

Recent advances in stem cell research for the treatment of diabetes

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Abstract

The success achieved over the last decade with islet transplantation has intensified interest in treating diabetes, not only by cell transplantation, but also by stem cells. The formation of insulin-producing cells from pancreatic duct, acinar, and liver cells is an active area of investigation. Protocols for the *in vitro* differentiation of embryonic stem (ES) cells based on normal developmental processes, have generated insulin-producing cells, though at low efficiency and without full responsiveness to extracellular levels of glucose. Induced pluripotent stem cells, which have been generated from somatic cells by introducing Oct3/4, Sox2, Klf4, and c-Myc, and which are similar to ES cells in morphology, gene expression, epigenetic status and differentiation, can also differentiate into insulin-producing cells. Overexpression of embryonic transcription factors in stem cells could efficiently induce their differentiation into insulin-expressing cells. The purpose of this review is to demonstrate recent progress in the research for new sources of β -cells, and to discuss strategies for the treatment of diabetes.

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INTRODUCTION

The pancreas is a mixed exocrine and endocrine gland that controls many homeostatic functions. The exocrine pancreas consists of acinar cells and the ductal epithelium, while the endocrine pancreas consists of four cell types (α -, β -, δ -, and pancreatic-polypeptide cells). The pancreas controls body metabolism, including the digestion of foods by exocrine enzymes secreted from acinar cells and the regulation of blood glucose levels by insulin secreted from β -cells. Clinical studies have shown that transplantation of a pancreas or purified pancreatic islets can support glucose homeostasis in type 1 diabetic individuals, in whom the β -cells have been destroyed by an autoimmune reaction^[1-5]. Islet transplantation carries the special advantages of being less invasive and resulting in fewer complications compared with the traditional pancreas or pancreas-kidney transplantation. However, islet transplantation efforts have limitations including the short supply of donor pancreata, the paucity of experienced islet isolation teams, side effects of immunosuppressants and poor long-term results^[6]. These limitations have led to the search for other stem/progenitor cell sources of β -cells and intense interest in how the differentiation of such progenitors can be directed, or “programmed”, efficiently.

The programming efforts are based on understanding how β -cells are normally generated in the embryo and how they arise during regeneration in adults, in response to tissue damage and disease. Here, we review recent studies on β -cell development and regeneration, and highlight unresolved issues in the field.

PANCREATIC β -CELL DEVELOPMENT

The pancreas is specified from the endoderm germ layer and develops from a dorsal and ventral protrusion of the primitive gut epithelium^[7-9]. These two pancreatic buds grow, branch, and fuse to form the definitive pancreas. Initially, broad suppression of mesodermal Wnt and fibroblast growth factor (FGF) signaling in the foregut enables pancreas induction, whereas active mesodermal Wnt signaling in the posterior gut suppresses these tissue fates^[10,11]. Retinoic acid signaling, apparently from paraxial mesoderm cells, helps to further refine the anterior-posterior position, in which the liver and pancreas can develop from the gut endoderm^[12-15]. Subsequently, in the ventral foregut, FGF from the cardiac mesoderm and bone morphogenetic protein/transforming growth factor- β (BMP/TGF- β) from septum transversum mesenchyme cells coordinately induce the liver program and suppress the pancreas program^[16-19]. In the dorsal foregut, signals from the notochord, including activin and FGF, suppress sonic hedgehog (shh) signaling within the endoderm and allow the pancreatic program^[20,21].

