Stem cell therapy for type 1 diabetes mellitus

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Abstract | The use of stem cells in regenerative medicine holds great promise for the cure of many diseases, including type 1 diabetes mellitus (T1DM). Any potential stem-cell-based cure for T1DM should address the need for β-cell replacement, as well as control of the autoimmune response to cells which express insulin. The *ex vivo* generation of β cells suitable for transplantation to reconstitute a functional β-cell mass has used pluripotent cells from diverse sources, as well as organ-specific facultative progenitor cells from the liver and the pancreas. The most effective protocols to date have produced cells that express insulin and have molecular characteristics that closely resemble *bona fide* insulin-secreting cells; however, these cells are often unresponsive to glucose, a characteristic that should be addressed in future protocols. The use of mesenchymal stromal cells or umbilical cord blood to modulate the immune response is already in clinical trials; however, definitive results are still pending. This Review focuses on current strategies to obtain cells which express insulin from different progenitor sources and highlights the main pathways and genes involved, as well as the different approaches for the modulation of the immune response in patients with T1DM.

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Introduction

Type 1 diabetes mellitus (T1DM) is characterized by the autoimmune destruction of the insulin-expressing, pancreatic β cells; as a result, patients with T1DM are dependent on exogenous insulin for their blood glucose control. Although the discovery of insulin has changed the outlook and survival of this group of individuals for almost a century,[1] patients with T1DM are still not exempt from the development of diabetic complications.[2,3] These complications are thought to result, in part, from the lack of physiological oscillations in insulin secretion and higher than normal glucose levels; thus, the search for effective ways to reestablish a functional β-cell mass in patients with T1DM is important. Transplantation of whole human pancreas or isolated, cadaveric human islets[4,5] has allowed patients with T1DM to become insulin-independent and has thereby shown proof of concept for β-cell replacement therapies. The shortage of donor pancreata and islets,[6] however, results in the search for alternative cell sources, and differentiation of stem cells (Box 1) into functional β cells is an attractive alternative.

A definitive cure for T1DM needs to address both the β-cell deficit and the autoimmune response to cells that express insulin. As clearly shown by the transplantation of segmental pancreatic grafts from identical twins, the autoimmune response remains many years after disease onset.[7] Thus, any β-cell replacement therapy will need some modulation of the immune system, whether by drugs or cell-based approaches.

This Review focuses mainly on the different approaches to obtain functional, insulin-producing cells from various types of progenitor cells. Although the goal of the field is to direct differentiation of human stem cells into functional β cells, some animal studies are included here, as they could lay the framework for successful future interventions in humans. Although stem cell-derived, insulin-positive cells have not yet reached clinical trial, clinical data from stem cell-derived therapies that target the autoimmune response have been reported and are discussed.

Competing interests
The authors declare no competing interests.

Many roads lead from stem cells to β cells

Efforts have been directed at the development of efficient protocols for the differentiation of stem cells of either embryonic (Figure 1) or adult origin (Figure 2) into functional cells that secrete insulin. A caveat to many of these studies is that some developmental pathways generate insulin-expressing cells that will never become true β cells, for example, cells of the fetal liver, yolk sac and brain.[8–10] A β cell should be defined by its ability to store large amounts of insulin and to secrete it in a regulated manner in response to a demand, such as glucose stimulation.[11]

Human embryonic stem cells

Efforts to generate β cells from human embryonic stem cells (hESCs) have focused on the fundamentals of normal embryonic development as a basis to understand the early stages of endoderm formation. The first step in the differentiation of hESCs into β cells is the production of definitive endoderm[12–17] rather than visceral endoderm, which expresses the same markers (for example, sex determining region Y box [SOX] 17 and forkhead box protein A2 [FOXA2]) but forms different tissues.[18]

Current protocols for hESC differentiation into β cells aim to reproduce the developmentally active signaling pathways Wnt and transforming growth factor β (TGFβ) (Box 2).[17,19–22] Growth factors, such as Activin A, fibroblast

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Key points

- A definitive cure for type 1 diabetes mellitus (T1DM) will address both the β-cell deficit and the autoimmune response to cells that express insulin
- Cells that express insulin have been obtained through differentiation of stem cells of either embryonic or adult origin, as well as genetically reprogrammed and transdifferentiated cells
- The most effective differentiation protocols for the derivation of cells that express insulin recapitulate normal embryonic development
- Knowledge of the transcription factors that regulate development of the embryonic pancreas has aided the evaluation of different strategies used to obtain β cells
- Clinical trials have used bone marrow-derived mesenchymal stromal cells and umbilical cord blood cells to suppress the immune response in patients with T1DM
- Even though challenges remain, the possibility of an effective stem-cell therapy for T1DM is a realistic goal for the foreseeable future

Box 1 | Definition of stem cell concepts

Stem cell
Unspecialized cells that are capable of renewing themselves through cell division but can be induced to become tissue-specific or organ-specific specialized cells under certain physiologic or experimental conditions.

