Gene Therapy (2015) **22**, 1–8

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REVIEW

The use of β-cell transcription factors in engineering artificial β cells from non-pancreatic tissue

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Type 1 diabetes results from the autoimmune destruction of the insulin-producing pancreatic beta (β) cells. Patients with type 1 diabetes control their blood glucose levels using several daily injections of exogenous insulin; however, this does not eliminate the long-term complications of hyperglycaemia. Currently, the only clinically viable treatments for type 1 diabetes are whole pancreas and islet transplantation. As a result, there is an urgent need to develop alternative therapies. Recently, cell and gene therapy have shown promise as a potential cure for type 1 diabetes through the genetic engineering of 'artificial' β cells to regulate blood glucose levels without adverse side effects and the need for immunosuppression. This review compares putative target cells and the use of pancreatic transcription factors for gene modification, with the ultimate goal of engineering a glucose-responsive 'artificial' β cell that mimics the function of pancreatic β cells, while avoiding autoimmune destruction.

Gene Therapy (2015) **22,** 1–8; doi:10.1038/gt.2014.93; published online 23 October 2014

INTRODUCTION

Type 1 diabetes (T1D) results from the autoimmune destruction of the insulin-producing pancreatic beta (β) cells, resulting in hyperglycaemia.[1] Currently, patients with T1D control their blood glucose levels using several daily injections of exogenous insulin;[2] however, this does not mimic the exquisite metabolic responsiveness of the β cell. Insulin therapy delays, but does not eliminate hyperglycaemic episodes and chronic complications associated with extended periods of hyperglycaemia.[3,4] Of equal, if not more, concern to the patient are the life-threatening hypoglycaemic episodes that are exacerbated because of hypoglycaemia unawareness, a phenomenon that worsens with both disease duration and maintenance of blood glucose levels close to normal values.[4]

Currently, whole pancreas or islet transplantation are the only clinically viable treatments for T1D. However, they are limited by a shortage of pancreas donors and the requirement for lifelong immunosuppression, which carries adverse side effects and can compromise the survival of transplanted tissue.[5] Developing cell and gene therapy strategies show immense promise as alternate therapies, potentially avoiding both the requirement for immunosuppression and recurrent autoimmunity.

Over the past decade, there have been several attempts to generate 'artificial' β cells that produce insulin in response to glucose in a regulated manner. Several approaches have been explored including: (i) the dedifferentiation and directed transdifferentiation of autologous cells[6–9] *ex vivo* followed by transplantation, (ii) *in vivo* transdifferentiation via the direct delivery of viral vectors to target organs; and (iii) the genetic modification and expansion of various stem cells *ex vivo* that can then be transplanted. The production of a functional 'artificial' β cell, via genetic manipulation, requires a comprehensive understanding of the pancreatic developmental process. The temporal expression of the various pancreatic transcription factors, their role in determining endocrine cell fate and their involvement in mature β-cell function are key factors for consideration in the design of an artificial β cell. The types of vectors used for gene delivery and the selection of an ideal candidate cell type for differentiation towards a β-cell phenotype are key considerations for achieving this objective. The cell type of choice for the gene therapy of diabetes is not the β cell. β Cells are reduced or absent in patients with T1D, because of autoimmune destruction. This fact will actively work against gene therapists trying to derive surrogate β cells from some stem cells and islet regeneration studies. The ultimate goal for the gene therapy of T1D is to produce a cell that has the ability to process, store and secrete insulin and maintain normal glucose tolerance in response to fluctuating blood glucose concentrations, while avoiding autoimmune attack. This review will compare putative non-pancreatic target cells, viral vectors and pancreatic transcription factors for gene modification to achieve this goal.

