD8(+) Foxp3(+) Tregs in the periphery and induced sustained normoglycemia, thereby establishing a tissue microenvironment that supported β-cell activation/survival in the pancreas (Boumaza et al., 2009). While an increase in pancreatic islets and β-cells producing mouse insulin was observed with intracardiac infusion of human MSCs into STZ-induced diabetic NOD/scid mice, nearly 3% of the infused human MSCs engrafted into the pancreas and 11% in the kidney, the two organs sustaining most damage, effectively demonstrating the homing and tissue repair potential of MSCs (Lee et al., 2006). A recent study (Y. Zhang et al., 2010) demonstrated the effective differentiation of human first-trimester fetal BM-MSCs under a novel four-step induction procedure into functional pancreatic islet-like cell clusters (ILCs) that contained 62±14% IPCs, expressed a broad gene profile related to pancreatic islet β-cell development, and released high levels of insulin (2.245±0.222 pmol/100 clusters per 30 min) and C-peptide (2.200 ± 0.468 pmol/100 clusters per 30 min) in response to glucose challenge *in vitro*. The pancreatic ILCs normalized the blood glucose level of diabetic model mice for at least 9 weeks when xenografted and examination of the grafts indicated that the transplanted cells survived in recipients and produced human insulin and C-peptide *in situ*. Most studies suggest the requirement of *in vivo* hyperglycemia or transplantation into a diabetic model for BM-MSCs to differentiate into IPCs able to produce and release insulin in a glucose-dependent manner and normalize hyperglycemia (Chen et al., 2004; Karnieli et al., 2007; Tang et al., 2004). An added benefit is that MSC and islet co-transplantation has been shown to improve islet graft function and survival in diabetic rats by promoting graft revascularization, suppressing inflammation and maintaining islet organization and morphology (Figliuzzi et al., 2009; Ito et al., 2010; Rackham et al., 2011). In fact, engraftment might not be a necessary prerequisite for human MSCs to enhance tissue repair as MSCs

embolized in the lung were shown to improve myocardial infarction by secreting anti-inflammatory factors (Lee et al., 2009). Due to these findings, MSCs offer an attractive avenue of research in diabetic stem cell therapy and may have the potential to enhance β-cell repair mechanisms even if the infused cells do not contribute significantly to the β-cell pool. However, in order to efficiently use MSCs therapeutically, problems of poor engraftment, limited differentiation under *in vivo* conditions and possible malignant transformation needs to be overcome.

3.1.4 Umbilical cord blood (UCB)-derived stem cells: Potential therapeutic tool in β-cell regeneration

Human UCB-derived stem cells represent a readily available source of MSCs and blood stem cells, both of which are potential sources of IPCs. hUCB-derived mononuclear cells (MNCs) possess MSC-like characteristics (expression of CD44, CD90 and CD105) (McGuckin et al., 2005) and have the ability to differentiate into mature adipocytes, osteoblasts, chondrocytes, skeletal myocytes, cardiomyocytes, neurons, and endothelial cells (Fan et al., 2011; Kogler et al., 2004; Parekh et al., 2009; Yoshida et al., 2005). *In vitro* differentiation of UCB-derived stem cells into insulin- and C-peptide-producing cells has been demonstrated using different approaches (Koblas et al., 2009; Gao et al., 2008). While some differentiated Oct-4 and SSEA-4 expressing hUCB cells by a protocol consisting of nicotinamide and extracellular matrix proteins (ECMs) laminin and fibronectin (Szymczak et al., 2010), others used RA, nicotinamide, exendin-4 and extracellular matrix proteins to differentiate hUCB-MSCs (Gao et al, 2008). In fact recently, 'cryopreserved' human UCB-derived MSCs were induced to differentiate into ILCs expressing nestin, Pdx-1, Ngn3, Ils-1, Pax6, Pax4, Nkx2.2, Nkx6.1, Glut-2 and insulin genes (Phuc et al., 2011). Unfortunately, despite differentiation into insulin producing cells, these cells seemed immature in terms of glucose sensing and/or secretory machinery required for glucose stimulated insulin secretion (GSIS) (D'Amour et al., 2006). *In vivo* differentiation of hUCB cells into β-cells has also been demonstrated following transplantation into STZ-induced diabetic immunocompromised (Kogler et al., 2004; Parekh et al., 2009; Zhao et al., 2006) or NOD mice (Koblas et al.,2009; Yoshida et al.,2005), though at a low rate. Detailed characterization of freshly isolated hUCB-MNCs indicate the ability to maintain as islet-progenitor cells expressing pdx1 and GLP1R for at least 5 passages of *in vitro* expansion. Transplantation of these cells into immune-incompetent mice resulted in their differentiation into insulin producing cells, more frequently during partial pancreatectomy, that were immature in terms of GSIS response (Parekh et al., 2009). Thus, it appears that the diabetic status, wherein a demand for neogenesis of insulin producing cells is induced in response to the decreased β-cells mass, results in a higher rate of compensatory hUCB cell differentiation (Yoshida et al., 2005). Kadam et al reported islet neogenesis from constitutively nestin-expressing human umbilical cord matrix stem cells (hUC-MSCs) (Kadam & Bhonde, 2010). These hUC-MSCs expressed CD29, CD44, CD73, CD90, CD105, smooth muscle actin, nestin, vimentin, proliferation marker Ki67 and embryonic markers Oct4, SSEA4, exhibited high proliferating capacity for an extended period and when subjected to a cocktail of specific differentiating factors, differentiated into fat, cartilage, bone, neurons and ILCs. These ILCs stained positive for DTZ, expressed human C-peptide, insulin and glucagon and demonstrated abundance of Pdx-1, Ngn3, insulin, glucagon and somatostatin transcripts. Transplantation into diabetic mice restored normoglycemia and exhibited normal glucose tolerance test, demonstrating

the potential of constitutively-expressing nestin-positive progenitors from hUC as a novel source for islet neogenesis and their usage in cell replacement therapy for diabetes. Interestingly, treatment with CD4(+)CD62L(+) Tregs that were modulated by hUCB derived stem cells was able to simultaneously overcome autoimmunity via systemic and local immunomodulations as well as the shortage of insulin producing cells via stimulation of β-cell regeneration (Zhao et al., 2010). A number of studies indicate the use of these cells for autologous transplantantion in type 1 diabetes subjects. An initial trial with promising results of autologous cord blood has been attempted for diabetes reversal in patients with new onset diabetes who had banked cord blood (Haller et al., 2008). However, although the potential of UCB derived stem cells in the future of T1D interventional therapies is immense, the reality remains that multiple therapeutic avenues need to be combined in order to achieve the dream of permanently reversing/preventing T1D.

3.1.5 Generation of insulin producing β-cells from induced pluripotent stem cells

Efforts to create pluripotent stem cells (iPSCs) that are molecularly and functionally similar to ES cells by reprogramming somatic cells have tremendous therapeutic potential. Fibroblasts, B lymphocytes, liver, stomach epithelial cells, UCBs, human fetal and newborn epithelia etc. have all been dedifferentiated by the stable genomic integration and overexpression of various combinations of defined transcription factors that participate in determining pluripotency in cells, for instance, oct3/4, sox2, klf4, c-myc or oct3/4, sox2, nanog, lin28 (Hochedlinger & Plath, 2009). However, the use of oncogenes (c-myc, klf4) and retroviral and lentiviral vectors have raised concerns about the risk of potential tumorigenicity. Various novel alternatives directed towards making reprogrammed cells safer and more practical for therapeutic use have been explored. For e.g. iPSCs generated by viral integration of only three transcription factors, Oct4/Sox2/Klf4 have exhibited reduced tumorigenicity in chimeras and progeny mice (Nakagawa et al., 2008), albeit with substantially lower reprogramming efficiency. Interestingly, the addition of two factors p53 siRNA and UTF1 enhanced the efficiency of iPSC generation upto 100-fold (Zhao et al., 2008). Furthermore, a combination of two small molecules, BIX-01294 (a G9a histone methyltransferase inhibitor) and BayK8644 (a L-channel calcium agonist) was able to compensate for viral transduction of Sox2, enabling reprogramming of Oct4/Klf4-transduced mouse embryonic fibroblasts, which do not endogenously express the factors essential for reprogramming (Shi et al., 2008). Similarly, valproic acid (VPA), a histone deacetylase inhibitor has been shown to enable reprogramming of primary human fibroblasts with only two factors, Oct4 and Sox2, without the need for the oncogenes c-Myc or Klf4 (Huangfu et al., 2008). In fact, the generation of iPSCs from hUCBs with only Oct4 and Sox2 has also been demonstrated recently (Giorgetti et al.,2010). These two factor-induced human iPSCs resemble human ES cells in pluripotency, global gene expression profiles and epigenetic states, highlighting an important new trend in this field, the replacement of viral-mediated gene transfer with drug therapies such as small molecule gene inducers. However, finding small molecules that induce specified gene targets is not always possible. Alternatively, it is possible to engineer cell-penetrating protein therapies wherein a protein of interest can directly be transferred into the target cell. This approach has been applied in a study that reported the generation of protein-induced pluripotent stem cells (piPSCs) from murine embryonic fibroblasts using recombinant cell-penetrating reprogramming proteins (Zhou et al.,2009). These piPSCs were capable of long-term self-

renewal and were pluripotent *in vitro* and *in vivo*. Addition of a transportation tag consisting of 11 linked copies of arginine to four proteins c-Myc, Klf4, Oct4 and Sox2 has also been shown to facilitate translocation across cell and nuclear membranes (Baker, 2009) with addition of valproic acid further boosting reprogramming rates. In fact, when proteins were administered four times over six days at 36-hour intervals, cells over 30 passages were morphologically indistinguishable from ES cells and expressed similar markers. Mixing in these cells with normal mouse embryos and allowing development in a surrogate mother resulted in the reprogrammed cells contributing to the germlayers in 13.5-day-old embryos. Interestingly, UCB-derived iPSCs have also been generated by lentiviral overexpression of oct4, sox2, nanog and lin28 with reprogramming efficiency similar to that of keratinocytes and fibroblasts. Towards development of a reliable protocol for induction of mature insulin-producing cells from iPSCs, a highly efficient strategy involving a) the use of activin A and wortmannin to induce definitive endoderm formation followed by b) priming with FGF10, KAAD-cyclopamine and c) addition of RA, NOGGIN and FGF7 to induce pancreatic specialization and d) EGF to regulate progenitor expansion has been demonstrated (Zhang et al., 2009). Using this stepwise induction strategy, most human iPSC lines could be induced into Pdx1-positive progenitor cells and further differentiated into cells expressing islet cell specific marker genes including Pdx1, MafA, Glut2 and insulin. Various marker genes were expressed at different induction stages: Sox17 on day 4, Pdx1 on day 8, Sox9 on day 13, amylase, Pdx1 and insulin on day 20. Additionally, co-expression of Pdx1 and C-peptide further confirmed insulin-producing cells suggesting that human iPSCs could be efficiently differentiated into pancreatic lineage cells. In a very recent study, a similar stepwise induction protocol was utilized with the exception that the pancreatogenic cocktail was enriched with Indolactam V/GLP-1 (Thatava et al., 2011). Under feeder-free conditions, fate specification of human iPSCs was initiated with activin A and Wnt3a that triggered engagement into definitive endoderm, followed by priming with FGF10 and KAAD-cyclopamine. Addition of RA, boosted by the pancreatic endoderm inducer indolactam V (ILV), yielded pancreatic progenitors expressing Pdx1, Ngn3 and NeuroD1 markers. Further differentiation under IGF-1, HGF and N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT) was enhanced by GLP-1 to generate islet-like cells that expressed pancreas-specific markers including insulin and glucagon. Sustained expression of Pdx and functional responsiveness to glucose challenge, secreting up to 230pM of C-peptide, was observed in progeny. Thus, a proficient means for lineage spefication of human iPSCs into functional glucose-responsive hormone-producing progeny has further refined the development of a personalized platform for islet-like cell generation. Whilst these studies generate proof of concept that iPSCs may be an appropriate source for the generation of clinically compliant, immunologically quiescent, therapeutic β-cells from an autologous, non-embryonic origin, progress towards clinical application is hampered by a number of factors. Determining the somatic cell candidate for dedifferentiation depends on factors such as relative availability and ease of isolation, definitive identification and characterization markers, *ex vivo* expansion potential as well as safety upon transplantation. For future consideration, extensive characterization of *in vitro* differentiated iPSC will be critical and must be investigated. While the use of small molecule gene inducers and cell-penetrating protein therapies address the issues of poor efficiency and slow kinetics of the reprogramming process by viral transfection of the nuclear factors, these methodologies require substantial improvement before they could be used in clinical applications.

Similarly, the use of non-viral gene delivery vectors that can obviate safety concerns regarding the risk of mutagenesis, terratoma and tumor formation that prevail in the use of oncogenes and viral transduction need to be stringently addressed. Last but not least, studies thus far have demonstrated that the induction of the insulin-secreting phenotype is still incomplete with differentiated cells making multiple hormones or inadequate amounts of insulin or lacking response to glucose challenge to be clinically useful. Extensive, in-depth investigation into the underlying mechanisms involved is therefore of critical importance. Thus, while iPSCs derived from autologous somatic cells represent an exciting and important avenue to replenish islet supply while simultaneously obviating immune concerns such as rejection and chronic immunosuppression, until the accompanying risks are completely obviated, other avenues of β-cell replacement need to be judiciously explored.

3.2 Identifying stem cells with insulin-generating capability and tracking differentiated β-cells - the necessity for cellular markers.