The newly specified pancreatic endoderm is initially marked by the expression of the pancreatic and duodenal homeobox gene 1 (Pdx1; also known as Ipfl1) and then by the pancreas specific transcription factor 1a (Ptf1a)^[22,23]. Both proteins are crucial for pancreatic development. Pdx1 marks all pancreatic and midgut progenitors^[22] and is crucial for development after the bud stage^[24,25]. Pdx1 levels also help to control the balance between the endocrine and exocrine (acinar and duct) progenitors that differentiate within the pancreas^[26]. Notch signaling also helps to regulate the balance of exocrine and endocrine cells, probably by allowing the expansion of an undifferentiated pancreatic-progenitor population^[27-29]. Loss of Notch signaling allows the endocrine lineage to develop, which is marked by and requires the basic helix-loop-helix (bHLH) transcription factor, neurogenin 3 (Ngn3)^[22,27,30]. Ngn3 directly influences the expression of another islet specific bHLH gene, neurogenic differentiation (NeuroD; also known as BETA2)^[31]. A loss of function assay of NeuroD/BETA2 implicates a phenotype similar to, but less severe than, Ngn3, leading to a diminished number of all endocrine cell types^[32]. Then, definitive β cells are generated under the influence of the v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MafA) transcription factor^[33,34].

EMBRYONIC STEM (ES) CELLS

ES cells, which are pluripotent diploid cells and can be induced to differentiate into cells of all three germ layers both *in vivo* and *in vitro*^[35,36], are a potentially abundant

source of β -cells. It has been reported that ES cells from mouse^[37-40], monkey^[41], and human^[38,42] were able to differentiate into insulin-positive cells, a potential source of new β -cells. Numerous groups have been developing ES cell differentiation protocols that attempt to mimic normal embryonic development. The first step of pancreatic development is the induction of a definitive endoderm using high concentrations of activin A treatment^[43,44]. Further treatment in sequential stages with keratinocyte growth factor (KGF), retinoic acid, Noggin, and cyclopamine (the hedgehog-signaling inhibitor) can then direct definitive endoderm toward Pdx1-expressing posterior foregut endoderm cells^[45,46]. Treatment with DAPT and exendin-4 recruited the Pdx1-expressing posterior foregut endoderm cells to the pancreatic and endocrine lineages, which expressed Pdx1, Nkx6-1, Nkx2-2, Ngn3, and/or Pax4. After treatment with exendin-4, IGF-1, and HGF, endocrine cells expressing the pancreatic hormones insulin, glucagon, somatostatin, pancreatic polypeptide and ghrelin are produced (Figure 1).

Melton's group recently reported small molecules that efficiently direct endodermal differentiation of mouse and human embryonic stem cells^[47]. In a screen of 4000 compounds, they identified two cell-permeable small molecules that direct differentiation of ES cells into the endodermal lineage. The efficiency of differentiation into definitive endoderm using these compounds was higher than that achieved by Activin A or Nodal, which commonly used protein inducers of endoderm. The definitive endoderm induced by these compounds was able to participate in normal development when injected into developing embryos, and was able to form pancreatic progenitors. These small molecules could induce reproducible and efficient differentiation of ES cells into endoderm.

On the other hand, a significant number of problems remain unsolved in terms of clinical application of ES cells, such as the risk of tumorigenicity and immunosuppression after transplantation. The ethical issue is another major obstacle to the clinical use of ES cells.

INDUCED PLURIPOTENT STEM (iPS) CELLS

iPS cells are also pluripotent diploid cells that can be induced to differentiate into cells of all three germ layers both *in vivo* and *in vitro*. Moreover, iPS cells have fewer ethical issues compared with ES cells, because iPS cells can be established from somatic cells. Initial iPS cells have been generated from mouse and human somatic cells by introducing Oct3/4 and Sox2 with either Klf4 and c-Myc or Nanog and Lin28, using retroviruses^[48-51]. Recently, it has been reported that valproic acid (VPA), a histone deacetylase inhibitor, enables reprogramming of primary human fibroblasts with only two factors, Oct4 and Sox2, without the need for the c-Myc or Klf4^[52]. The results support the possibility of reprogramming by chemical means, which would make therapeutic use of reprogrammed cells safer and more practical. Another