SELECTING AN IDEAL TARGET CELL

The first trials of insulin gene therapy were performed using somatic cells, such as monkey kidney cells and fibroblasts.[10,11] However, these cells were unable to produce biologically active insulin, as they did not express proinsulin-processing enzymes. As a result, attention turned to autologous cells that are derived from an endodermal origin and possess characteristics similar to those of β cells[12] (Table 1). The ideal surrogate for β-cell engineering would be able to sense minute changes in glucose, process proinsulin to insulin and c-peptide and store this mature insulin for later secretion.

Endocrine cells have been examined, in particular, pituitary cells that contain both proinsulin-processing enzymes and

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Received 6 June 2014; revised 4 September 2014; accepted 18 September 2014; published online 23 October 2014

Table 1. Summary of target cells and the degree of β-cell similarity

Characteristics	*Target cell*				
	Liver	*Pituitary*	*Muscle*	*K cells*	*BMSC*
Endodermal origin	+	−	−	−	+
Possession of β-cell transcription factors	−	−	−	−	+
Glucose-sensing system	+	−	−	+	−
Processing enzymes	−	+	−	+	−
Glucose-regulatable promoter	+	−	−	+	−
Exocytosis system	−	+	−	+	−
Autologous use	+	+	+	+	+
Allogeneic use	−	−	−	−	+

Abbreviation: BMSC, bone marrow-derived mesenchymal stem cell.

secretory granules. A murine pituitary cell line (AtT20MtIns-1.4) transfected *in vitro* with a recombinant plasmid containing human preproinsulin cDNA produced biologically active insulin; however, glucose responsiveness was absent in these cells.[13] After transfection with both glucose transporter 2 and glucokinase, the AtT20Ins cells became glucose responsive, at subphysiologic levels. Also, the *in vivo* secretion of adenocorticotropic hormone stimulated glucocorticoid synthesis, inhibiting insulin function and therefore limiting their therapeutic efficacy.[14]

Muscle cells have been only sparingly studied because of their lack of insulin proconvertases and storage vesicles. As a result, they require intensive genetic manipulation to produce functional β cells, which is not optimal for therapeutic applications. Nonetheless, muscle-targeted gene therapy for the treatment of T1D has been explored. Implantation of vascular smooth muscle cells transduced with furin-cleavable insulin under the control of a glucose-regulatable promoter into spontaneously diabetic congenic BioBreeding rats has been attempted. This resulted in the reduction of blood glucose levels in two of the eight rats for a period of 6 weeks; however, insulin therapy was still required.[15] Regulated insulin secretion resulted in markedly lower exogenous insulin requirements to sustain normal growth without any hypoglycaemic episodes. Another study indicated that it was possible to reverse diabetes in streptozotocin (STZ) mice for >4 months following the dual expression of insulin and glucokinase in muscle.[16] A synergenic action in the skeletal muscle between the insulin produced and the increased phosphorylation of glucokinase was established, preventing hyperglycaemia.

Liver cells are derived from the same endodermal origin as the pancreas and consequently are more amenable to pancreatic transdifferentiation when compared with other cell types.[17] Similar to pancreatic β cells, they express the key glucose-responsive elements glucose transporter 2 and glucokinase, making hepatocytes an attractive target cell for engineering artificial β cells. Although hepatocytes do not contain proinsulin-processing enzymes and lack secretory granules, this function may be induced via expression of insulin analogues cleaved by furin in the liver.[7,8] However, truly regulated secretion to a glucose stimulus requires the presence of insulin storage granules. Within seconds of being exposed to glucose, the cell transports glucose across the membrane and metabolizes it. The secretory granule migrates to the surface of the cell, fuses with the membrane and secretes its contents, thus regulating blood glucose levels. The result in many studies that have simply expressed either insulin or insulin analogues in liver cell lines or animal livers[18–20] has been the synthesis and constitutive release of insulin, but not its storage or regulated secretion. By comparison, our laboratory has shown that the expression of insulin in a liver cell line that had endogenous expression of β-cell transcription factors led to pancreatic transdifferentiation, formation of secretory granules and a regulated response to glucose with reversal of diabetes.[7]