Nestin, which is detected at low levels in human pancreatic tissue *in vitro*, has long since been debated as a cellular marker for ESC differentiation into IPCs (Baharvand et al., 2006). The existence of multipotential nestin-positive cells with unusually extended proliferative capacity that might participate in neogenesis of islet endocrine cells has been demonstrated in rat and human pancreatic islets and ducts (Zulewski et al., 2001). Upon confluence, these cells differentiated into cells that expressed liver and exocrine pancreas markers such as α-fetoprotein and pancreatic amylase, and displayed a ductal/endocrine phenotype with expression of CK19, neural-specific cell adhesion molecule, insulin, glucagon, and the pancreas/duodenum specific homeodomain transcription factor, IDX-1. While others dispute that nestin-positive cells residing in the pancreas are a potential source for regenerating or expanding islets (Gao et al., 2003), multipotential stem cells with β-cell generative capacity may be identified by this marker. Very recently, Kadam et al. isolated and characterized hUC-MSCs that constitutively expressed nestin and differentiated into functional ILCs capable of restoring normoglycemia in experimental diabetic mice (Kadam & Bhonde 2010). An effective multistep protocol to efficiently induce pancreatic ductal and β-cell differentiation *in vitro* from multipotent, nestin-positive rat bone-marrow stem cells in a serum-free system has recently been described (Milanesi et al., 2011). In this procedure, trichostatin A, a regulator of chromatin remodeling and 5-aza 2' deoxycytidine, an inhibitor of DNA methylase were used to enhance the induction and differentiation toward pancreatic lineage, followed by all-trans RA to promote pancreatic differentiation. Important transcription factor genes, such as Pdx1, Ngn3, and Pax6 were sequentially induced following the *in vivo* development timeline. Lastly, nicotinamide, was used to induce expression of islet and ductal-specific markers. The differentiated cells displayed glucose-responsive insulin secretion and expressed Glut2, indicating the clinical relevance of 'nestin-positive' bone-marrow stem cells. In further characterization of candidate nestin-positive islet progenitors (NIPs), Lechner *et al.* proposed that some islet cell progenitors possess characteristics of both neural origin, exemplified by nestin, as well as of bone-marrow progenitors, exemplified by the so-called side-positive (SP) cell phenotype (Lechner et al., 2002). This phenotype is characterized by the ability to exclude the vital dye Hoechst through the action of the ATP-binding cassette transporter ABCG2 (BCRP1). The presence of this molecular marker potentially allows for purification of these cells thereby providing an

efficient progenitor cell for islet differentiation. These studies prompted investigation into the possibility that pancreatic SP cells possessed β-cell regenerative potential (Zhang et al., 2005). SP cells were shown to be capable of insulin, glucagon, Glut2 and Pdx1 expression induced by a variety of growth factors, concomitant with a decreased expression of progenitor cell markers such as nestin and Ngn3. These differentiated cells also released insulin in response to high glucose concentrations, though in notably lesser amounts when compared to primary β-cells, calling for continued investigation into standardizing the the conditions required for inducing complete differentiation of SP cells into fully functional β-cells.

As necessary as it is to have markers that identify progenitor cells amenable to the islet pathway, it is of equal importance to have makers that can rapidly identify the small number of successfully differentiated cells. With an aim to improve the efficiency of analyzing and sorting β-like insulin-producing cells from undifferentiated cells, Fukazawa *et al.* designed a novel β-cell specific and glucose-responsive artificial promoter system designated pGL3.hINS-363 3x (Fukazawa et al., 2006). This system exhibited significant luciferase activity not only in insulin-producing MIN6 m9 cells but also in isolated human islets. The pGL3.hINS-363 3x construct showed no activity in non-insulin producing cells in low glucose conditions (2mM glucose) but demonstrated significant activity with β-cell specificity in high glucose conditions (16mM glucose). Furthermore, pGL3.hINS-363 3x showed significant promoter activity in differentiated AR42J cells that could produce insulin after activin A and betacellulin treatment. Detection of small numbers of newly formed β-cells by this approach could significantly enhance the production of differentiated, insulin-positive, β-cells for transplantation. Growing evidence suggests that microRNAs (miRNA) play an important role in insulin production, secretion and action and that diabetes changes miRNA expression profiles in many tissues (Baroukh et al., 2007; Correa-Medina et al.,2009; Joglekar et al.,2007,2009; Li et al., 2009; Poy et al., 2004,2009; Tang et al., 2009). miRNAs are a novel group of highly conserved, endogenous, 22–23 nucleotide non-coding RNAs that regulate biological functions very precisely by negatively modulating the gene expression either by promoting mRNA degradation or through translational repression of proteins. Very recently, Chen et al. have described an approach wherein they isolated miRNAs from human embryonic stem cell (hES-T3 cells) -derived pancreatic ILCs (T3Pi) that expressed insulin, glucagon and somatostatin and showed that these miRNAs negatively regulated the expression of protein-coding mRNAs (Chen et al., 2011; Sebastiani et al., 2011). T3pi clusters showed very high expression of miRNAs, miR-186, miR-199a and miR-339, that down-regulated the expression of LIN28, PRDM1, CALB1, GCNT2, RBM47, PLEKHH1, RBPMS2 and PAK6 indicating that these miRNAs and their target genes very likely played important regulatory roles in the development of pancreas and/or differentiation of islet cells. Using this approach, differentiated T3Pi cells with high miRNA expression can be identified and miRNAs may possibly even be manipulated to increase the proportion of β-cells and insulin synthesis for cellular therapy in type I diabetics. Another recent study indicates that miR-24, miR-26,miR-182 or miR-148 act as positive regulators of insulin transcription in cultured β-cells or in isolated primary islets by reducing the expression of insulin transcriptional repressors (Melkman-Zehavi et al.,2011). MiRNAs may also provide a new class of biomarkers for diabetes and progress in the development and use of synthetic miRNAs such as antagomirs (Krutzfeldt et al., 2005) to silence miRNAs such as miR-375 in case of diabetes may provide a novel therapeutic tool for the treatment of diabetes and other diseases in the

future. Furthermore, mechanistic studies on the role of miRNAs in the modulation of the immune system may help identify potential therapeutic targets to ameliorate responses to islet transplantation.

4. Role of transcription factors, growth factors and other cellular targets in enhancing β-cell differentiation and proliferation

Pancreatic development includes the generation of endoderm/gut endothelium, pancreatic differentiation, endocrine specification, and ultimately β-cell differentiation. Therefore, in order to efficiently promote β-cell differentiation and expansion, it is of vital importance to understand the factors that contribute to islet development. A number of signaling molecules and transcriptional regulators including Wnt, TGF-β, FGF, Notch and Hedgehog control various aspects of pancreas and endocrine cell development, proliferation and differentiation (Evans-Molina et al., 2009; Jun et al.,2010; Mishra et al., 2010). During development, it is the definitive endoderm that gives rise to the pancreas and genes such as Wnt/β -catenin, Nodal, GATA4/6, FoxA, Sox17 and Mix amongst others participate in its formation. Pdx1 represents a marker of all pancreatic lineages (endocrine, exocrine and ductal), mediating β-cell function, growth, and proliferation and its inactivation prevents both islet and acinar cell differentiation. Pdx1 regulation is mediated by the IRS2/Akt/FoxO1 pathway, maintaining the ability of β-cells to proliferate and function properly. The notch signaling pathway in turn regulates the expansion and differentiation of the pancreatic progenitor cells by the expression of the 'pro-endocrine' gene, Ngn3. Transcription factors such as Pdx-1, Isl1, Ngn-3, Nkx2.2, Nkx6.1, NeuroD, Hlxβ9, Pax-4, MafA and Pax-6 all participate in islet differentiation with Ngn-3 acting as a key transcription factor required for islet cell development. Amongst these transcription factors, Pdx-1 is the most extensively utilized factor, driving both β-cell neogenesis as well as transdifferentation of pancreatic and extra-pancreatic cells into insulin-producing β-cells.

Various factors such as pregnancy, diabetogenic stimuli, growth factors such as HGF, a combination of epidermal growth factor (EGF) and gastrin, clonophylline, betacellulin, GLP-1 or its long-lasting homolog exendin-4, members of the regenerating protein family such as Reg protein and INGAP can stimulate replication and proliferation of β-cells (Jun et al., 2010; Mishra et al., 2010). Herein, we describe a few of the candidates that are used to attain maximal β-cell expansion by targeting pathways that augment mature β-cell proliferation, inhibit apoptosis, or simultaneously target both. Betacellulin is a member of the EGF family, and is known to increase the rate of native β-cell self-duplication and enhance Pdx1-positive progenitor differentiation. It also induces proliferation and differentiation of insulinoma cells as well as converts an exocrine pancreatic cell line (AR42J) into an insulin-expressing phenotype when combined with activin A. This combined treatment potentiates β-cell self-duplication, ductal cell proliferation and δ-cell transdifferentation into new β-cells (Li et al., 2004). Similarly, activin promotes regeneration and differentiation of newly formed β-cells and together with growth differentiation factors participates in endocrine and exocrine lineage specification (Mishra et al., 2010). The incretin GLP-1 is produced from gut endocrine cells and has been shown to stimulate β-cell proliferation and neogenesis, inhibit β-cell apoptosis and have a profound effect on stimulating the release of insulin and suppression of glucagon from the pancreas. Rapid degradation by dipeptidyl peptidase IV (DPPIV) is responsible for its short biological half life. To overcome this,

exenatide/Exendin-4 (AC2993), a long acting GLP-1 receptor (GLP-1R) agonist and other such stable GLP-1 analogs that are resistant to degradation (liraglutide) and inhibitors of DPPIV(sitagliptin, vidaglitpin) are being investigated for treatment of diabetes (Geelhoed-Duijvestijn, 2007). Injections of GLP-1 or exendin-4, have been shown to increase the β-cell mass and improve hyperglycemia. Combination of GLP-1 and gastrin or EGF with gastrin restores β-cell mass and inhibits autoimmune destruction of islet cells (Suarez-Pinzon et al., 2005, 2008). Administration of GLP-1 and gastrin restores normoglycemia by expanding β-cell mass and by downregulating immune response in autoimmune diabetic NOD mice. Diabetic immunodeficient NOD*scid* mice recipients treated with this combination therapy together with transplantation of human islets demonstrated expanded β-cell mass largely derived from cytokeratin 19-positive pancreatic duct cells. Several growth factors play important roles in β-cell differentiation, proliferation and islet survival. For instance, HGF acts by targeting the protein kinase B/Akt involved in an intracellular signaling cascade linked to improving islet cell survival and function. It also upregulates Glut-2, glucokinase, and insulin gene expression in β-cells and has been shown to increase longitudinal and functional graft survival. Keratinocyte growth factor promotes differentiation of IPCs and VEGF regulates insulin gene expression and β-cell proliferation through laminin and maintains adult islet function. IGF1 expression in β-cells of transgenic mice regenerates the endocrine pancreas during type 1 diabetes. Though the IGF-I-mediated mechanism(s) of restoring β-cell mass is not fully understood, early studies indicate that IGF-I modulates cell cycle proteins and increases replication of pre-existing β-cells after damage (Agudo et al., 2008). The Reg gene family belongs to the calcium-dependent lectin (C-type lectin) gene superfamily and is divided into subclasses based on the primary structures of the encoded proteins. Several Reg family genes, such as Reg2, -3α, and -3β, are upregulated to induce β-cell regeneration, growth and protection and also during the progression of autoimmune diabetes. INGAP, one of the Reg family gene products has been used in numerous studies to facilitate β-cell differentiation and expansion. In addition, heparan sulfate, by regulating ligand-receptor interactions, plays an important role in embryonic development and regulation of postnatal islet maturation. Dicer1 (a ribonuclease required by the RNA interference and miRNA pathways to produce the active small RNA component that represses gene expression) maintains the adult pancreas and regulates the differentiation of endocrine precursor cells. Interestingly, Dicer1-deficient β-cells mostly retain their identity including the expression of typical β-cell markers involved in glucose sensing and insulin transcription but show an increase in repressors of insulin transcription (Melkman-Zehavi et al., 2011). Similarly miRNAs reinforce insulin expression by reducing the expression of insulin transcriptional repressors. Before utilizing these factors as treatment options, safety concerns regarding the risk of cancerous transformation and participation in other cellular processes should be investigated in depth.

β-cell proliferation is not only affected by factors that that induce expansion and differentiation, but is also tightly regulated by factors that impede/inhibit proliferation. Modulation of these factors therefore represents another alternative to enhance β-cell proliferation. For instance deletion of p27Kip1, a key cell cycle inhibitor, increased the frequency of cellular replication and proliferation during β-cell development and in the early neonatal period (Rachdi et al., 2006). Improved glucose tolerance and hyperinsulinemia associated with increased islet mass and proliferation was observed in p27-deficient mice, while induction of p27 expression resulted in severe glucose intolerance

and reduced β-cell mass. Additionally, p27(-/-) mice showed decreased susceptibility to develop STZ-induced diabetes compared to controls that displayed elevated blood glucose levels (Georgia & Bhushan, 2006). In mice that developed STZ-induced diabetes, β-cells retained the ability to reenter the cell cycle at a far greater frequency in the p27(-/-) mice than in wild-type littermates. These studies establish the role of p27 in maintaining the quiescent state of newly differentiated β-cells generated during embryogenesis and indicate p27 as a key regulator in the establishment of β-cell mass and thus as an important target in regenerative therapies for diabetes. The success of this therapy however would depend on the ability of the cell to reactivate p27 to stop uncontrolled proliferation when metabolic demands for insulin are met. Apart from the risk of tumorigenesis, another drawback is the dedifferentiation and loss of insulin secretion commonly observed with repeated cell division of β-cells. Another key regulator of β-cell replication and expansion is glycogen synthase kinase-3 (GSK3), known to negatively regulate insulin-mediated glycogen synthesis and glucose homeostasis (Rayasam et al 2009). Islet β-cell growth is controlled by endogenous GSK-3β activity via feedback inhibition of the insulin receptor/PI3K/Akt signalling pathway (Liu et al., 2010). In fact, glucose regulates steady-state levels of Pdx1 via the reciprocal actions of GSK3 and AKT kinases. Glucose-stimulated activation of AKT and inhibition of GSK3 decreased Pdx1 phosphorylation and delayed Pdx1 degradation demonstrating the important role of AKT-GSK3 axis in glucose modulation of Pdx1 stability (Humphrey et al., 2010). Similarly, direct pharmacologic inhibition of AKT destabilized, while inhibition of GSK3 increased Pdx1 protein stability. Increased expression and activity of GSK3 has been reported in type II diabetics and obese animal models and consequently, inhibitors of GSK3 have been demonstrated to have anti-diabetic effects *in vitro* and in animal models. However, the use of GSK3 inhibitors poses a challenge as achieving selectivity of an over achieving kinase involved in various pathways with multiple substrates may lead to side effects and toxicity.