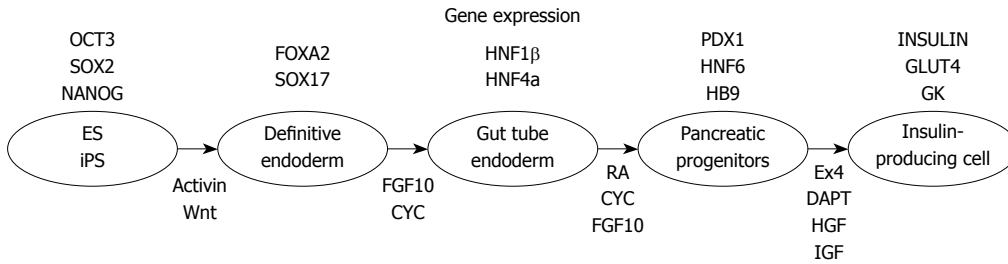


Figure 1 Schematic representation of the stepwise differentiation of embryonic stem (ES) and induced pluripotent stem (iPS) cells to insulin-producing cells. CYC: KAAD-cyclopamine; RA: All-trans retinoic acid; DAPT: γ -secretase inhibitor; Ex4: Exendin-4; FGF: Fibroblast growth factor.

group showed that adult mouse neural stem cells (NSCs) expressed higher endogenous levels of Sox2 and c-Myc than embryonic stem cells^[53] and that exogenous expression of the germline-specific transcription factor Oct4 was sufficient to generate pluripotent stem cells from adult mouse NSCs^[54]. These data suggest that, in inducing pluripotency, the number of reprogramming factors can be reduced when using somatic cells that endogenously express appropriate levels of complementing factors.

On the other hand, retroviral integration of the transcription factors might activate or inactivate host genes, resulting in tumorigenicity, as was the case in some patients who underwent gene therapy. Yamanaka's group reported the generation of mouse iPS cells with transient expressions of Oct3/4, Sox2, Klf4, and c-Myc from plasmids. Repeated transfection into mouse embryonic fibroblasts of two expression plasmids, one containing complementary DNAs (cDNAs) for Oct3/4, Sox2, and Klf4 and the other containing the c-Myc cDNA, resulted in iPS cells without evidence of plasmid integration^[55]. At the same time, another group demonstrated the generation of mouse iPS cells from fibroblasts and liver cells using non-integrating adenoviruses transiently expressing Oct4, Sox2, Klf4, and c-Myc^[56]. Moreover, Ding's group reported generation of protein-induced pluripotent stem cells (piPSCs) from murine embryonic fibroblasts using recombinant cell-penetrating reprogramming proteins without transfection of any genes^[57]. For efficient transduction of four reprogramming factors, Oct4, Sox2, Klf4, and c-Myc, into cells, they used protein transduction technology^[58-61]. A poly-arginine (11R) protein transduction domain (PTD) fused to the C terminus of these reprogramming factors efficiently delivered the proteins into cells and induced iPS cells, which demonstrated long-term self-renewal and were pluripotent *in vitro* and *in vivo*. These reports provide strong evidence that insertional mutagenesis is not required for *in vitro* reprogramming. The production of iPS cells without integration into the host genome addresses a critical safety concern for potential use of iPS cells in regenerative medicine.

Although some papers have shown the generation of insulin-secreting islet-like clusters from human iPS cells^[62,63], the efficiency of the method seems low. The method, as detailed in this review in the ES cells section,

might represent a critical step in the development of insulin-producing cells from iPS cells (Figure 1). Indeed, Melton's group recently reported generation of iPS cells from patients with type 1 diabetes and differentiation from the iPS cells into insulin-producing cells using this method^[64].