Owing to the extensive immunomodulatory capacity of mesenchymal stem cells (MSCs),[21–24] their therapeutic potential as a treatment for T1D in an autologous or allogeneic setting has been pursued, with some of these studies currently under human clinical trials.[25] Although the use of native MSC transplantation in animal models of diabetes has been undertaken,[26,27] the majority of clinical MSC research has focused on the *in vitro* production of insulin-producing cells (IPCs), via the application of differentiation protocols to upregulate the expression of β-cell transcription factors.[28]

Native bone marrow-derived mesenchymal stem cells (BMSCs) do express some β-cell transcription factors, and therefore have a potential predisposition for differentiation towards a β-cell phenotype. IPCs can be obtained from BMSCs via the use of a high glucose culture medium[29] or nicotinamide-enriched medium to induce differentiation.[30] The resulting differentiated cells express insulin, at both the mRNA and protein level, and ameliorate hyperglycaemia in STZ diabetic rats.[30] Similarly, it is possible to induce IPC differentiation from BMSCs *in vitro* using a three-step protocol, which results in high expression levels of *Pdx-1*, insulin and glucagon, and glucose-responsive production of insulin.[31] More recently, the combination of a gene and cell therapy successfully produced IPCs from BMSCs. Retroviral transduction of BMSCs with *Pdx-1* resulted in the production of insulin in response to increasing glucose concentrations, and when these cells were transplanted under the renal capsule of STZ diabetic severe-combined immunodeficient mice, the mice showed reduced blood glucose concentrations beginning 12 days posttransplantation, and normal glucose tolerance until 6–8 weeks posttransplantation.[32]

VECTOR CHOICE FOR GENE THERAPY

By exploiting the natural ability of viruses to infect and deliver genes into cells, engineered viral vectors that do not replicate and efficiently transduce genes into infected target cells have been developed. Their suitability for gene transfer into target cells is determined by whether they are integrating or non-integrating, the target cell type and the nature of gene expression required (Table 2). Ideally, β-cell engineering would use integrating viral vectors to provide sustained gene transfer in daughter cells over the life of the patient resulting in a sustained therapeutic benefit.

Retroviral

Retroviral vectors are the most widely used gene delivery vector, and are derived from disabled murine viruses.[33] The advantage of integration into the host's genome is however overshadowed by the risk of insertional mutagenesis.[34] This was first recognized in 1999 following the treatment of nine severe-combined immunodeficiency patients with a retroviral vector, which resulted in the development of leukaemia in four of the nine patients.[35] This study showed that retroviruses in general have site-specific integration preferences in close proximity to the transcriptional regulatory sequences of proto-oncogenes.[36] Retroviral gene transfer is also limited by their ability to only transduce dividing cells, and can therefore only be targeted towards selected cell or tissue types. This ultimately becomes a challenge when the target tissue is composed predominantly of non-dividing cells, such as the liver. Xu *et al.*[37] studied the retroviral transduction of BMSCs with an insulin gene under the control of the cytomegalovirus promoter, and the ability of these transduced cells to restore normoglycaemia in STZ diabetic mice. It was found that the BMSCs successfully expressed insulin and were able to maintain

Table 2. Viral vectors for gene delivery

Viral vector	*Packaging capacity (kb)*	*Advantages*	*Disadvantages*
Retroviral	8–10	Integrates into host genome No expression of viral proteins Long-term expression	Only transduces dividing cells Limited insertion capacity Random integration into host genome
Adenoviral	< 8	Transduces dividing and non-dividing cells Produces high titres of virus High levels of expression	Immune response to viral proteins No integration into host genome Short-term expression
Adeno-associated	< 5	Transduces dividing and non-dividing cells Non-pathogenic and low immunogenicity Long-term expression Broad host range	Requires helper virus Can generate neutralizing antibodies Limited insertion size
Lentiviral	< 10	Transduces dividing and non-dividing cells Stable, long-term expression Can be generated into a self-inactivating vector	Possible toxicity because of viral proteins in packaging construct Random integration into host genome

normoglycaemia for at least 42 days. In addition, the transduced BMSCs were able to evade autoimmune destruction that ordinarily targets pancreatic islets.