5. Prolonging long term survival, growth and function of β-cells *in vitro*

Despite the promising results of β-cell regenerative strategies, maintaining functional islet cells in long-term cultures with persistent insulin-secreting capabilities has proven challenging. The efficacy of various cell culture medium supplements in prolonging the survival and function of newly generated β-cells and enhancing their survival in long-term cultures for optimal expansion has been investigated. For e.g. human islets were cultured *in vitro* for more than a year during which time, using multilabeling immunohistochemical and immunoelectron microscopic analyses with islet cell markers (antibodies to hormones, neuron-specific enolase, chromogranin A) and ductal cell markers (cytokeratins 7 and 19, carbonic anhydrase II, DU-PAN2, CA19-9, and MUC1), islet cells gradually transdifferentiated into ductal, acinar, and undifferentiated cells, considered pancreatic precursor (stem) cells (Schmied et al., 2001). Although endocrine cells remained detectable at day 60, hormone secretion ceased after day 28. Eventually all endocrine and exocrine cells were replaced by undifferentiated cells that expressed neuron-specific enolase, chromogranin A, laminin, vimentin, cytokeratin 7 and 19, α-1-antitrypsin, TGF-α, and EGF-receptor. While this study successfully demonstrated the possibility of culturing human islets for long periods, it brings into focus the formidable challenge of overcoming the loss of

insulin-secreting capabilities and transdifferentiation of β-cells. The efficacy of supplementing culture medium with growth hormone (GH) and prolactin (PRL) as well as co-culturing islets with fibroblasts on stimulating long term cell proliferation as well as maintenance of insulin-synthesizing and -secreting capacity has also been investigated (Gartner et al., 2006). The supportive role of FGF2 in maintaining functional β-cells in culture has been previously demonstrated (Hardikar et al, 2003). FGF2, acting as a paracrine chemoattractant, stimulates clustering of human islet-derived precursor cells, leading to islet-like cell aggregate formation necessary for the early stages of islet cell differentiation. An impressive protocol that combined bFGF, leukemia inhibitory factor (LIF), and bone morphogenetic protein-4 (BMP-4) proved that islet progenitor-like cells can be derived from islet-enriched fractions under serum-free defined culture conditions and induced to stably express high levels of Pdx1 and Notch pathway-associated genes, characteristic of embryonic pancreatic progenitor cells, for more than 6 months and maintain endodermal and pancreatic phenotypes. Unfortunately, insulin expression remained minimal. Based on the hypothesis that loss of the trophic support provided by surrounding non-endocrine pancreatic cell populations underlies the decline in β-cell mass and insulin secretory function observed in human islets following isolation and culture, the effect of co-culturing islets with ductal epithelial cells on islet structural integrity, β-cell mass and insulin secretory capacity was investigated (Murray et al., 2009). Ten days following isolation, the results showed that co-culturing islets with ductal epithelial cells led to preserved islet morphology and sustained β-cell function, with the presence of ductal epithelial cells beneficial for maintenance of β-cell mass. While reinforcing the possibility of maintaining islets in long-term cultures under appropriate cell culture conditions, the study highlights the necessity for further characterization of regulatory influences in order to realize the promise of its therapeutic potential. The importance of a three-dimensional (3D) environment incorporating extracellular matrix (ECM) components in providing favorable conditions to preserve human islets in long-term culture has also been demonstrated (Daoud et al., 2010). The loss of the ECM basement membrane during isolation contributes to eventual apoptosis *in vitro*. While collagen I/IV and fibronectin induce adhesion, fibronectin is the only ECM protein capable of maintaining islet structural integrity and insulin content distribution in cultures. Although in this study islet phenotype was eventually lost, insulin gene expression was highest in islets cultured on collagen I and IV. Insulin release peaked with fibronectin along with a decrease in SUR1 expression, while glucose metabolism along with Glut2 and GCK expression was highest on collagen I and IV surfaces. A very recent study consisting of long-term and highly uniform human islet culture within a micro-fabricated scaffold with collagen 1 gel supplemented with ECM components fibronectin and collagen IV with controlled pore structures, displayed an insulin release profile similar to freshly isolated islets, yielding a stimulation index of approximately 1.8 (Daoud et al., 2011). Gene expression was markedly increased for all pancreatic genes, giving approximately 50-fold elevation of insulin gene expression with respect to suspension culture. The level of pancreatic hormones was also highly elevated. These findings provide the groundwork for, 1) establishing a modified 3D construct to culture pancreatic islets, and 2) for understanding the underlying mechanisms of islet interactions with its surroundings, in order to provide a platform for long-term maintenance and preservation of human pancreatic islets *in vitro*.

6. Hepatocytes and Xenogeneic islets - prospects and barriers as alternative sources

Reprogramming adult mammalian cells is an attractive approach for generating β-cells for replacement therapy. The common embryonic origin of liver and pancreas, the similarity of glucose-sensing systems, the large group of mutually expressed, specific transcription factors and the high level of developmental plasticity exhibited by adult human liver cells suggest that liver stem cells/hepatocytes are a potential source of pancreatic progenitor tissue. For instance, persistent expression of the Pdx1 or its super-active form Pdx1-VP16 fusion protein in hepatic cells reprograms these cells into pancreatic β-cell precursors under condition of hyperglycemia or hepatic regeneration (Yang et al., 2006). Also, most hepatocytes of Ad-pdx-1-infected mice demonstrated positivity for Pdx-1 expression but expressed insulin and somatostatin only in STZ-treated or in STZ-treated plus partial hepatectomy mice vs. nontreated mice. A corresponding amelioration of hyperglycemia and along with expression of other β-cell markers like Glut2 glucokinase was also observed (Kim etal., 2007). Similarly *ex vivo* lentiviral-mediated Pdx1 expression in isolated adult liver cells resulted in expression of insulin in the transduced cells at both mRNA and protein levels, dependent on the presence of glucose and sulfonylurea (Fodor etal., 2007). Expression of β-cell genes, including those encoding solute carrier family 2 (facilitated glucose transporter), member 2 (Slc2a2), glucokinase (Gck), ATP-binding cassette, sub-family C (CFTR/MRP), member 8 (Abcc8), the potassium inwardly-rectifying channel, subfamily J, member 11 (Kcnj11) and proprotein convertase subtilisin/kexin type 1 (Pcsk1) were also observed. The PDX1-transduced hepatocytes expressed several pancreatic transcription factors related to early pancreatic endocrine development (endogenous Pdx1, NeuroD1, and Nkx6.1) as well as the late-stage pancreatic transcription factors (Pax4, Pax6, and MafA). Transplantation of these transdifferentiated liver cells in STZ-induced diabetic *scid* mice resulted in improvement in hyperglycemia. This capacity of Pdx-1 to activate the pancreatic lineage in mature liver has been independently confirmed in numerous studies (Imai et al., 2005, Horb et al., 2003, Li et al., 2005; Miyatsuka et al., 2003; Zalzman et al., 2005). Also the effects of additional pancreatic transcription factors alone or combined with Pdx-1 expression on this process have been analyzed (Sapir et al., 2005; Kaneto et al., 2005a, 2005b; Kojima et al., 2003; Meivar-Levy et al., 2006). For instance, a recent study indicates the dominant role of Nkx6.1, expressed in mature β-cells, in altering the hepatic developmental fate along the pancreatic lineage and function (Gefen-Halevi et al., 2010; Wang, 2007). Nkx6.1 activated immature pancreatic markers such as Ngn-3 and Isl-1 but not pancreatic hormones gene expression in human liver cells, suggesting a potential role for Nkx6.1 in promoting Pdx-1 reprogrammed cells maturation along the β-cell-like lineage. Complementation of Nkx6.1 by ectopic Pdx-1 expression substantially and specifically promoted insulin expression while addition of glucose augmented processed hormone secretion without increasing the number of reprogrammed cells. Other studies demonstrate that combinations of overexpressed MafA with NeuroD and Ngn3 or NeuroD and betacellulin in diabetic mice markedly induced insulin gene transcription and ameliorated glucose tolerance without producing hepatitis (Kaneto et al.., 2005a, 2005b; Kojima et al., 2003). Transient transfection of human hepatoma cells (Li et al., 2005) or hepatic WB cells (Tang et al., 2006) with super-active version Pdx1-VP16 resulted in transdifferentiation into pancreatic cells, characterized by induction of both pancreatic exocrine cells (by detection of amylase protein) and endocrine cells (by detecting insulin, glucagon and somatostatin proteins) and suppression of the hepatic phenotype.

Transdifferentiated β-cells were responsive to physiological stimuli, exhibiting 1) functional β-cell markers including prohormone convertase 1/3 (PC1/3), insulin C-peptide and GLP-1R, 2) increased insulin mRNA expression after treatment of cells with GLP-1 and betacellulin and c) elevated insulin secretion after glucose challenge. Thus, reprogramming with multiple pancreatic transcription factors using first generation, non-toxic, transiently-expressed adenoviral vectors along with appropriate conditions (hyperglycemia and/or partial hepatectomy) can reprogram liver stem/adult liver cells into functional insulin-producing cells. The use of adult human liver cells for generating functional insulin-producing tissue may pave the way to autologous transplantation, circumventing both the shortage in tissue availability, the need for immunosuppressive therapy and the ethical issues associated with ESC therapy. Additionally the ability of the liver to regenerate itself after surgical removal of a portion to use for transdifferentiation without significant harm to the patient is an added bonus.

Xenotransplantation using porcine cells, tissues, or organs may offer a potential solution for the shortage of allogeneic human organs (Denner et al., 2009). However, prior to their clinical use the hurdles of immunologic rejection and risk of transmission of porcine pathogens needs to be overcome. Immunologic rejection in pig to primate xenotransplants consists of hyperacute rejection, acute humoral xenograft rejection (AHXR), acute cellular rejection and chronic rejection (Elliott et al., 2011). Hyperacute rejection results from the activation of the complement cascade that converts graft endothelial cells from an anticoagulant to a procoagulant phenotype. Apart from old world primates and man, all animals possess a cell surface antigen containing the epitope – Galα1–3Galβ1–4GlcNAc-R ('α-gal') to which humans have complement fixing antibodies that cause immediate rejection of animal cells when transplanted into humans. This can be prevented by reducing or inhibiting functional 'α-gal' activity using knock-out or knock-in procedures involving human alpha-1, 2-Ft or GnT-111 gene expression as well as by depletion of anti-pig antibodies or complement from serum. Adult porcine islets do not express Gal, reducing the antibody-mediated response to them after transplantation. However, fetal and neonatal islets do, and therefore the use GT knockout (α1,3-galactosyltransferase gene-knockout [GTKO]) pigs as the as the sources of all islets (fetal, neonatal and adult) is likely to be advantageous. Similarly, genetically-engineered pigs expressing thrombomodulin, tissue factor (TF) pathway inhibitor, CD39 or other mechanisms that prevent the coagulation dysfunction; or expressing human complement-regulatory proteins, CD46 [membrane cofactor protein] or CD55 [human decayacceleratingfactor] or CD59 or all three in pig islets (Ekser & Cooper, 2010); or expressing anticoagulant and antiplatelet molecules within the graft, may afford some protection (Cowan & d'Apice, 2008). Recombinant antithrombin III may also ameliorate both early graft damage and the development of systemic coagulation disorders in pig-to-human xenotransplantation. This strategy, in parallel with physical methods such as encasing islets in a protective layer, holds promise for reducing the thrombogenicity of pig islet xenografts. Ideally, genetically engineered GTKO/CD46 pigs whose organs and cells are protected from the coagulation dysregulation that include modifications that prevent tissue factor activity on the graft and as well as activation of recipient platelets to express TF and initiate consumptive coagulopathy are required. AHXR or delayed xenograft rejection results from vascular endothelial cell activation and injury caused by the complement and cellular components of the innate immune system. There is increasing evidence that primate neutrophils, natural killer (NK) cells and macrophages

play a role in AHXR, particularly seen following the development of a T-cell dependent elicited antibody response. Administration of CD39, heme oxygenase, thrombomodulin and TF pathway inhibitor have been used in its treatment (Cowan & d'Apice, 2008). Acute cellular rejection involving T- and B-cell infiltration of the graft, T-cell activation and a T-cell-dependent elicited antibody response is believed to be stronger following xenotransplantation than the alloresponse. However, potent pharmacologic agents can largely prevent acute cellular rejection, which is therefore typically not observed with intense immunosuppressive drug regimens. Ekser et al. have obtained promising results using an immunosuppressive regimen consisting of induction therapy with antithymocyte globulin (ATG), and maintenance with an anti-CD154 monoclonal antibody and Mycophenolate mofetil (MMF) (Ekser & Cooper, 2010). A clinically acceptable regimen of ATG, CTLA4-Ig and MMF has also been found to be particularly effective in preventing T-cell activation in the xenotransplantation setting. Chronic rejection symptoms in the form of chronic vasculopathy has been observed in pig to primate graft that survived for more than a few weeks, similar to the chronic rejection seen in long-surviving allografts. The risk of development of xenozoonosis in the recipient of a pig graft (particularly with regard to porcine endogenous retroviruses (PERV), is of concern. However, the stable long-term expression of anti-PERV siRNAs has been shown to be effective in knocking down PERV expression in cells. Breeding of designated pathogen-free pigs can prevent transmission of most porcine microbes (Dieckhoff et al., 2008; Ramsoondar et al., 2009). Another hurdle includes sensitization to pig antigens (e.g., swine leukocyte antigens), resulting in an increase in antibodies to HLA. Fortunately current evidence indicates that antibodies that develop after exposure to a pig xenograft are not crossreactive against HLA, and so would not be detrimental to a subsequent allograft (Cooper et al., 2004). However this does not preclude patients with a high level of HLA-reactive antibodies who may still be at greater risk of rejecting a pig xenograft. Clinical attempts to treat type 1 diabetes with implanted porcine islets are underway and showing promising early results. Immune-suppression, sertoli cell co-transplantation and intraperitoneal implantation of micro-encapsulated neonatal islets have been tried in type 1 diabetic humans with some clinical benefit reported from the latter two. In a very recent study by Elliot et al using the microencapsulation technique, transitory insulin independence of several months duration was observed (Elliott et al., 2011). Interestingly, the treatment appeared to significantly decrease severe hypoglycemic episodes and reduce/abolish hypoglycemic unawareness episodes, even in the absence of insulin independence. Evidence of xenosis in the xenotransplants recipients though diligently sought could not be found, given the credentials of the designated pathogen free source herd used. Virus safety in xenotransplantation is a fast-developing field, and new findings may contribute to improved outcomes.