PANCREATIC STEM/PROGENITOR CELLS

Although it is clear that the majority of new β -cells derive from pre-existing insulin-expressing cells after surgical injury^[65,66], several *in vitro* studies have shown that insulin-producing cells can be generated from adult pancreatic ductal tissues^[67-71]. A recent study has shown that duct ligation can activate Ngn3-positive β -cell precursors in the ductal epithelium^[72]. The Edmonton group has shown that, in clinical islet transplantation, a significant positive correlation exists between the number of ductal-epithelial cells transplanted and long-term metabolic success, as assessed by an intravenous glucose tolerance test at approximately two years post-transplantation. No significant correlation was observed between the total islet equivalents and long-term metabolic success^[73]. Cells in the pancreatic anlage migrate from the ducts while differentiating to form clusters that will eventually become islets during embryonic development^[74]; therefore, the post-natal pancreatic duct might harbor islet precursor/stem cells. Inada *et al*^[75] generated transgenic mice expressing human carbonic anhydrase II (CA II) promoter-Cre recombinase or inducible CreER to cross with ROSA26 loxP-Stop-loxP LacZ reporter mice. CA II-expressing cells within the pancreas act as progenitors that give rise to both new islets and acini normally after birth and after injury (ductal ligation). This identification of a differentiated pancreatic cell type as an *in vivo* progenitor of all differentiated pancreatic cell types has implications for a potential expandable source of new islets for replenishment therapy for diabetes^[75]. Such interesting results suggest the possibility of multipotent progenitors in adult pancreatic ducts.

Mouse pancreatic stem cells have been isolated from duct-rich population, which are capable of self-renewal and multipotency^[76,77]. On the other hand, human cells from the duct-rich population were unable to divide after 30 d under several culture conditions, although the cells were able to differentiate into insulin-producing cells^[78]. There are some differences between the methodologies

used by the two groups, such as culture conditions, isolation stresses, and/or species themselves. The ability of β -cells to expand is limited, especially in the adult, and the partial growth ability is insufficient to permit recovery from cell loss in type 1 diabetes^[79]. Therefore, it is important to isolate human pancreatic “stem” cells comprising a sufficient number of β -cells for the treatment of diabetes.

The transdifferentiation of acinar cells to islets has also been proposed^[80-82]. Melton’s group showed *in vivo* reprogramming of adult pancreatic exocrine cells to β -cells by viral delivery of the developmental transcription factors Pdx1, Ngn3, and MafA^[83]. Pancreatic exocrine cells greatly outnumber β -cells; therefore, the transdifferentiation of acinar cells to β -cells is also an interesting possibility.

MESENCHYMAL STEM CELLS

Another interesting stem cell in this field is the mesenchymal stem cell (MSC). It has been reported that marginal mass islet transplantation with autologous MSCs promotes long-term islet allograft survival and sustained normoglycemia^[84]. MSCs also prevent the rejection of fully allogeneic islet grafts by the immunosuppressive activity of matrix metalloproteinase-2 and -9^[85], and protect NOD mice from diabetes by inducing regulatory T cells^[86].

PERSPECTIVES

Several reports have suggested that epitopic transdifferentiation is also possible. *In vivo* transduction of mice with an adenovirus expressing Pdx-1^[87,88], and both betacellulin and NeuroD^[89], or a modified form of Pdx-1 carrying the VP16 transcriptional activation domain^[90], or MafA together with Pdx-1 and NeuroD^[91], markedly increased insulin biosynthesis and induced various pancreas-related factors in the liver. The existence of potential β -cell precursors in the adult liver is of obvious medical interest. Moreover, overexpression of embryonic transcription factors in stem cells could efficiently induce their differentiation into insulin-expressing cells. We reported that transduction of Pdx-1 and NeuroD proteins induces insulin gene expression^[67,92,93]. Other groups also showed that transduction of NeuroD *in vivo* or TAT-Ngn3 fused TAT-PTD induced insulin-producing cells^[94,95]. The production of insulin-producing cells using protein transduction technology without gene transduction addresses a critical safety concern for potential use of the cells in regenerative medicine. Further investigations to induce differentiation of stem/progenitor cells into insulin-producing cells will help to establish cell-based therapies in diabetes.

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