Adenoviral

Adenoviral vectors were initially studied owing to their ability to transduce non-dividing cells with high efficiency. However, these vectors transfer their genes episomally and subsequently provide only transient gene expression.[33,38] In addition, immune responses against the viral proteins and in some cases the transgene itself have been reported.[39,40] To overcome the immunogenicity of the viral capsid proteins, a 'gutless' adenovirus was developed, in which the majority of the viral genes were removed.[41] Although reduced immunogenicity was observed with the new-generation adenoviral vectors, immunosuppressants are still required to manage immune responses activated following treatment.[42] The prevalence of pre-existing immunity to adenovirus in humans would also limit the multiple administrations of vector required to maintain long-term therapeutic effects. These characteristics actively work against choosing adenoviral vectors to produce an artificial β cell.

Adeno-associated

Adeno-associated viral vectors are replication-defective parvoviruses able to transduce both dividing and non-dividing cells. Although they show a limited gene cargo capacity (< 5 kb), they preferentially integrate into the host genome at a specific site on chromosome 19.[43] This renders adeno-associated viral vectors a safe and attractive gene delivery candidate as their insertion sites can be predicted and potentially oncogenic consequences avoided. Adeno-associated viral vectors have been used in the treatment of T1D, specifically to deliver directly the preproinsulin gene to livers of chemically STZ mice,[44] where proinsulin was produced in the liver and blood glucose levels were transiently reduced. This study supports the utility of adeno-associated viral vectors for insulin gene transfer to non-dividing hepatocytes.

Lentiviral

Lentiviral vectors are retroviruses with the ability to transduce non-dividing cells as well as dividing cells, which makes them attractive candidates for the transduction of a variety of cell lineages.[45] Lentiviral vectors are derived from human immunodeficiency virus, and, accordingly, biosafety was initially a concern surrounding their suitability as human therapeutics. Construction of self-inactivating human immunodeficiency virus-derived vectors, with deletions in the long terminal repeat promoter, decreases the likelihood of generating a replication-competent virus,[46] and subsequently provides greater safety for clinical application. As a result, lentiviral vectors have shown promise for corrective gene therapy and are currently the gene transfer vector of choice within our laboratory. We have successfully used a lentiviral vector (HMD) to deliver furin-cleavable insulin to the livers of STZ diabetic rats,[8] non-obese diabetic mice[47] and pancreatectomized Westran pigs.[48] In these animal models, we have seen spontaneous expression of an array of β-cell transcription factors, formation of granules and regulated and permanent correction of diabetes.[8,47,48] Liver to pancreas transdifferentiation is relatively common in other situations,[49] especially when the liver is insulted. The pancreatic transdifferentiation in our studies was undoubtedly related to the combination of the surgical procedure used, which isolates the liver from the circulation, the lentiviral vector and the microenvironment of diabetic hyperglycaemia, which collectively insulted the liver cells. By comparison, the simple injection of insulin into the portal circulation led to unregulated constitutive insulin release, no pancreatic transdifferentiation and an abnormal glucose response[47] as seen in a similar study by Elsner *et al.*[19] Although lentiviral expression of insulin has become a popular choice for gene therapy in some rodent models showing amelioration of hyperglycaemia, normal glucose tolerance was not achieved because of an absence of β-cell transcription factor expression and resultant pancreatic transdifferentiation.[50,51]

β-CELL TRANSCRIPTION FACTORS

The pancreas as a whole organ is derived from the endoderm during embryonic development. Transcription factors have a significant role in pancreatic embryogenesis, particularly in determining islet cell differentiation (Figure 1). During adult life, transcription factors regulate the expression of islet cell hormones.[52]