7. The role of gene therapy in enhancing islet function

While not routinely achieving long-term normoglycemia, islet transplantation does afford substantial benefits in the form of reduced incidences of debilitating hypoglycemic episodes and hypoglycemic unawareness, lower daily insulin requirements, a detectable level of c-peptide, and improved A1c levels. The major limitation of this procedure is the inadequate numbers of donor islets available for transplantation. While we have discussed the various approaches to generate and expand islet β-cells, strategies aimed at increasing the efficiency

of islet function in terms of insulin synthesis and secretion are of equal importance since it would reduce the number of islets required for restoring glucose homeostasis following transplantation. Towards this end, gene therapy has been proposed. A simple conceptual approach to create hyper-functional islets would consist of augmentation of the regulated insulin secretory capacity of an islet graft by insulin gene transfer to the graft before transplantation. Deng et al. demonstrated that Ad-Ins-transduced islets showed superior function in terms of insulin production and secretion as well as GSIS (Deng et al., 2003). The amount of basal insulin secretion and the overall pattern of insulin secretion from the Ad-Ins-transduced islet appeared completely normal and following transplantation, recipients remained normoglycemic for more than 100 days without evidence of deterioration in graft function. Histological examination of the grafts showed normal islet graft morphology and the presence of abundant insulin. Interestingly, although transfer of an exogenous insulin gene under control of a powerful viral promoter forced β-cells to produce more insulin, transplantation of a large number of these islets did not result in recipient hypoglycemia, confirming that Ad-Ins-transduced islets secreted insulin in an appropriately regulated manner. Furthermore, only 25% of the previously needed islet mass was required to reverse diabetes suggesting that four times as many transplants could be performed compared to the unmodified islets. The use of insulin promoter instead of the CMV promoter would have further increased insulin secretion from Ad-Ins-transduced islets in response to changes in blood glucose concentration in a highly regulated manner. Furthermore, the islet gene transfer was carried out using an islet-virus co-culture technique resulting in only partial islet transduction (37% expressing transgene). The authors speculate that Ad-Ins-vector delivery by vascular perfusion of the pancreas, would achieve maximal transgene expression in nearly all islet endocrine cells making it possible to reverse diabetes with islets isolated from only a portion of the perfused pancreas. The consideration of live donors as a source of islets is not unreasonable since laparoscopic-assisted distal pancreatectomy and nephrectomy has been performed successfully in three living donors since December 2007 with minimal peri-operative mortality and acceptable morbidity and the distal pancreas has been used successfully as a segmental vascularised graft (Gruessner et al., 2001; Maruyama et al., 2010; Tan et al., 2005). The uneventful postoperative course and normal functioning of the grafts indicate the technique to be minimally invasive and safe, making the consideration of live related donors for providing islet tissue supply a reasonable solution to the problem of organ shortage until other strategies such as stem-cell derivation of β-cells or xenogeneic sources of islet tissue can be refined. In order to avoid the potential side effects associated with the use of viral vectors, Chen et al. demonstrated targeting of plasmid DNA to the pancreas *in vivo* using ultrasound-targeted microbubble destruction (UTMD) (Chen et al., 2010). Intravenous microbubbles carrying plasmids were destroyed within the pancreatic microcirculation by ultrasound, achieving local gene expression that was further targeted to β-cells by a modified rat insulin promoter (RIP3.1). Delivery of RIP3.1-NeuroD1 two days after STZ-induced diabetes to rats pretreated with the JNK inhibitor SP600125 successfully blocked β-cell apoptosis and resulted in *in vivo* islet regeneration, restoration of β-cell mass and normalization of blood glucose level, insulin and C-peptide in rats. The key pancreatic transcription factor Pdx1, possesses a protein transduction domain (PTD) that facilitates its entry into cells (Koya et al., 2008). Reversal of STZ-induced diabetes in mice by cellular transduction with recombinant Pdx1 represents a novel protein transduction domain-based therapy. The potential use of a non-viral, systemic, carrier-based delivery of Fas siRNA gene therapy through intravenous administration using a polymeric carrier, polyethylenimine

(PEI) offers yet another novel approach (Jeong et al., 2010). Another alternative for obtaining hyper-functional islets lies in hyperinsulinemia. Genetic conditions that include mutations in the potassium channel, the sulfonylurea receptor and the energy-sensing mechanisms of β-cells result in hyperinsulinemia. In diffuse congenital hyperinsulinism of infancy, near-total pancreatectomy (98%) has to be performed as a life-saving procedure in order to achieve glycemic control, demonstrating the extreme potency of these cells when compared to a normal islet (Lovvorn et al., 1999; Pierro et al., 2011). Use of these hyperfunctional islets in islet transplantation, preferably along with normal islet tissue, is an interesting proposition, although the use of diseased tissue would raise several concerns and challenges.

8. Increasing post-transplant islet survival - an ummunological approach

From 1999 to 2007, clinical islet transplantation at established centers has resulted in a remarkable reduction in the occurrence of severe hypoglycemia and a success rate of 70% in achievement of insulin independence persisting for 2 years or more in 50% of those achieving insulin independence, or 35% of all islet alone graft recipients. These results are consistent throughout the 8 years of follow-up included in the Collaborative Islet Transplant Registry (Alejandro et al., 2008). Approximately 60% of transplanted islets are lost in the first 10–14 days post-transplantation (Evgenov et al., 2006) most likely due to local hypoxic injury caused by lack of islet vascularity and the deleterious effects of the innate immune response which induces apoptosis, necrosis, coagulation, and complement fixation (Huang et al., 2008). This non-specific inflammatory response results in the production and release of a number of proinflammatory cytokines (e.g. TNF-α, IL-1β, IFN-γ) that further enhance local inflammatory activity, stimulate adaptive immunity and exert deleterious effects on islet β-cell function by inducing apoptosis and cell death (Donath et al., 2008). Islet cells themselves, upon transplantation, possess the ability to receive signals that result in the activation of multiple signaling factors including STAT1, AP-1 and NFkB (Eizirik & Mandrup-Poulsen, 2001). Lisofylline has demonstrated significant ability in down-regulating the systemic inflammatory response by interference with STAT4 signaling resulting in improved islet transplant survival (Yang et al., 2005). Suppression of these pathways has also been attempted through gene therapy approaches (Moore et al., 2006). Currently, islet-directed anti-inflammatory therapy mostly focuses on Toll-like receptor (TLR) signaling pathways within the islets (Huang et al., 2008). Fortifying the β-cell with protection against islet-destructive cytokines represents another avenue to defend against the immune response. For instance, adenoviral transduction of islet cells with a construct expressing IRAP, the interleukin-1 receptor antagonist protein, resulted in improved islet survival and replication caused mainly due to interference in the activation of the IL-1 mediated apoptotic pathway (Tellez et al., 2005). Other strategies that overexpress anti-apoptotic proteins such as the TNF-α inducible transcription factor A20 or bcl-2 demonstrate beneficial effects on islet survival. While immunomodulatory therapies (e.g. monoclonal antibody therapies, CTLA4Ig, anti-thymocyte globulin (ATG), IL-1 receptor antagonist therapy, cellular therapies, etc.) act by either providing immunoregulatory cytokines such as IL-4, IL-10 or TGF-β or by altering the balance between TH1 and TH2 cells, immunosuppressive regimens act by either suppressing the immune response by binding to specific cytoplasmic proteins that inhibit IL-2 secretion and subsequent T cell expansion (Calcineurin inhibitors CNIs; cyclosporine and tacrolimus) or by suppressing IL-2R

signaling thereby inactivating T cells (sirolimus) or by suppressing cell division of lymphocytes (Azathioprine) (Winter & Schatz, 2003). Therefore, combinations of CNIs and steroid-sparing or -free regimens with drugs that demonstrate powerful immunosuppressive/anitinflammatory potency in the absence of nephrotoxicity and diabetogenicity are being investigated. A triple therapy approach that combined rapamycin plus agonist IL-2-related and antagonist-type mutant IL-15-related Ig cytolytic fusion proteins(IL-2.Ig and mutIL-15.Ig) demonstrated a striking ability to reverse diabetes and ameliorate inflammation, mainly due to augmentation of the pro-regulatory effects of IL-2 and inhibition of the proinflammatory mediator IL-15, creating a favorable balance between regulation and inflammation (Koulmanda et al., 2007). Currently, targeted antigen specific/non-specific and antibody-specific immunotherapies that readjust the underlying immunologic imbalance in order to stop or reverse the β-cell-specific autoimmune and inflammatory process within islets and maintain immune tolerance are being combined with islet regeneration therapies in a variety of clinical studies and hold great therapeutic promise for islet transplantation outcomes. These include islet transplantation followed with ATG /alemtuzumab (Campath -1H, monoclonal anti CD52 Ab) / hOKT3 γ induction therapy / anti-CD25 (daclizumab) induction therapy along with a sirolimus-based, prednisone-free maintenance regimen in combination with MMF and low Tacrolimus (Bellin et al., 2008; Gillard et al., 2010; Herold et al. 2005; Magliocca & Knechtle, 2006). Short term ATG treatment in T1DM of recent onset has been shown to contribute to the preservation of residual C-peptide production and to lower insulin requirement following diagnosis. Because Tregs strongly suppress the immune response in syngeneic islet transplantation and improve graft survival and function, several approaches are now emerging to induce/increase host Tregs activity in the transplant setting, including amongst others, systemic TGF-β1 therapy (W. Zhang et al., 2010). Other β-cell therapies currently in Phase II or III stages of development include Otelixizumab (anti-CD3), Teplizumab (anti-CD3), rituximab(anti-CD20), abatacept (CTLA4Ig), DiapPep 277 (heat shock protein) and GAD, Oral Insulin amongst others. Parenteral administration of anti-CD3 mAb for transplantation in humans and for treatment of autoimmune diabetes has been approved. Various combination interventions such as costimulatory blockade with anti-CD4 monoclonal antibodies plus CTLA4Ig and ATG plus CTLA4Ig (Suzuki et al., 2010) as well as combinations of anti-lymphocyte serum (ALS) plus anti-CD3mAb are under investigation. As discussed earlier, GLP-1R agonists like exendin-4 stimulate β-cell proliferation and neogenesis and inhibit β-cell apoptosis while DPPIV inhibitors increase cell insulin content, and therefore are of immense benefit in the above mentioned combination therapies (GLP-1 agonists plus anti-CD3mAB, anti-CD3mAb plus exendin-4, ALS plus exendin-4 etc.) for preserving and expanding β-cell mass following transplantation. Other avenues include encapsulating islets with nanofibre scaffolds or biomatrices synthesized to contain immunosuppressive drugs or drugs that stimulate vasculogenesis/angiogenesis as well as 'bioartificial pancreas'. Stem cells of embryonic, mesenchymal (prochymal), cord blood and haematopoietic (and some neural stem cell) origin, besides their use for regenerative purposes, also possess potent immunomodulatory functions and have great therapeutic potential in increasing post-transplant survival either alone or in combination with the therapies discussed above. Thus, depending on the time of the therapeutic intervention, various immunological approaches may be employed as monotherapy or in combination with short-term tolerance promoting immunoregulatory drugs or drugs promoting preservation, differentiation, expansion or insulin secretion to increase the functional and

longitudinal survival of islets post-transplantation. However, while assessing the efficacy of these therapies, it is important to keep in mind that both CNIs as well as corticosteroids contribute to an increased risk of developing post-transplant hyperglycemia and to the differential diagnosis of graft rejection (Cantarovich &Vistoli, 2011; Egidi et al., 2005). Conducting large randomized trials to establish guidelines that minimize the adverse effects of immunosuppressive regimens for pancreas transplantation will be useful in achieving long-term, functional graft survival.

9. Conclusion

The possibility of transplanting sufficient quantities of functional, viable islets to induce life-long euglycemia depends on the successful outcomes of the various strategies discussed herein, namely regeneration of β-cells utilizing every kind cell from the pancreas, stem cells as well as cells from alternate sources, preserving and expanding their number *ex vivo*, and ensuring their maximal survival after transplantation through protection from hypoxic and immune insults. The possibility of generating a β-cell with a hyperfunctional phenotype would also significantly expand the functional β-cell pool. The evidence presented here indicates that while the challenges are many and accomplishing this task seems formidable, considerable inroads have already been made in each step highlighted in this review, drawing us a step closer to the possibility of widespread applicability of islet transplantation for the reversal of diabetes. Islet transplantation represents a definitive intervention for patients with Type 1 diabetes and with the significant progress achieved in the fields of stem cell therapy, immunomodulation, and gene therapy, the prospect of translating this new found knowledge into clinical applications that promote successful long term outcomes of islet transplantation is looking very promising.

10. References

Aguayo-Mazzucato C, Bonner-Weir S.(2010). Stem cell therapy for type 1 diabetes mellitus. *Nat Rev Endocrinol*, Vol.6, No.3, pp.139-148, ISSN 1759-5037.

Agudo J, Ayuso E, Jimenez V, Salavert A, Casellas A, Tafuro S, Haurigot V, Ruberte J, Segovia JC, Bueren J, Bosch F. (2008). IGF-I mediates regeneration of endocrine pancreas by increasing beta cell replication through cell cycle protein modulation in mice. *Diabetologia,* Vol.51, No.10, pp.1862-1872, ISSN 0012-186X.

Alejandro R, Barton FB, Hering BJ, Wease S; Collaborative Islet Transplant Registry Investigators. (2008). 2008 Update from the Collaborative Islet Transplant Registry.*Transplantation*, Vol.86, No.12, pp.1783-1788, ISSN1534-6080.

Baeyens L, Bouwens L. (2008).Can beta-cells be derived from exocrine pancreas? *Diabetes Obes Metab,* Vol. 10, Suppl. 4, pp.170-178, ISSN 1463-1326.

Baeyens L, De Breuck S, Lardon J, Mfopou JK, Rooman I, Bouwens L. (2005). In vitro generation of insulin-producing beta cells from adult exocrine pancreatic cells. *Diabetologia*, Vol.48, No.1, pp. 49-57, ISSN 0012-186X.

Baharvand H, Jafary H, Massumi M, Ashtiani SK. (2006). Generation of insulin-secreting cells from human embryonic stem cells. *Dev Growth Differ*, Vol.48, No.5, No.323-332, ISSN 0012-1592

Baker M. (2009). Stem cells: Fast and furious. *Nature*,Vol.458, No.7241, pp.962-965, ISSN 1476-4687.

Bai MD, Rong LQ, Wang LC, Xu H, Fan RF, Wang P, Chen XP, Shi LB, Peng SY. (2008). Experimental study on operative methods of pancreaticojejunostomy with reference to anastomotic patency and postoperative pancreatic exocrine function. *World J Gastroenterol*, Vol.14, No.3, pp. 441-447, ISSN 1007-9327.

Banerjee M & Bhonde RR. (2003). Islet generation from intra islet precursor cells of diabetic pancreas: in vitro studies depicting in vivo differentiation. *JOP*, Vol.4, No.4, pp. 137-145, ISSN 1590-8577.

Baroukh N, Ravier MA, Loder MK, Hill EV, Bounacer A, Scharfmann R, Rutter GA, Van Obberghen E. (2007). MicroRNA-124a regulates Foxa2 expression and intracellular signaling in pancreatic beta-cell lines. *J Biol Chem*, Vol.282, No.27, pp. 19575-19588, ISSN 0021-9258.

Bellin MD, Kandaswamy R, Parkey J, Zhang HJ, Liu B, Ihm SH, Ansite JD, Witson J, Bansal-Pakala P, Balamurugan AN, Papas KK, Sutherland DE, Moran A, Hering BJ.(2008).Prolonged insulin independence after islet allotransplants in recipients with type 1 diabetes. *Am J Transplant*, Vol.8, No.11, pp.2463-2470, ISSN 1600-6143.

Bernard-Kargar C and Ktorza A. (2001). Endocrine pancreas plasticity under physiological and pathological conditions. *Diabetes* Vol.50, Suppl.1, pp.S30-35, ISSN 0012-1797.

Blaine SA, Ray KC, Anunobi R, Gannon MA, Washington MK, Means AL. (2010). Adult pancreatic acinar cells give rise to ducts but not endocrine cells in response to growth factor signaling. *Development*, Vol.137, No.14, pp. 2289-2296, ISSN 1477-9129.

Bonner-Weir S, Deery D, Leahy JL, Weir GC.(1989). Compensatory growth of pancreatic beta-cells in adult rats after short-term glucose infusion. Diabetes, Vol.38, No.1, pp.49-53, ISSN 0012-1797.

Bonner-Weir S, Inada A, Yatoh S, Li WC, Aye T, Toschi E, Sharma A. (2008). Transdifferentiation of pancreatic ductal cells to endocrine beta-cells. *Biochem Soc Trans*, Vol.36, Pt 3, pp. 353-356, ISSN 0300-5127.

Bonner-Weir S, Li WC, Ouziel-Yahalom L, Guo L, Weir GC, Sharma A. (2010). Beta-cell growth and regeneration: replication is only part of the story. *Diabetes*, Vol.59, No.10, pp. 2340-2348. ISSN 1939-327X.

Bonner-Weir S, Sharma A.(2006). Are there pancreatic progenitor cells from which new islets form after birth? *Nat Clin Pract Endocrinol Metab*, Vol.2, No.5, pp.240-241, ISSN 1745-8366.

Bonner-Weir S, Toschi E, Inada A, Reitz P, Fonseca SY, Aye T, Sharma A. (2004). The pancreatic ductal epithelium serves as a potential pool of progenitor cells. Pediatr Diabetes, Vol.5,Suppl. 2, pp.16-22, ISSN 1399-543X.

Bonner-Weir S, Weir GC. (2005). New sources of pancreatic beta-cells.*Nat Biotechnol*, Vol.23, No.7, pp.857-861, ISSN 1087-0156.

Borowiak M, Maehr R, Chen S, Chen AE, Tang W, Fox JL, Schreiber SL, Melton DA. (2009).Small molecules efficiently direct endodermal differentiation of mouse and human embryonic stem cells. *Cell Stem Cell*,Vol.4, No.4, pp.348-358, ISSN 1875-9777.

Boumaza I, Srinivasan S, Witt WT, Feghali-Bostwick C, Dai Y, Garcia-Ocana A, Feili-Hariri M. (2009). Autologous bone marrow-derived rat mesenchymal stem cells promote PDX-1 and insulin expression in the islets, alter T cell cytokine pattern and

preserve regulatory T cells in the periphery and induce sustained normoglycemia. *J Autoimmun*, Vol.32, No.1, pp. 33-42, ISSN 0896-8411.

Brennand K, Huangfu D, Melton D. (2007). All beta cells contribute equally to islet growth and maintenance. *PLoS Biol,* Vol. 5, No.7, pp.e163, ISSN 1545-7885.

Brennand K, Melton D.(2009). Slow and steady is the key to beta-cell replication. *J Cell Mol Med*, Vol.13, No.3, pp.472-487, ISSN 1582-4934.

Bretzel RG, Jahr H, Eckhard M, Martin I, Winter D, Brendel MD. (2007). Islet cell transplantation today. *Langenbecks Arch Surg.* Vol. 392, No.3, pp. 239-253, ISSN 1435-2443.

Butler AE, Cao-Minh L, Galasso R, Rizza RA, Corradin A, Cobelli C, Butler PC. (2010).Adaptive changes in pancreatic beta cell fractional area and beta cell turnover in human pregnancy. *Diabetologia,* Vol.53, No.10, pp.2167-2176, ISSN 1432-0428.

Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. (2003). Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes*, Vol.52, No.1, pp.102-110, ISSN 0012-1797.

Cantarovich D, Vistoli F. (2009). Minimization protocols in pancreas transplantation. *Transpl Int*, Vol.22, No.1, pp.61-68, ISSN 0934-0874.

Carlotti F, Zaldumbide A, Loomans CJ, van Rossenberg E, Engelse M, de Koning EJ, Hoeben RC. (2010). Isolated human islets contain a distinct population of mesenchymal stem cells. Islets, Vol.2, No.3, pp.164-173, ISSN 1938-2022.

Chhabra P, Mirmira RG, Brayman KL.(2009). Regenerative medicine and tissue engineering: contribution of stem cells in organ transplantation. *Curr Opin Organ Transplant,* Vol.14, No.1, pp.46-50, ISSN 1531-7013.

Chen BZ, Yu SL, Singh S, Kao LP, Tsai ZY, Yang PC, Chen BH, Shoei-Lung Li S. (2011). Identification of microRNAs expressed highly in pancreatic islet-like cell clusters differentiated from human embryonic stem cells. *Cell Biol Int*, Vol.35, No.1, pp. 29-37, ISSN 1095-8355.

Chen LB, Jiang XB and Yang L (2004). Differentiation of rat marrow mesenchymal stem cells into pancreatic islet beta-cells. *World J Gastroenterol*, Vol.10, No.20, pp. 3016-3020, ISSN 1007-9327.

Chen S, Borowiak M, Fox JL, Maehr R, Osafune K, Davidow L, Lam K, Peng LF, Schreiber SL, Rubin LL, Melton D.(2009). A small molecule that directs differentiation of human ESCs into the pancreatic lineage. *Nat Chem Biol*,Vol.5, No.4, pp.258-265, ISSN 1552-4469.

Chen S, Shimoda M, Wang MY, Ding J, Noguchi H, Matsumoto S, Grayburn PA. (2010). Regeneration of pancreatic islets in vivo by ultrasound-targeted gene therapy. *Gene Ther*, Vol.17, No.11, pp.1411-1420, ISSN 1476-5462.

Chung CH, Hao E, Piran R, Keinan E, Levine F. (2010). Pancreatic beta-cell neogenesis by direct conversion from mature alpha-cells. *Stem Cells,* Vol.28, No.9, pp. 1630-1638, ISSN 1549-4918.

Chung CH & Levine F. (2010). Adult pancreatic alpha-cells: a new source of cells for beta-cell regeneration. *Rev Diabet Stud,* Vol.7, No.2, pp. 124-131, ISSN 1614-0575.

Collombat P, Xu X, Ravassard P, Sosa-Pineda B, Dussaud S, Billestrup N, Madsen OD, Serup P, Heimberg H, Mansouri A. (2009). The ectopic expression of Pax4 in the mouse

pancreas converts progenitor cells into alpha and subsequently beta cells. *Cell*, Vol.138, No.3, pp. 449-462, ISSN 1097-4172.

Cooper DKC, Tseng Y-L, Saidman SL. (2004). Alloantibody and xenoantibody cross-reactivity in transplantation. *Transplantation*, pp. 77:1–5, ISSN 0041-1337.

Correa-Medina M, Bravo-Egana V, Rosero S, Ricordi C, Edlund H, Diez J, Pastori RL. (2009). MicroRNA miR-7 is preferentially expressed in endocrine cells of the developing and adult human pancreas. *Gene Expr Patterns*, Vol.9, No.4, pp. 193-199, ISSN 1567-133X.

Cowan PJ, d'Apice AJ. (2008)The coagulation barrier in xenotransplantation: incompatibilities and strategies to overcome them. *Curr Opin Organ Transplant*, Vol.13, No.2, pp.178-183, ISSN 1531-7013.

D'Amour KA, Bang AG, Eliazer S, Kelly OG, Agulnick AD, Smart NG, Moorman MA, Kroon E, Carpenter MK, Baetge EE, (2006) Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol*, Vol.24, No.11, pp. 1392-1401, ISSN 1087-0156.

Daoud J, Petropavlovskaia M, Rosenberg L, Tabrizian M. (2010). The effect of extracellular matrix components on the preservation of human islet function in vitro. *Biomaterials*, Vol.31, No. 7, pp. 1676-1682, ISSN 1878-5905.

Daoud JT, Petropavlovskaia MS, Patapas JM, Degrandpré CE, Diraddo RW, Rosenberg L, Tabrizian M. (2011). Long-term in vitro human pancreatic islet culture using three-dimensional microfabricated scaffolds. *Biomaterials*, Vol.32, No.6,pp. 1536-1542, ISSN 1878-5905.

Deng S, Vatamaniuk M, Lian MM, Doliba N, Wang J, Bell E, Wolf B, Raper S,Matschinsky FM, Markmann JF. (2003). Insulin gene transfer enhances the function of human islet grafts. *Diabetologia*, Vol.46, No.3, pp.386-393, ISSN 0012-186X.

Denner J, Schuurman HJ, Patience C. (2009). The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes--chapter 5: Strategies to prevent transmission of porcine endogenous retroviruses. *Xenotransplantation*, Vol.16, No.4, pp.239-248, ISSN 1399-3089.

Desai BM, Oliver-Krasinski J, De Leon DD, Farzad C, Hong N, Leach SD, Stoffers DA. (2007). Preexisting pancreatic acinar cells contribute to acinar cell, but not islet beta cell, regeneration. *J Clin Invest*, Vol.117, No.4, pp.971-977, ISSN 0021-9738.

Dieckhoff B, Petersen B, Kues WA, Kurth R, Niemann H, Denner J. (2008). Knockdown of porcine endogenous retrovirus (PERV) expression by PERV-specific siRNA in transgenic pigs. *Xenotransplantation*, Vol.15, pp.36–45, ISSN 1399-3089.

Donath MY, Størling J, Berchtold LA, Billestrup N, Mandrup-Poulsen T.(2008). Cytokines and beta-cell biology: from concept to clinical translation. *Endocr Rev*, Vol.29,No.3, pp.334-350, ISSN 0163-769X.

Dor Y, Brown J, Martinez OI, Melton DA. (2004). Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature*, Vol.429, No.6987, pp. 41-46, ISSN 1476-4687.

Dor Y & Melton DA (2004). How important are adult stem cells for tissue maintenance? *Cell Cycle*, Vol. 3, No. 9, pp. 1104-1106, ISSN 1551-4005.

Egidi FM.(2005). Management of hyperglycaemia after pancreas transplantation: are new immunosuppressants the answer? *Drugs*, Vol.65, No.2,pp.153-166, ISSN 0012-6667.

Eizirik DL, Mandrup-Poulsen T.(2001).A choice of death--the signal-transduction of immune-mediated beta-cell apoptosis. *Diabetologia*, Vol.44, No.12, pp.2115-2133, ISSN 0012-186X

Ekser B, Cooper DK.(2010). Overcoming the barriers to xenotransplantation: prospects for the future. *Expert Rev Clin Immunol*, Vol.6, No.2, pp.219-230, ISSN 1744-666X.

Elliott RB. (2011). Towards Zenotransplantation of pig islets in the clinic. *Curr Opin Organ Transplant,* Vol.16, No.2, pp.195-200, ISSN 1531-7013.

Evans-Molina C, Vestermark GL, Mirmira RG.(2009). Development of insulin-producing cells from primitive biologic precursors. *Curr Opin Organ Transplant*, Vol.14, No.1, pp.56-63, ISSN1531-7013.

Evgenov NV, Medarova Z, Pratt J, Pantazopoulos P, Leyting S, Bonner-Weir S, Moore A.(2006). In vivo imaging of immune rejection in transplanted pancreatic islets. *Diabetes*, Vol.55, No.9,pp.2419-2428, ISSN 0012-1797.

Fan CG, Zhang QJ, Zhou JR. (2011). Therapeutic potentials of mesenchymal stem cells derived from human umbilical cord. *Stem Cell Rev,* Vol.7, No.1, pp. 195-207, ISSN 1558-6804.

Fändrich F, Ungefroren H. (2010). Customized cell-based treatment options to combat autoimmunity and restore beta-cell function in type 1 diabetes mellitus: current protocols and future perspectives. *Adv Exp Med Biol.* Vol.654, pp. 641-665, ISSN 0065-2598.

Fanjul M, Gmyr V, Sengenès C, Ratovo G, Dufresne M, Lefebvre B, Kerr-Conte J, Hollande E. (2010). Evidence for epithelial-mesenchymal transition in adult human pancreatic exocrine cells. *J Histochem Cytochem,* Vol.58, No.9, pp. 807-823, ISSN 1551-5044.

Fiaschi-Taesch NM, Salim F, Kleinberger J, Troxell R, Cozar-Castellano I, Selk K, Cherok E, Takane KK, Scott DK, Stewart AF.(2010). Induction of human beta-cell proliferation and engraftment using a single G1/S regulatory molecule, cdk6. *Diabetes*, Vol.59, No.8, pp.1926-1936, ISSN 1939-327X.

Figliuzzi M, Cornolti R, Perico N, Rota C, Morigi M, Remuzzi G, Remuzzi A, Benigni A. (2009). Bone marrow-derived mesenchymal stem cells improve islet graft function in diabetic rats. *Transplant Proc,* Vol.41, No.5, pp. 1797-1800, ISSN 1873-2623.

Fodor A, Harel C, Fodor L, Armoni M, Salmon P, Trono D, Karnieli E. (2007). Adult rat liver cells transdifferentiated with lentiviral IPF1 vectors reverse diabetes in mice: an ex vivo gene therapy approach. *Diabetologia*,Vol.50, No.1, pp.121-130, ISSN 0012-186X .

Fukazawa T, Matsuoka J, Naomoto Y, Nakai T, Durbin ML, Kojima I, Lakey JR, Tanaka N. (2006). Development of a novel beta-cell specific promoter system for the identification of insulin-producing cells in in vitro cell cultures. *Exp Cell Res,* Vol.312, No.17, pp. 3404-3412, ISSN 0014-4827.

Furth ME & Atala A (2009). Stem cell sources to treat diabetes. *J Cell Biochem,* Vol.106, No.4, pp. 507-511, ISSN 1097-4644.

Gao R, Ustinov J, Pulkkinen MA, Lundin K, Korsgren O, Otonkoski T. (2003). Characterization of endocrine progenitor cells and critical factors for their differentiation in human adult pancreatic cell culture. *Diabetes,* Vol.52, No.8, pp. 2007-2015, ISSN 0012-1797.

Gao R, Ustinov J, Korsgren O, Otonkoski T.(2005). In vitro neogenesis of human islets reflects the plasticity of differentiated human pancreatic cells. *Diabetologia*, Vol.48, No.11, pp.2296-2304, ISSN 0012-186X.

Gao F, Wu DQ, Hu YH, Jin GX, Li GD, Sun TW, Li FJ.(2008).In vitro cultivation of islet-like cell clusters from human umbilical cord blood-derived mesenchymal stem cells. *Transl Res*,Vol.151, No.6, pp.293-302, ISSN 1931-5244.

Gartner W, Koc F, Nabokikh A, Daneva T, Niederle B, Luger A, Wagner L. (2006). Long-term in vitro growth of human insulin-secreting insulinoma cells. *Neuroendocrinology*, Vol. 83,No.2, pp.123-130, ISSN 0028-3835.

Geelhoed-Duijvestijn PH.(2007). Incretins: a new treatment option for type 2 diabetes? *Neth J Med*. Vol.65, No.2, pp.60-64, ISSN0300-2977.