Forkhead box factor *FoxA1* and *FoxA2* expression is directly implicated in endodermal formation, with *FoxA2* deletions resulting in the disruption of endoderm formation in mouse models.[53] Homeobox factor *Pdx-1* is considered the 'master regulator' as it has a significant role in the early development of the pancreas, being expressed in both the endoderm and pancreatic buds.[52,54] *Pdx-1* expression levels are regulated by the interaction between the transcription factors, hepatocyte nuclear factor-3β, hepatocyte nuclear factor-1α and SP1/3, and *Pdx-1* itself.[55] Homozygous *Pdx-1 −/−* mice are apancreatic, and while they survive foetal development, they die a few days following birth.[56,57] This confirms the necessity of embryonic *Pdx-1* expression for successful pancreatic development.

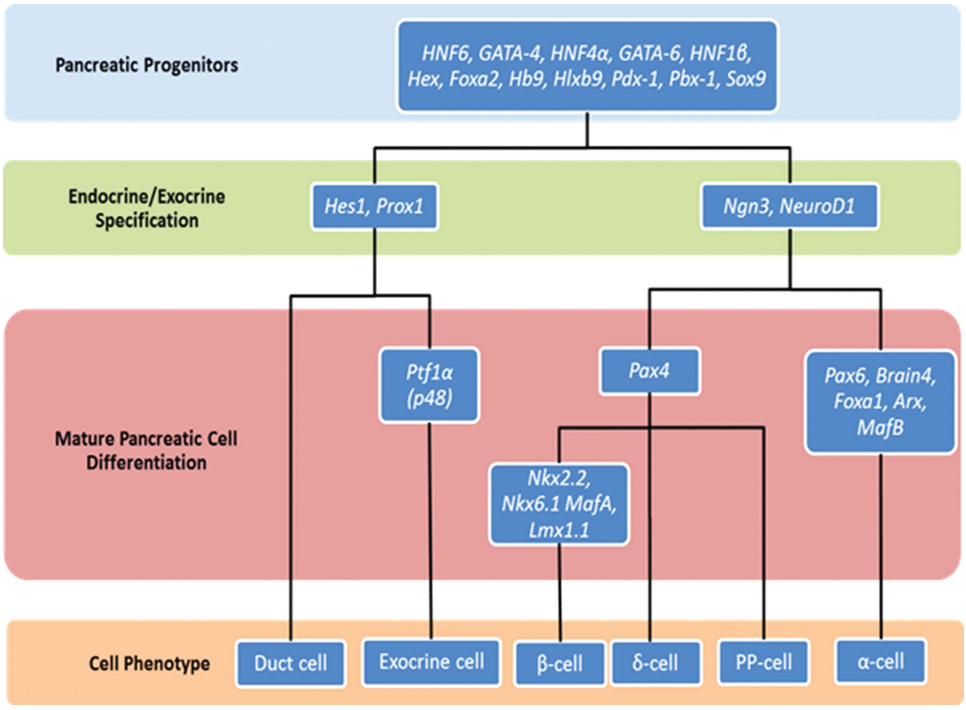

Figure 1. Schematic representation of the transcription factor hierarchy involved in pancreatic development and cellular differentiation. The early endoderm of the gut receives signals from the mesenchyme and notochord to initiate formation of the pancreatic buds, with expression of *FoxA2* and *Hb9* detectable in the pancreatic precursors. *Pdx-1* then drives the subsequent differentiation of the pancreatic precursor cells by interacting with *Pbx-1*. *Neurog3* and *Hes-1* mediate endocrine and exocrine differentiation, respectively, with *NeuroD1* functioning to maintain the endocrine cell fate. *Pax6* and *Pax4* then mediate the differentiation of α and β cells, respectively, with the final differentiation process towards a β-cell phenotype being governed by *Nkx2.2* and *Nkx6.1*.