Gefen-Halevi S, Rachmut IH, Molakandov K, Berneman D, Mor E, Meivar-Levy I, Ferber S. (2010). NKX6.1 promotes PDX-1-induced liver to pancreatic β-cells reprogramming. *Cell Reprogram*, Vol.12, No.6,pp.655-664, ISSN 2152-4998.

Georgia S, Bhushan A. (2006). p27 Regulates the transition of beta-cells from quiescence to proliferation. *Diabetes*, Vol.55, No.11, pp.2950-2956, ISSN 0012-1797.

Gianani R. (2011). Beta cell regeneration in human pancreas. Semin Immunopathol, Vol.33, No.1, pp.23-27,ISSN1863-2300.

Gianani R, Putnam A, Still T, Yu L, Miao D, Gill RG, Beilke J, Supon P, Valentine A, Iveson A, Dunn S, Eisenbarth GS, Hutton J, Gottlieb P, Wiseman A. (2006). Initial results of screening of nondiabetic organ donors for expression of islet autoantibodies. *J Clin Endocrinol Metab*, Vol.91, No.5, pp.1855-1861, ISSN 0021-972X.

Gillard P, Keymeulen B, Mathieu C.(2010). Beta-cell transplantation in type 1 diabetic patients: a work in progress to cure.*Verh K Acad Geneeskd Belg*, Vol.72No.1-2, pp.71-98, ISSN 0302-6469.

Gillard P, Mathieu C. (2011) .[Epub ahead of print] Immune and cell therapy in type 1 diabetes: too little too late? *Expert Opin Biol Ther,* ISSN 1744-7682.

Giorgetti A, Montserrat N, Rodriguez-Piza I, Azqueta C, Veiga A, Izpisúa Belmonte JC. Generation of induced pluripotent stem cells from human cord blood cells with only two factors: Oct4 and Sox2. Nat Protoc, Vol.5, No.4, pp.811-820, ISSN 1750-2799.

Granger A, Kushner JA. (2009). Cellular origins of beta-cell regeneration: a legacy view of historical controversies. *J Intern Med.* Vol.266, No.4, pp.325-338, ISSN1365-2796.

Gruessner RW, Kandaswamy R, Denny R. (2001). Laparoscopic simultaneous nephrectomy and distal pancreatectomy from a live donor. *J Am Coll Surg*, Vol.193, No.3, pp.333-337, ISSN 1072-7515.

Gu G, Dubauskaite J, Melton DA. (2002). Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. *Development*, Vol.129, No.10, pp. 2447-2457, ISSN 0950-1991.

Guo T & Hebrok M (2009). Stem cells to pancreatic beta-cells: new sources for diabetes cell therapy. *Endocr Rev.* Vol.30, No.3, pp. 214-227. ISSN 1945-7189.

Guz Y, Nasir I, Teitelman G. (2001). Regeneration of pancreatic beta cells from intra-islet precursor cells in an experimental model of diabetes. *Endocrinology*,Vol.142, No.11, pp. 4956-4968, ISSN 0013-7227.

Haller MJ, Viener HL, Wasserfall C, Brusko T, Atkinson MA, Schatz DA.(2008) Autologous umbilical cord blood infusion for type 1 diabetes. *Exp. Hematol*, Vol.36, No.6, pp.710-715, ISSN 0301-472X.

Hanley S & Rosenberg L. (2009). Islet-derived progenitors as a source of in vitro islet regeneration. *Methods Mol Biol*, Vol.482:371-385, ISSN 1064-3745.

Hardikar AA, Marcus-Samuels B, Geras-Raaka E, Raaka BM, Gershengorn MC.(2003). Human pancreatic precursor cells secrete FGF2 to stimulate clustering into hormone-expressing islet-like cell aggregates. *Proc Natl Acad Sci U S A*, Vol.100, No.12, pp. 7117-7122, ISSN 0027-8424.

Herold KC, Gitelman SE, Masharani U, Hagopian W, Bisikirska B, Donaldson D, Rother K, Diamond B, Harlan DM, Bluestone JA.(2005).A single course of anti-CD3 monoclonal antibody hOKT3gamma1(Ala-Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes. *Diabetes*,Vol.54, No.6, pp.1763-1769, ISSN 0012-1797.

Hochedlinger K, Plath K.(2009). Epigenetic reprogramming and induced Pluripotency. *Development*,Vol.136, NO.4, pp.509-523, ISSN 0950-1991.

Horb ME, Shen CN, Tosh D, Slack JM. (2003). Experimental conversion of liver to pancreas. *Curr Biol*, Vol.13, No.2, pp.105–115, ISSN 0960-9822.

Huang X, Moore DJ, Ketchum RJ, Nunemaker CS, Kovatchev B, McCall AL, Brayman KL. (2008). Resolving the conundrum of islet transplantation by linking metabolic dysregulation, inflammation, and immune regulation. *Endocr Rev*, Vol.29, No.5, pp.603-630, ISSN 0163-769X.

Huangfu D, Osafune K, Maehr R, Guo W, Eijkelenboom A, Chen S, Muhlestein W, Melton DA. (2008). Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nat Biotechnol*, Vol.26, No.11, pp.1269-1275, ISSN 1546-1696.

Humphrey RK, Yu SM, Flores LE, Jhala US.(2010) Glucose regulates steady-state levels of PDX1 via the reciprocal actions of GSK3 and AKT kinases. *J Biol Chem*, Vol.285, No.5, pp.3406-3416, ISSN 1083-351X.

Imai J, Katagiri H, Yamada T, Ishigaki Y, Ogihara T,Uno K, Hasegawa Y, Gao J,Ishihara H, Sasano H, Mizuguchi H, Asano T, Oka Y. (2005). Constitutively active PDX1induced efficient insulin production in adult murine liver. *Biochem Biophys Res Commun*, Vol.326, No.2,pp. 402–409, ISSN 0006-291X.

Inada A, Nienaber C, Katsuta H, Fujitani Y, Levine J, Morita R, Sharma A, Bonner-Weir S. (2008). Carbonic anhydrase II-positive pancreatic cells are progenitors for both endocrine and exocrine pancreas after birth. *Proc Natl Acad Sci U S A*, Vol.105, No.50, pp. 19915-19919. ISSN 1091-6490.

Ito T, Itakura S, Todorov I, Rawson J, Asari S, Shintaku J, Nair I, Ferreri K, Kandeel F, Mullen Y. (2010). Mesenchymal stem cell and islet co-transplantation promotes graft revascularization and function. *Transplantation*, Vol.89, No.12, pp. 1438-1445, ISSN 1534-6080.

Jeong JH, Kim SH, Lee M, Kim WJ, Park TG, Ko KS, Kim SW. (2010). Non-viral systemic delivery of Fas siRNA suppresses cyclophosphamide-induced diabetes in NOD mice. *J Control Release*, Vol.143, No.1, pp.88-94, ISSN 1873-4995.

Joglekar MV, Joglekar VM, Hardikar AA. (2009). Expression of islet-specific microRNAs during human pancreatic development. *Gene Expr Patterns,* Vol.9, No.2, pp. 109-113, ISSN 1567-133X.

Joglekar MV, Parekh VS, Mehta S, Bhonde RR, & Hardikar AA. (2007). MicroRNA profiling of developing and regenerating pancreas reveal post-transcriptional regulation of neurogenin3. *Dev Biol,* Vol.311, No.2, pp. 603-612, ISSN 1095-564X.

Juhl K, Bonner-Weir S, Sharma A. (2010). Regenerating pancreatic beta-cells: plasticity of adult pancreatic cells and the feasibility of in-vivo neogenesis. *Curr Opin Organ Transplant,* Vol.15, No.1, pp. 79-85, ISSN1531-7013.

Jun HS. (2010). In vivo regeneration of insulin-producing beta-cells. *Adv Exp Med Biol*, Vol.654, pp.627-640, ISSN 0065-2598.

Kadam SS & Bhonde RR. (2010). Islet neogenesis from the constitutively nestin expressing human umbilical cord matrix derived mesenchymal stem cells. *Islets,* Vol.2, No.2, pp. 112-120. ISSN 1938-2022.

Kaneto H, Matsuoka TA, Nakatani Y, Miyatsuka T, Matsuhisa M,Hori M,Yamasaki Y. (2005a). A crucial role of MafA as a novel therapeutic target for diabetes. *J Biol Chem* , Vol.280, No.15, pp.15047–15052, ISSN 0021-9258.

Kaneto H, Nakatani Y, Miyatsuka T, Matsuoka TA, Matsuhisa M,Hori M,Yamasaki Y. (2005b). PDX-1/VP16 fusion protein, together with NeuroD or Ngn3, markedly induces insulin gene transcription and ameliorates glucose tolerance. *Diabetes,* Vol.54, No.4,pp.1009–1022, ISSN 0012-1797.

Karnieli O, Izhar-Prato Y, Bulvik S, Efrat S. (2007). Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation. *Stem Cells,* Vol.25, No.11, pp. 2837-2844, ISSN 1549-4918.

Karnik SK, Chen H, McLean GW, Heit JJ, Gu X, Zhang AY, Fontaine M, Yen MH,Kim SK. (2007). Menin controls growth of pancreatic beta-cells in pregnant mice andpromotes gestational diabetes mellitus. *Science*, Vol.318, No.5851, pp.806-809, ISSN 1095-9203.

Kaung HL.(1994). Growth dynamics of pancreatic islet cell populations during fetal and neonatal development of the rat. *Dev Dyn*, Vol.200, No.2,163-175, ISSN 1058-8388.

Kayali AG, Flores LE, Lopez AD, Kutlu B, Baetge E, Kitamura R, Hao E, Beattie GM, Hayek A.(2007). Limited capacity of human adult islets expanded in vitro to redifferentiate into insulin-producing beta-cells.*Diabetes,* Vol.56, No.3, pp703-708, ISSN 0012-1797.

Kikugawa R, Katsuta H, Akashi T, Yatoh S, Weir GC, Sharma A, Bonner-Weir S. (2009). Differentiation of COPAS-sorted non-endocrine pancreatic cells into insulin-positive cells in the mouse. *Diabetologia,* Vol.52, No.4, pp. 645-652, ISSN1432-0428.

Kim S, Shin JS, Kim HJ, Fisher RC, Lee MJ, Kim CW. (2007). Streptozotocin-induced diabetes can be reversed by hepatic oval cell activation through hepatic transdifferentiation and pancreatic islet regeneration. *Lab Invest*, Vol.87, No.7, pp.702-712, ISSN 0023-6837.

Knip M. (1997) Disease-associated autoimmunity and prevention of insulin-dependent diabetes mellitus. *Ann Med*, Vol. 29, No.5, pp. 447-451, ISSN 0785-3890.

Koblas T, Zacharovová K, Berková Z, Leontovic I, Dovolilová E, Zámecník L, Saudek F.(2009). In vivo differentiation of human umbilical cord blood-derived cells into

insulin-producing beta cells. *Folia Biol (Praha)*, Vol.55, No.6, pp.224-232, ISSN 0015-5500.

Kodama S, Toyonaga T, Kondo T, Matsumoto K, Tsuruzoe K, Kawashima J, Goto H, Kume K, Kume S, Sakakida M, Araki E. (2005). Enhanced expression of PDX-1 and Ngn3 by exendin-4 during beta cell regeneration in STZ-treated mice. *Biochem Biophys Res Commun*, Vol.327, No.4, pp. 1170-1178, ISSN 0006-291X.

Kogler G, Sensken S, Airey JA, Trapp T, Muschen M, Feldhahn N, Liedtke S, Sorg RV, Fischer J, Rosenbaum C, Greschat S, Knipper A, Bender J, Degistirici O, Gao J, Caplan AI, Colletti EJ, Almeida-Porada G, Müller HW, Zanjani E, Wernet P. (2004). A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential. *J Exp Med*, Vol.200, No.2, pp. 123-135, ISSN 0022-1007

Kojima H, Fujimiya M, Matsumura K, Younan P,Imaeda H, Maeda M,Chan, L. (2003). NeuroD-betacellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice. *Nat Med*, Vol.9, No.5, pp.596–603, ISSN 1078-8956.

Kopinke D & Murtaugh LC. (2010). Exocrine-to-endocrine differentiation is detectable only prior to birth in the uninjured mouse pancreas. *BMC Dev Biol*, Vol.10, pp. 38, ISSN 1471-213X.

Koulmanda M, Budo E, Bonner-Weir S, Qipo A, Putheti P, Degauque N, Shi H, Fan Z, Flier JS, Auchincloss H Jr, Zheng XX, Strom TB.(2007). Modification of adverse inflammation is required to cure new-onset type 1 diabetic hosts. *Proc Natl Acad Sci U S A*, Vol.104, No.32, pp.13074-13079, ISSN 0027-8424.

Koya V, Lu S, Sun YP, Purich DL, Atkinson MA, Li SW, Yang LJ. (2008). Reversal of streptozotocin-induced diabetes in mice by cellular transduction with recombinant pancreatic transcription factor pancreatic duodenal homeobox-1: a novel protein transduction domain-based therapy. *Diabetes*, Vol.57, No.3, pp.757-769, ISSN 1939-327X.

Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S, Young H, Richardson M, Smart NG, Cunningham J, Agulnick AD, D'Amour KA, Carpenter MK, Baetge EE. (2008). Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nat Biotechnol*, Vol.26, No.4, pp. 443-452, ISSN 1546-1696.

Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, Stoffel M. (2005). Silencing of microRNAs in vivo with 'antagomirs'. *Nature*, Vol.438, No. 7068, pp. 685-689, ISSN 1476-4687.

Kushner JA, Weir GC, Bonner-Weir S. (2010). Ductal origin hypothesis of pancreatic regeneration under attack. Cell Metab. Vol.11, No.1, pp.:2-3, ISSN 932-7420.

Langer RM. (2010) Islet transplantation: lessons learned since the Edmonton breakthrough. *Transplant Proc*, Vol.42, No.5, pp. 1421-1424, ISSN 1873-2623.

Lardon J, De Breuck S, Rooman I, Van Lommel L, Kruhøffer M, Orntoft T, Schuit F, Bouwens L.(2004). Plasticity in the adult rat pancreas: transdifferentiation of exocrine to hepatocyte-like cells in primary culture. *Hepatology*,Vol.39, No.6, pp.1499-1507, ISSN 0270-9139.

Lechner A, Leech CA, Abraham EJ, Nolan AL, & Habener JF. (2002). Nestin-positive progenitor cells derived from adult human pancreatic islets of Langerhans contain side population (SP) cells defined by expression of the ABCG2 (BCRP1) ATP-

binding cassette transporter. *Biochem Biophys Res Commun,* Vol.293, No.2, pp. 670-674, ISSN 0006-291X.

Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, Semprun-Prieto L, Delafontaine P, Prockop DJ. (2009). Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. *Cell Stem Cell,* Vol.5, No.1, pp. 54-63, ISSN 1875-9777.