Following the fusion of the dorsal and ventral buds, the differentiation of exocrine and endocrine pancreatic compartments occurs rapidly, with the basic helix–loop–helix factors, *Hes-1* and *Neurog3*, in the pancreatic precursor directing the respective compartmental fates via notch signalling.[58] The notch signalling pathway has a major role in the control of cell identity, proliferation, differentiation and apoptosis.[59] Subsequent differentiation of the various islet cellvtypes (α, β, γ and pancreatic polypeptide) is directed by the hierarchical expression of various transcription factors.[52] Lineage analyses have shown that all hormone-producing cells express *Neurog3*.[58] In mice, *Neurog3* deletions result in the absence of endocrine cells,[60] testament to its necessity in endocrine cell development. *Neurog3* activates the persistent expression of *NeuroD1* that maintains the endocrine cell differentiation programme.[61] In fact, *NeuroD1* −/− mice show a reduction in the development of insulin-producing β cells.[62]

As soon as the endocrine cell fate has been programmed, the paired homeobox factors, *Pax4* and *Pax6*, direct the fates of individual hormone-producing cells.[52] Studies in mice show that homozygous *Pax4* deficiency results in a lack of β and γ cells,[52,54] and mice that are homozygous *Pax6* deficient lack α cells.[63] Collectively, these studies suggest that *Pax4* is required for determining β- and γ-cell fate, whereas *Pax6* is required for determining α-cell fate.

Differentiation into β cells is ultimately driven by the NK homeobox factors, *Nkx2.2* and *Nkx6.1*. Knocking out the *Nkx6.1* gene in mice results in the absence of β cells;[64] however, all other cell types develop. Interestingly, *Nkx2.2* is expressed in other cell types including α and pancreatic polypeptide cells; however when *Nkx2.2* is knocked out, insulin-producing cells are absent.[65] Consequently, *Nkx2.2* and *Nkx6.1* are imperative in β-cell differentiation. More recently, a leucine zipper transcription factor (*MafA*) has been discovered downstream of *Nkx6.1* in the lineage analysis and was found to be involved in the maintenance of β-cell function, specifically through interactions with *Pdx-1* and *NeuroD1* that modulated insulin transcription.[66] The modification of transcription and subcellular localization of fundamental transcription factors, such as *FoxA2*, *MafA*, *NeuroD1* and *Pdx-1*, will alter important cellular processes such as β-cell differentiation, cell cycle arrest and function, and accordingly represents an interesting potential target for the development of an artificial β cell.[67]

DIRECT TRANSFER OF PANCREATIC TRANSCRIPTION FACTORS

Genetically engineering other cells within the body to produce insulin is an attractive alternative to pancreas and islet transplantation as these cells would not likely express the same autoantigens that caused β-cell destruction in the first instance. Owing to the common origin of the liver and pancreas,[12] the ability to transdifferentiate tissues from the liver to the pancreas has been studied to a greater extent than other tissues types, particularly for the generation of IPCs through the transfer of pancreatic transcription factors.

Pdx-1

Ferber *et al.*[9] demonstrated the potential of transcription factors to be directly delivered to liver tissue, via a recombinant adenovirus-mediated transfer of *Pdx-1*. Considering *Pdx-1* has a significant role in early embryonic development of the pancreas, an attempt to demonstrate the ability of adenovirus-mediated delivery of *Pdx-1* to ameliorate hyperglycaemia by inducing expression of endogenous insulin was performed. Results showed that expression of *Pdx-1* in livers of diabetic mice induced insulin expression and secretion leading to restoration of normoglycaemia. However,

normoglycaemia was transient and only maintained for 8 days. In addition, exocrine differentiation of liver to pancreatic tissue resulted in the development of hepatitis and an increased likelihood of autoimmune destruction.[9,68]

Similarly, Kojima *et al*.[68] used a helper-dependent adenovirus to deliver *Pdx-1* to the livers of STZ diabetic mice. These mice developed hepatitis because of unexpected exocrine differentiation of the transduced cells. This was likely related to the use of a potent ubiquitously expressed elongation factor-1α promoter, which resulted in continuous unrestricted expression of *Pdx-1* at high levels. To date, several studies in which *Pdx-1* has been transferred to hepatocytes to induce the process of transdifferentiation have been performed.[69–73] In addition, direct delivery of *Pdx-1* has been achieved in a variety of other differentiated cell types, including mouse pancreas via the bile duct,[74] rat intestinal epithelium-derived cells (IEC-6)[75] and primary duct cells.[76] One study showed how combinations of pancreatic transcription factors (*Pdx-1*, *Neurog3* and *MafA*) were successful in converting pancreatic exocrine cells *in vivo* to closely resemble β cells,[77] and thus provided evidence for the use of transcription factor combinations. The newly generated IPCs are indistinguishable from normal islets and display all the characteristic hallmarks of normal β cells; however, the limited number of successfully converted exocrine cells and the fact they did not organize themselves into islet structures limited their effectiveness.

Pdx-1 continues to be used as a mediator of IPC production because of its proven ability to induce pancreatic transdifferentiation. The move to stem cells as targets has come as no surprise considering their plasticity and regenerative capabilities. *Pdx-1* has been delivered to MSCs from a variety of sources, including BMSC,[32,78–82] umbilical cord MSC[83] and adipose-derived MSC[84–86] with varying success in the generation of glucose-responsive IPCs. Despite the obvious attraction of MSCs as targets for *Pdx-1* delivery, embryonic stem cells (ESCs) have also been pursued as a potential target. A study by Miyazaki *et al*.[87] showed that a murine ESC line (EB3) could be induced to differentiate into IPCs following transfection with *Pdx-1*; however, transdifferentiation was not substantial enough for therapeutic use owing to a lack of expression of the insulin 1, glucagon, pancreatic polypeptide gene or glucose transporter 2 genes, which are all specific to the endocrine pancreas *in vivo*. This was followed by a number of studies in other ESC lines[88–90] showing their capacity to differentiate into IPCs.

Neurog3

The role of *Neurog3* in defining endocrine cell fate would logically point towards its more frequent use in gene therapy; however this is not the case. Most studies have reported low levels of insulin production after delivery of *Neurog3*.[71,76,91–93] Of the few reported attempts at using *Neurog3* for β-cell engineering, a study using adenoviral transfer of *Neurog3* and betacellulin to hepatic progenitor cells (oval cells) resulted in the production of insulin and transdifferentiation of the oval cell population.[94] However, as mentioned previously, the most successful use of *Neurog3* delivery was observed using a combinatorial approach.[77]

NeuroD1

To prevent *Pdx-1*-induced exocrine differentiation, Kojima *et al*.[68] expressed *NeuroD1* in conjunction with betacellulin within a helper-dependent adenovirus vector to the livers of STZ-treated diabetic mice. It was found that *NeuroD1*-betacellulin delivery restored and maintained normoglycaemia for >120 days, with associated upregulation of the upstream and downstream pancreatic transcription factors, including *Neurog3*, *Pax6*, *Pax4*, *Nkx2.2* and *Nkx6.1*. However, while the use of betacellulin would not likely be acceptable for clinical application, the use of *NeuroD1* resulted in no significant hepatotoxicity or development of hepatitis, marking *NeuroD1* as a worthy alternative transcription factor for directing differentiation to a β-cell-like phenotype. *NeuroD1* is also an ideal alternative as it has been shown to be the strongest inducer of insulin expression (as compared with *Pdx-1*, *Neurog3* and *Pax4*) in primary duct cells.[76] Similar studies showing the ability of *NeuroD1* to induce insulin expression have been performed using hepatocytes.[95]