Lee RH, Seo MJ, Reger RL, Spees JL, Pulin AA, Olson SD, Prockop DJ. (2006). Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice. *Proc Natl Acad Sci U S A,* Vol.103, No.46, pp. 17438-17443, ISSN 0027-8424.

Leitão CB, Tharavanij T, Cure P, Pileggi A, Baidal DA, Ricordi C, Alejandro R. (2008). Restoration of hypoglycemia awareness after islet transplantation. *Diabetes Care*, Vol.31, No.11, pp.2113-2115, ISSN 1935-5548.

Levetan C. (2010). Distinctions between islet neogenesis and β-cell replication: implications for reversal of Type 1 and 2 diabetes. *J Diabetes.* Vol.2, No.2, pp.76-84, ISSN 1753-0407.

Li L, Seno M, Yamada H, Kojima I. (2003). Betacellulin improves glucose metabolism by promoting conversion of intraislet precursor cells to beta-cells in streptozotocin-treated mice. *Am J Physiol Endocrinol Metab,* Vol.285, No.3, pp. E577-583, ISSN 0193-1849.

Li L, Yi Z, Seno M, Kojima I.(2004). Activin A and betacellulin: Effect on regeneration of pancreatic beta-cells in neonatal streptozotocin-treated rats. *Diabetes,* Vol.53,No.3,pp.608-615, ISSN 0012-1797.

Li WC, Horb ME, Tosh D, Slack JM. (2005). In vitro transdifferentiation of hepatoma cells into functional pancreatic cells. *Mech Dev,* Vol. 122, No.6, pp. 835–847, ISSN 0925-4773.

Li Y, Song YH, Li F, Yang T, Lu YW, Geng YJ. (2009). MicroRNA-221 regulates high glucose-induced endothelial dysfunction. *Biochem Biophys Res Commun,* Vol.381, No.1, pp. 81-83, ISSN 1090-2104.

Lipsett MA, Castellarin ML, Rosenberg L. (2007). Acinar plasticity: development of a novel in vitro model to study human acinar-to-duct-to-islet differentiation. *Pancreas,* Vol.34, No.4, pp.452-457, ISSN 1536-4828.

Liu Y, Tanabe K, Baronnier D, Patel S, Woodgett J, Cras-Méneur C, Permutt MA. (2010). Conditional ablation of Gsk-3β in islet beta cells results in expanded mass and resistance to fat feeding-induced diabetes in mice. *Diabetologia*, Vol.53, No.12, pp. 2600-2610, ISSN 1432-0428.

Lovvorn HN 3rd, Nance ML, Ferry RJ Jr, Stolte L, Baker L, O'Neill JA Jr, Schnaufer L, Stanley CA, Adzick NS. (1999). Congenital hyperinsulinism and the surgeon: lessons learned over 35 years. *J Pediatr Surg.* Vol.34, No.5, pp.786-792; discussion 792-793, ISSN 0022-3468.

Lu J, Herrera PL, Carreira C, Bonnavion R, Seigne C, Calender A, Bertolino P, Zhang CX. (2010). Alpha cell-specific Men1 ablation triggers the transdifferentiation of glucagon-expressing cells and insulinoma development. *Gastroenterology,* Vol.138, No.5, pp. 1954-1965, ISSN 1528-0012.

Magliocca JF, Knechtle SJ. (2006).The evolving role of alemtuzumab (Campath-1H) for immunosuppressive therapy in organ transplantation. *Transpl Int*, Vol.19, No.9,pp.705-714, ISSN 0934-0874.

Martín J, Hunt SL, Dubus P, Sotillo R, Néhmé-Pélluard F, Magnuson MA, Parlow AF, Malumbres M, Ortega S, Barbacid M. (2003). Genetic rescue of Cdk4 null mice restores pancreatic beta-cell proliferation but not homeostatic cell number. *Oncogene,* Vol.22, No.34, pp. 5261-5269, ISSN 0950-9232.

Martin-Pagola A, Sisino G, Allende G, Dominguez-Bendala J, Gianani R, Reijonen H, Nepom GT, Ricordi C, Ruiz P, Sageshima J, Ciancio G, Burke GW, Pugliese A.(2008). Insulin protein and proliferation in ductal cells in the transplanted pancreas of patients with type 1 diabetes and recurrence of autoimmunity. *Diabetologia*, Vol.51,No.10,pp.1803-1813, ISSN 0012-186X

Maruyama M, Kenmochi T, Akutsu N, Saigo K, Iwashita C, Otsuki K, Ito T, Asano T. (2010). Laparoscopic-assisted distal pancreatectomy and nephrectomy from a live donor. *J Hepatobiliary Pancreat Sci*,Vol.17, No.2, pp.193-196, ISSN 1868-6982. McGuckin CP, Forraz N, Baradez MO, Navran S, Zhao J, Urban R, Tilton R, Denner L.(2005). Production of stem cells with embryonic characteristics from human umbilical cord blood. *Cell Prolif*. Vol.38, No.4, pp.245-55, ISSN 0960-7722.

Means AL, Meszoely IM, Suzuki K, Miyamoto Y, Rustgi AK, Coffey RJ Jr, Wright CV, Stoffers DA, Leach SD. (2005). Pancreatic epithelial plasticity mediated by acinar cell transdifferentiation and generation of nestin-positive intermediates. *Development,* Vol.132, No.16, pp. 3767-377,. ISSN 0950-1991.

Meier JJ, Butler AE, Saisho Y, Monchamp T, Galasso R, Bhushan A, Rizza RA, Butler PC.(2008). Beta-cell replication is the primary mechanism subserving the postnatal expansion of beta-cell mass in humans. *Diabetes*, Vol.57, No.6, pp.1584-1594, ISSN1939-327X.

Meivar-Levy I, Ferber S. (2006). Regenerative medicine: using liver to generate pancreas for treating diabetes. *Isr Med Assoc J*, Vol.8, No.6, pp.430-434, ISSN 1565-1088.

Melkman-Zehavi T, Oren R, Kredo-Russo S, Shapira T, Mandelbaum AD, Rivkin N, Nir T, Lennox KA, Behlke MA, Dor Y, Hornstein E. (2011) miRNAs control insulin content in pancreatic β-cells via downregulation of transcriptional repressors. *EMBO J*, Vol.30,No. 5, pp.835-845, ISSN1460-2075.

Menge BA, Tannapfel A, Belyaev O, Drescher R, Müller C, Uhl W, Schmidt WE, Meier JJ.(2008). Partial pancreatectomy in adult humans does not provoke beta-cell regeneration. *Diabetes,* Vol.57, No.1,pp.142-149, ISSN 1939-327X.

Milanesi A, Lee JW, Xu Q, Perin L, Yu JS. (2011). Differentiation of Nestin-Positive Cells Derived from Bone Marrow into Pancreatic Endocrine and Ductal Cells in vitro. *J Endocrinol*, ISSN 1479-6805.

Minami K, Okuno M, Miyawaki K, Okumachi A, Ishizaki K, Oyama K, Kawaguchi M, Ishizuka N, Iwanaga T, Seino S. (2005). Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells. *Proc Natl Acad Sci U S A*, Vol.102, No.42, pp. 15116-15121, ISSN 0027-8424.

Minami K & Seino S. (2008). Pancreatic acinar-to-beta cell transdifferentiation in vitro. *Front Biosci*, Vol.13, pp.5824-5837, ISSN 1093-4715.

Mishra PK, Singh SR, Joshua IG, Tyagi SC.(2010). *Front Biosci*, Vol.15, pp.461-477, ISSN 1093-4715.

Miyatsuka T, Kaneto H, Kajimoto Y, et al. (2003). Ectopically expressed PDX-1 in liver initiates endocrine and exocrine pancreas differentiation but causes dysmorphogenesis. *Biochem Biophys Res Commun*, Vol.310, No.3, pp.1017–1025, ISSN0006-291X.

Montaña E, Bonner-Weir S, Weir GC. (1994). Transplanted beta cell response to increased metabolic demand. Changes in beta cell replication and mass. *J Clin Invest*, Vol.93, No.4, pp.1577-1582, ISSN.0021-9738.

Moore DJ, Markmann JF, Deng S.(2006). Avenues for immunomodulation and graft protection by gene therapy in transplantation. *Transpl Int*,Vol.19,No.6, pp.435-445, ISSN 0934-0874.

Murray HE, Paget MB, Bailey CJ, Downing R. (2009). Sustained insulin secretory response in human islets co-cultured with pancreatic duct-derived epithelial cells within a rotational cell culture system. *Diabetologia*, Vol.52, No.3, pp.477-485, ISSN 1432-0428.

Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, Okita K,Mochiduki Y, Takizawa N, Yamanaka S. (2008). Generation of induced pluripotent stem cells without myc from mouse and human fibroblasts. *Nat Biotechnol* ,Vol.26, No.1, pp.101-106, ISSN 1546-1696.

Nir T & Dor Y. (2005). How to make pancreatic beta cells--prospects for cell therapy in diabetes. *Curr Opin Biotechnol*, Vol.16, No.5, pp. 524-529, ISSN 0958-1669.

Nir T, Melton DA, Dor Y. (2007). Recovery from diabetes in mice by beta cell regeneration. *J Clin Invest* 117, No.9, pp. 2553-2561, ISSN 0021-9738.

Noguchi H. (2009). Pancreatic islet transplantation. *World J Gastrointest Surg.* Vol.1, No.1, pp.16-20, ISSN 1948-9366.

Okuno M, Minami K, Okumachi A, Miyawaki K, Yokoi N, Toyokuni S, Seino S. (2007). Generation of insulin-secreting cells from pancreatic acinar cells of animal models of type 1 diabetes. *Am J Physiol Endocrinol Metab*, Vol.292, No.1, pp.E158-65, ISSN 0193-1849.

Parekh VS, Joglekar MV, Hardikar AA. (2009). Differentiation of human umbilical cord blood-derived mononuclear cells to endocrine pancreatic lineage. *Differentiation,* Vol.78, No.4, pp. 232-240, ISSN 1432-0436.

Phuc PV, Nhung TH, Loan DT, Chung DC, Ngoc PK (2011). Differentiating of banked human umbilical cord blood-derived mesenchymal stem cells into insulin-secreting cells. *In Vitro Cell Dev Biol Anim,* Vol.47, No.1, pp. 54-63, ISSN 1543-706X.

Pierro A, Nah SA. (2011). Surgical management of congenital hyperinsulinism of infancy. *Semin Pediatr Surg*, Vol.20, No. 1, pp. 50-53, ISSN 1532-9453.

Pittenger GL, Taylor-Fishwick D, Vinik AI. (2009). A role for islet neogenesis in curing diabetes. *Diabetologia.* Vol.52, No.5, pp.735-738, ISSN 1432-0428.

Poy MN, Eliasson L, Krutzfeldt J, Kuwajima S, Ma X, Macdonald PE, Pfeffer S, Tuschl T, Rajewsky N, Rorsman P, Stoffel M. (2004). A pancreatic islet-specific microRNA regulates insulin secretion. *Nature,* Vol.432, No.7014, pp. 226-230, ISSN 1476-4687.

Poy MN, Hausser J, Trajkovski M, Braun M, Collins S, Rorsman P, Zavolan M, Stoffel M. (2009). miR-375 maintains normal pancreatic alpha- and beta-cell mass. *Proc Natl Acad Sci U S A,* Vol.106, No.14, pp. 5813-5818, ISSN 1091-6490.

Rachdi L, Balcazar N, Elghazi L, Barker DJ, Krits I, Kiyokawa H, Bernal-Mizrachi E. (2006). Differential effects of p27 in regulation of beta-cell mass during development,

neonatal period, and adult life. *Diabetes*, Vol.55, No.12, pp.3520-3528, ISSN 0012-1797.

Rackham CL, Chagastelles PC, Nardi NB, Hauge-Evans AC, Jones PM, King AJ. (2011). Co-transplantation of mesenchymal stem cells maintains islet organisation and morphology in mice. *Diabetologia*, ISSN 1432-0428.

Ramsoondar J, Vaught T, Ball S, et al. (2009). Production of transgenic pigs that express porcine endogenous retrovirus small interfering RNAs. *Xenotransplantation*, Vol.16, pp.164–180, ISSN 1399-3089.

Rane SG, Dubus P, Mettus RV, Galbreath EJ, Boden G, Reddy EP, Barbacid M. (1999). Loss of Cdk4 expression causes insulin-deficient diabetes and Cdk4 activation results in beta-islet cell hyperplasia. *Nat Genet*, Vol.22, No.1, pp. 44-52, ISSN 1061-4036.

Rayasam GV, Tulasi VK, Sodhi R, Davis JA, Ray A. (2009). Glycogen synthase kinase 3: more than a namesake. *Br J Pharmacol*, Vol.156, No.6, pp.885-898, ISSN 1476-5381.

Rieck S, Kaestner KH. (2010). Expansion of beta-cell mass in response to pregnancy. *Trends Endocrinol Metab*, Vol.21, No.3, pp.151-158, ISSN 1879-3061.

Rooman I, Heremans Y, Heimberg H, Bouwens L. (2000). Modulation of rat pancreatic acinoductal transdifferentiation and expression of PDX-1 in vitro. *Diabetologia*, Vol. 43, No.7, pp.907-914, ISSN 0012-186X.

Salpeter SJ, Klein AM, Huangfu D, Grimsby J, Dor Y. (2010). Glucose and aging control the quiescence period that follows pancreatic beta cell replication. *Development*, Vol.137, No.19, pp. 3205-3213, ISSN 1477-9129.

Santana A, Ensenat-Waser R, Arribas MI, Reig JA, Roche E (2006). Insulin-producing cells derived from stem cells: recent progress and future directions. *J Cell Mol Med*, Vol.10, No.4, pp. 866-883, ISSN 1582-1838.

Sapir T, Shternhall K, Meivar-Levy I, et al. (2005). From the cover: cell replacement therapy for diabetes. Generating functional insulinproducing tissue from adult human liver cells. *Proc Natl Acad Sci USA*, Vol.102, No.22, pp.7964–7969, ISSN 0027-8424.

Scaglia L, Smith FE, Bonner-Weir S. (1995). Apoptosis contributes to the involution of beta cell mass in the post partum rat pancreas. *Endocrinology*, Vol.136, No.12, pp.5461-5468, ISSN 0013-7227.

Schmied BM, Ulrich A, Matsuzaki H, Ding X, Ricordi C, Weide L, Moyer MP, Batra SK, Adrian TE, Pour PM. (2001). Transdifferentiation of human islet cells in a long-term culture. *Pancreas*. Vol.23, No.2, pp.157-171, ISSN 0885-3177.