Promising results using viral delivery of *NeuroD1* to hepatocytes have also been reported by our laboratory. Specifically, a genetically modified rat liver cell line (H4IIE), which does not express β-cell transcription factors, was engineered to express both insulin and *NeuroD1*.[96] The engineered cell line developed storage granules and reversed diabetes in non-obese diabetic/severe-combined immunodeficient mice following transplantation. It also stimulated the expression of the β-cell transcription factors, *Pdx-1*, *NeuroD1*, *Pax6*, *Nkx2.2* and *Nkx6.1*, in addition to rat insulin 1 and 2, glucagon, somatostatin, proconvertase 1 and 2 and pancreatic polypeptide. The engineered cells also regulated secretion of insulin in response to increasing concentrations of glucose. As a result, *NeuroD1* could potentially be used in future gene therapy protocols to induce safe differentiation of target tissues.

Pax4

As mentioned earlier, *Pax4* deficiencies in mice results in the lack of β and γ cells;[52,54] therefore, it can be deduced that *Pax4* is required for determining β-cell fate and could be used for the generation of IPCs. Overexpression of *Pax4* in mouse embryonic stem cells selected for nestin expression has been shown to drive differentiation to a β-cell phenotype,[97] with the resultant IPCs capable of maintaining normal blood glucose levels for 14 days. Similarly, Liew *et al*.[98] reported that overexpression of *Pax4* in human ESCs enhances their propensity to form putative β cells. However, the capacity for teratoma formation of undifferentiated ESCs limits their potential for clinical application.

Nkx6.1

The β-cell-specific expression of *Nkx6.1* theoretically makes it an ideal choice as a factor to drive β-cell differentiation. However, a study by Gefen-Halevi *et al*.[99] showed that ectopic expression of *Nkx6.1* alone, although capable of inducing the expression of immature pancreatic markers, such as *Neurog3* and *Isl-1*, was not capable of inducing expression of pancreatic hormones. In addition, *Nkx6.1* did not appear to be a strong inducer of expression of upper-hierarchy β-cell transcription factors such as *Pdx-1* and *NeuroD1*. In fact, only upon coexpression with ectopic *Pdx-1* was there substantial insulin expression and glucose-regulated processed hormone secretion. Consequently, the inability to induce expression of the full repertoire of β-cell transcription factors makes *Nkx6.1* an inferior choice for β-cell engineering.

CONCLUSIONS AND FUTURE DIRECTIONS

With respect to β-cell replacement strategies, direct delivery of β-cell transcription factors presents an alternative method of achieving a β-cell-like phenotype in autologous tissues. The generation of sufficient quantities of IPCs on a large scale and isolation of pure IPCs is of utmost importance for the success of any bench-to-clinic therapy of T1D. Although work on direct delivery of transcription factors has focused predominantly on hepatocytes as a target in the past decade, the emergence of stem cells and their suitability as a target should be further investigated in the future. Limiting the use of an autologous cell therapy is the considerable effort required to generate a single therapy for individuals. Furthermore, the potential development of a full repertoire of β-cell autoantigens could increase susceptibility of

the grafts to recurrent autoimmunity. It is also clear that the choice of transcription factor for direct delivery has a significant role in determining the success of the cell and gene therapy, as exocrine differentiation and true conversion to a pancreatic phenotype are potential deleterious outcomes. Ideally, an allogeneic cell therapy that is capable of circumventing the autoimmune response would overcome these limitations.

CONFLICT OF INTEREST

AMS is an inventor on patent 'Genetically modified cells and uses thereof', EP20080782908, AU 2008/001160 and US12/672 832. The other authors declare no conflict of interest.

ACKNOWLEDGEMENTS

DG is supported by an Australian Postgraduate Award and the Arrow Bone Marrow Transplant Foundation/Hawkesbury Canoe Classic Scholarship. Research conducted by BO'B, AMS and DG was supported by National Health and Medical Research Council of Australia Project Grants (352909 and 513100), project grants from Diabetes Australia Research Trust and Rebecca L Cooper Medical Research Foundation. We thank Richard Limburg for IT support.

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