Seaberg RM, Smukler SR, Kieffer TJ, Enikolopov G, Asghar Z, Wheeler MB, Korbutt G, van der Kooy D. (2004). Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages. *Nat Biotechnol*, Vol.22, No.9, pp. 1115-1124, ISSN 1087-0156.

Sebastiani G, Vendrame F, Dotta F. (2011). MicroRNAs as New Tools for Exploring Type 1 Diabetes: Relevance for Immunomodulation and Transplantation Therapy. *Transplant Proc*, Vol.43, No.1, pp.330-332, ISSN 1873-2623.

Shi M, Liu ZW, Wang FS.(2011). Immunomodulatory properties and therapeutic application of mesenchymal stem cells. *Clin Exp Immunol*, Vol.164, No.1,pp.1-8, ISSN 1365-2249.

Shi Y, Desponts C, Do JT, Hahm HS, Scholer HR, Ding S. (2008) Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. *Cell Stem Cell*, Vol.3, No.5, pp.568-574, ISSN 1875-9777.

Shimajiri Y, Kosaka Y, Scheel DW, Lynn FC, Kishimoto N, Wang J, Zhao S, German MS. (2011). A mouse model for monitoring islet cell genesis and developing therapies for diabetes. *Dis Model Mech,* Vol.4, No.2, pp. 268-276, ISSN 1754-8411.

Solar M, Cardalda C, Houbracken I, Martín M, Maestro MA, De Medts N, Xu X, Grau V, Heimberg H, Bouwens L, Ferrer J. (2009). Pancreatic exocrine duct cells give rise to insulin-producing beta cells during embryogenesis but not after birth. *Dev Cell,* Vol.17, No.6, pp. 849-860, ISSN 1878-1551.

Sordi V, Bertuzzi F, Piemonti L. (2008). Diabetes mellitus: an opportunity for therapy with stem cells? *Regen Med,* Vol.3, No.3, pp. 377-397, ISSN 1746-076X.

Stanley EG & Elefanty AG. (2008). Building better beta cells. *Cell Stem Cell,* Vol.2, No.4, pp. 300-301, ISSN 1875-9777.

Suarez-Pinzon WL, Lakey JR, Brand SJ, Rabinovitch A. (2005). Combination therapy with epidermal growth factor and gastrin induces neogenesis of huan islet {beta}-cells from pancreatic duct cells and an increase in functional {beta}-cell mass. *J Clin Endocrinol Metab,* Vol.90, No.6, pp. 3401-3409, ISSN 0021-972X.

Suarez-Pinzon WL, Power RF, Yan Y, Wasserfall C, Atkinson M, Rabinovitch A. (2008).Combination therapy with glucagon-like peptide-1 and gastrin restores normoglycemia in diabetic NOD mice. *Diabetes,*Vol.57,No.12, pp.3281-3288,ISSN1939-327X.

Sun Y, Chen L, Hou XG, Hou WK, Dong JJ, Sun L, Tang KX, Wang B, Song J, Li H, Wang KX. (2007). Differentiation of bone marrow-derived mesenchymal stem cells from diabetic patients into insulin-producing cells in vitro. *Chin Med J (Engl),* Vol.120, No.9, pp. 771-776, ISSN 0366-6999.

Suzuki A, Nakauchi H, Taniguchi H.(2004). Prospective isolation of multipotent pancreatic progenitors using flow-cytometric cell sorting. *Diabetes,*Vol.53, No.8, pp.2143-2152, ISSN 0012-1797.

Suzuki J, Ricordi C, Chen Z. (2010). Immune tolerance induction by integrating innate and adaptive immune regulators. *Cell Transplant,* Vol.19, No.3, pp.253-268, ISSN 1555-3892.

Szymczak P, Wojcik-Stanaszek L, Sypecka J, Sokolowska A, Zalewska T.(2010). Effect of matrix metalloproteinases inhibition on the proliferation and differentiation of HUCB-NSCs cultured in the presence of adhesive substrates. *Acta Neurobiol Exp (Wars),*Vol.70, No.4, pp.325-336, ISSN 1689-0035.

Tan M, Kandaswamy R, Sutherland DE, Gruessner RW. (2005). Laparoscopic donor distal pancreatectomy for living donor pancreas and pancreas-kidney transplantation. *Am J Transplant,*Vol.5, No.8, pp.1966-1970, ISSN 1600-6135.

Tang DQ, Cao LZ, Burkhardt BR, Xia CQ, Litherland SA, Atkinson MA, Yang LJ. (2004). In vivo and in vitro characterization of insulin-producing cells obtained from murine bone marrow. *Diabetes,* Vol.53, No.7, pp. 1721-1732, ISSN 0012-1797.

Tang DQ, Lu S, Sun YP, Rodrigues E, Chou W, Yang C, Cao LZ, Chang LJ, Yang LJ. (2006). Reprogramming liver-stem WB cells into functional insulin-producing cells by persistent expression of Pdx1- and Pdx1-VP16 mediated by lentiviral vectors. *Lab Invest,*Vol.86, No.1, pp.83-93, ISSN 0023-6837.

Tang X, Muniappan L, Tang G, Ozcan S (2009). Identification of glucose-regulated miRNAs from pancreatic {beta} cells reveals a role for miR-30d in insulin transcription. *RNA,* Vol.15, No.2, pp. 287-293. ISSN 1469-9001.

Tellez N, Montolio M, Biarnes M, Castano E, Soler J, Montanya E. (2005). Adenoviral overexpression of interleukin-1 receptor antagonist protein increases beta-cell replication in rat pancreatic islets. *Gene Ther*,Vol.12, No.2, pp.120-128, ISSN 0969-7128.

Teta M, Rankin MM, Long SY, Stein GM, & Kushner JA. (2007). Growth and regeneration of adult beta cells does not involve specialized progenitors. *Dev Cell,* Vol.12, No.5, pp. 817-826, ISSN 1534-5807.

Thatava T, Nelson TJ, Edukulla R, Sakuma T, Ohmine S, Tonne JM, Yamada S, Kudva Y, Terzic A, Ikeda Y. (2011). Indolactam V/GLP-1-mediated differentiation of human iPS cells into glucose-responsive insulin-secreting progeny. *Gene Ther,* Vol.18, No.3, pp.283-293, ISSN 476-5462.

Thorel F, Népote V, Avril I, Kohno K, Desgraz R, Chera S, Herrera PL. (2010). Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. *Nature,* Vol.464, No.7292, pp. 1149-1154, ISSN 1476-4687.

Uchida T, Nakamura T, Hashimoto N, Matsuda T, Kotani K, Sakaue H, Kido Y, Hayashi Y, Nakayama KI, White MF, Kasuga M. (2005). Deletion of Cdkn1b ameliorates hyperglycemia by maintaining compensatory hyperinsulinemia in diabetic mice. *Nat Med,* Vol.11, No.2, pp. 175-182, ISSN 1078-8956.

Uccelli A, Benvenuto F, Laroni A, Giunti D.(2011). Neuroprotective features of mesenchymal stem cells. Best Pract Res Clin Haematol. Vol.24, No.1, pp.59-64, ISSN 1532-1924.

van Belle TL, Coppieters KT, von Herrath MG. (2011). Type 1 diabetes: etiology, immunology, and therapeutic strategies. *Physiol Rev,* Vol.91, No.1 pp.79-118, ISSN 1522-1210.

Volarevic V, Arsenijevic N, Lukic ML, Stojkovic M (2011). Concise review: Mesenchymal stem cell treatment of the complications of diabetes mellitus. *Stem Cells,* Vol.29, No.1, pp. 5-10, ISSN 1549-4918.

Wang AY, Ehrhardt A, Xu H, Kay MA. (2007).Adenovirus transduction is required for the correction of diabetes using pdx-1 or neurogenin-3 in the liver. *Mol Ther*,Vol.15, No.2. pp.255-263, ISSN 1525-0016.

Winter WE, Schatz D. (2003). Prevention strategies for type 1 diabetes mellitus: current status and future directions. *BioDrugs*, Vol.17, No.1, pp.39-64, ISSN 1173-8804.

Xia B, Zhan XR, Yi R, Yang B. (2009). Can pancreatic duct-derived progenitors be a source of islet regeneration? *Biochem Biophys Res Commun*, Vol.383, No.4, pp. 383-385, ISSN 1090-2104.

Xie QP, Huang H, Xu B, Dong X, Gao SL, Zhang B, Wu YL. (2009). Human bone marrow mesenchymal stem cells differentiate into insulin-producing cells upon microenvironmental manipulation in vitro. *Differentiation*, Vol.77, No.5, pp. 483-491, ISSN 1432-0436

Xu X, D'Hoker J, Stangé G, Bonné S, De Leu N, Xiao X, Van de Casteele M, Mellitzer G, Ling Z, Pipeleers D, Bouwens L, Scharfmann R, Gradwohl G, Heimberg H. (2008). Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell,* Vol.132, No.2, pp. 197-207, ISSN 1097-4172.

Yang LJ. (2006). Liver stem cell-derived beta-cell surrogates for treatment of type 1 diabetes. *Autoimmun Rev*, Vol.5, No.6, pp.409-413, ISSN 1568-9972.

Yang Z, Chen M, Ellett JD, Carter JD, Brayman KL, Nadler JL.(2005).Inflammatory blockade improves human pancreatic islet function and viability. *Am J Transplant*, Vol.5, No.3, pp.475-483, ISSN 1600-6135.

Yoshida S, Ishikawa F, Kawano N, Shimoda K, Nagafuchi S, Shimoda S, Yasukawa M, Kanemaru T, Ishibashi H, Shultz LD, Harada M. (2005). Human cord blood-derived cells generate insulin-producing cells in vivo. *Stem Cells,* Vol.23, No.9, pp. 1409-1416, ISSN 1066-5099.

Zalzman M, Anker-Kitai L, Efrat S. (2005). Differentiation of human liver derived, insulin-producing cells toward the (beta)-cell phenotype.*Diabetes*,Vol.54, No.9, pp.2568–2575, ISSN 0012-1797.

Zhang D, Jiang W, Liu M, Sui X, Yin X, Chen S, Shi Y, Deng H. (2009). Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin producing cells. *Cell Res*,Vol.19, No.4, pp.429-438, ISSN 1748-7838.

Zhang L, Hu J, Hong TP, Liu YN, Wu YH, Li LS. (2005). Monoclonal side population progenitors isolated from human fetal pancreas. *Biochem Biophys Res Commun,* Vol.333, No.2, pp. 603-608, ISSN 0006-291X.

Zhang W, Zhang D, Shen M, Liu Y, Tian Y, Thomson AW, Lee WP, Zheng XX.(2010).Combined administration of a mutant TGF-beta1/Fc and rapamycin promotes induction of regulatory T cells and islet allograft tolerance. *J Immunol*,Vol.185, No.8,4750-4759, ISSN 1550-6606.

Zhang Y, Shen W, Hua J, Lei A, Lv C, Wang H, Yang C, Gao Z, Dou Z. (2010). Pancreatic islet-like clusters from bone marrow mesenchymal stem cells of human first-trimester abortus can cure streptozocin-induced mouse diabetes. *Rejuvenation Res,* Vol.13, No.6, pp. 695-706, ISSN 1557-8577.

Zhao Y, Lin B, Dingeldein M, Guo C, Hwang D, Holterman MJ.(2010). New type of human blood stem cell: a double-edged sword for the treatment of type 1 diabetes. *Transl Res*. Vol.155, No.5, pp.211-216, ISSN1878-1810.

Zhao Y, Wang H, Mazzone T. (2006). Identification of stem cells from human umbilical cord blood with embryonic and hematopoietic characteristics. *Exp Cell Res*, Vol.312, No.13, pp.2454-2464, ISSN 0014-4827.

Zhao Y, Yin X, Qin H, Zhu F, Liu H, Yang W, Zhang Q, Xiang C, Hou P, Song Z, Liu Y, Yong J, Zhang P, Cai J, Liu M, Li H, Li Y, Qu X, Cui K, Zhang W, Xiang T, Wu Y, Zhao Y, Liu C, Yu C, Yuan K, Lou J, Ding M, Deng H. (2008). Two supporting factors greatly improve the efficiency of human iPSC generation. *Cell Stem Cell*, Vol.3, No.5, pp. 475-479, ISSN 1875-9777.

Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T, Trauger S, Bien G, Yao S, Zhu Y,Siuzdak G, Scholer HR, Duan L, Ding S. (2009). Generation of induced pluripotent stem cells using recombinant proteins, *Cell Stem* Cell, Vol.4, No.5, ISSN 1875-9777.

Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA.(2008). In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature*, Vol.455, No.7213, pp.627-632, ISSN 1476-4687.

Zulewski H, Abraham EJ, Gerlach MJ, Daniel PB, Moritz W, Müller B, Vallejo M, Thomas MK, Habener JF. (2001). Multi-potential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. Diabetes, Vol.50, No.3, pp. 521-533, ISSN 0012-1797.
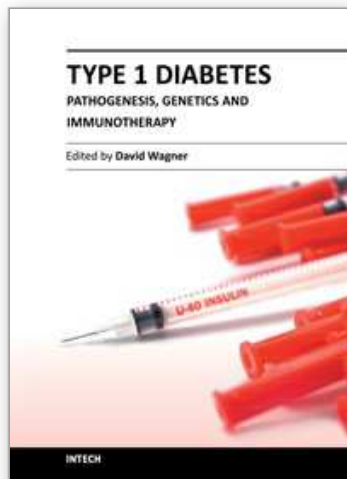
Type 1 Diabetes - Pathogenesis, Genetics and Immunotherapy
Edited by Prof. David Wagner

ISBN 978-953-307-362-0
Hard cover, 660 pages
Publisher InTech
Published online 25, November, 2011
Published in print edition November, 2011

This book is a compilation of reviews about the pathogenesis of Type 1 Diabetes. T1D is a classic autoimmune disease. Genetic factors are clearly determinant but cannot explain the rapid, even overwhelming expanse of this disease. Understanding etiology and pathogenesis of this disease is essential. A number of experts in the field have covered a range of topics for consideration that are applicable to researcher and clinician alike. This book provides apt descriptions of cutting edge technologies and applications in the ever going search for treatments and cure for diabetes. Areas including T cell development, innate immune responses, imaging of pancreata, potential viral initiators, etc. are considered.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Preeti Chhabra, Clark D. Kensinger, Daniel J. Moore and Kenneth L. Brayman (2011). Present Accomplishments and Future Prospects of Cell-Based Therapies for Type 1 Diabetes Mellitus, Type 1 Diabetes - Pathogenesis, Genetics and Immunotherapy, Prof. David Wagner (Ed.), ISBN: 978-953-307-362-0, InTech, Available from: http://www.intechopen.com/books/type-1-diabetes-pathogenesis-genetics-and-immunotherapy/present-accomplishments-and-future-prospects-of-cell-based-therapies-for-type-1-diabetes-mellitus

INTECH
open science | open minds