Progenitor cell
Often used synonymously with precursor cell, this term indicates a cell that gives rise to another more differentiated cell type. These cells have limited self-renewal capacity and can give rise to several, but not all, cell types.

Pluripotency
The ability of a stem cell to differentiate into any of the three germ layers (endoderm, mesoderm or ectoderm) and to give rise to any fetal or adult cell type.

Multipotency
The ability of a progenitor cell to give rise to cells of multiple but limited cell types.

Clonogenic potential
The capacity of a single stem cell to give rise to a colony of genetically identical cells, or clones, that have the same properties as the original cell.

Transdifferentiation
The direct conversion of one mature cell phenotype to another.

Dedifferentiation
The conversion of a fully differentiated cell to a progenitor-like phenotype before potential redifferentiation to a different cell type.

growth factor (FGF) 10 and retinoic acid, have been used to drive the differentiation of hESCs into cells which express the transcription factor PDX1 (Box 3). Other markers used to identify definitive endoderm include SOX17, brachyury protein, FOXA2, CXC-chemokine receptor (CXCR) 4 and cerberus.

Efforts have been directed at the identification of small molecules to control hESC differentiation by the modulation of signal transduction pathways, gene expression or metabolism. Two molecules—induce definitive endoderm (IDE) 1 and 2—have been identified that successfully induce the formation of definitive endoderm from mouse and human ESCs with a 70–80% efficiency, which is higher than the differentiation induced by Activin A or Nodal.[23] With these small molecules, large amounts of endoderm were formed that expressed multiple endodermal markers. Ideally, to direct differentiation, small-molecule inducers would be less expensive, more easily controlled and more efficient than growth factors.

The next step, *in vitro*, is to reproduce the formation of the pancreatic dorsal anlage that depends on simultaneous retinoic acid signaling and inhibition of Hedgehog signaling, both of which have been effectively reproduced.[24,25] Endoderm cells induced by IDE1 and IDE2 were able to develop into pancreatic progenitors *in vitro* in response to FGF10, retinoic acid and inhibitors of Hedgehog signaling. Moreover, the application of the small molecule indolactam V to endoderm cells induced pancreatic progenitor cells that express PDX1 at >45% efficiency.[26] Indolactam V activates protein kinase C signaling, the same pathway activated after treatment with WNT3a, Activin A, FGF10, cyclopamine and retinoic acid.

The production of cells capable of synthesizing insulin, glucagon, somatostatin, pancreatic polypeptide and ghrelin has been achieved.[27,28] A protocol that used serum-free culture medium without a feeder layer of stromal cells to provide growth factors[29] yielded 2–8% of cells positive for C-peptide that when transplanted into streptozotocin-induced diabetic mice[30] prolonged survival of the recipients. Although the insulin content of these hESC-derived cells is similar to that of human islets, these cells lack the key function of glucose-stimulated insulin secretion *in vitro*. Lack of responsiveness to glucose is one of the main limitations of current *in vitro* differentiation protocols of hESC-derived β cells and is reminiscent of fetal and neonatal β cells, which also express insulin but are unresponsive to glucose. Nevertheless, hESCs have been shown to secrete insulin in response to glucose several months after transplantation into immunodeficient mice[27]—an exciting finding for the field. Additional studies have also shown the need of *in vivo* differentiation to derive functionally mature β cells from hESCs, which suggests that the presence of *in vivo* factors is important for the final stages of maturation.[31,32] To determine such factors should lead to effective *in vitro* differentiation protocols that could result in the production of sufficient amounts of functional β cells.

Induced pluripotent stem cells

Induced pluripotent stem (iPS) cells have become an exciting potential alternative to hESCs,[33,34] the use of which is still restricted in many countries in both research and clinical settings. The use of defined factors has made it possible to derive pluripotent cells from human somatic cells. iPS cells have been generated from mouse and human somatic cells by introducing POU domain class 5 transcription factor 1 (also known as Octamer-binding transcription factor [OCT] 4), and SOX2 with combinations of Kruppel-like factor 4 (KLF4), Myc proto-oncogene protein (c-MYC), NANOG and lin-28 homolog A (LIN28). The use of the oncogenes c-MYC and KLF4 raised concerns about potential tumor formation by iPS cells. This concern has been addressed by the use of valproic acid, a histone deacetylase inhibitor, which enables reprogramming of primary human fibroblasts with only

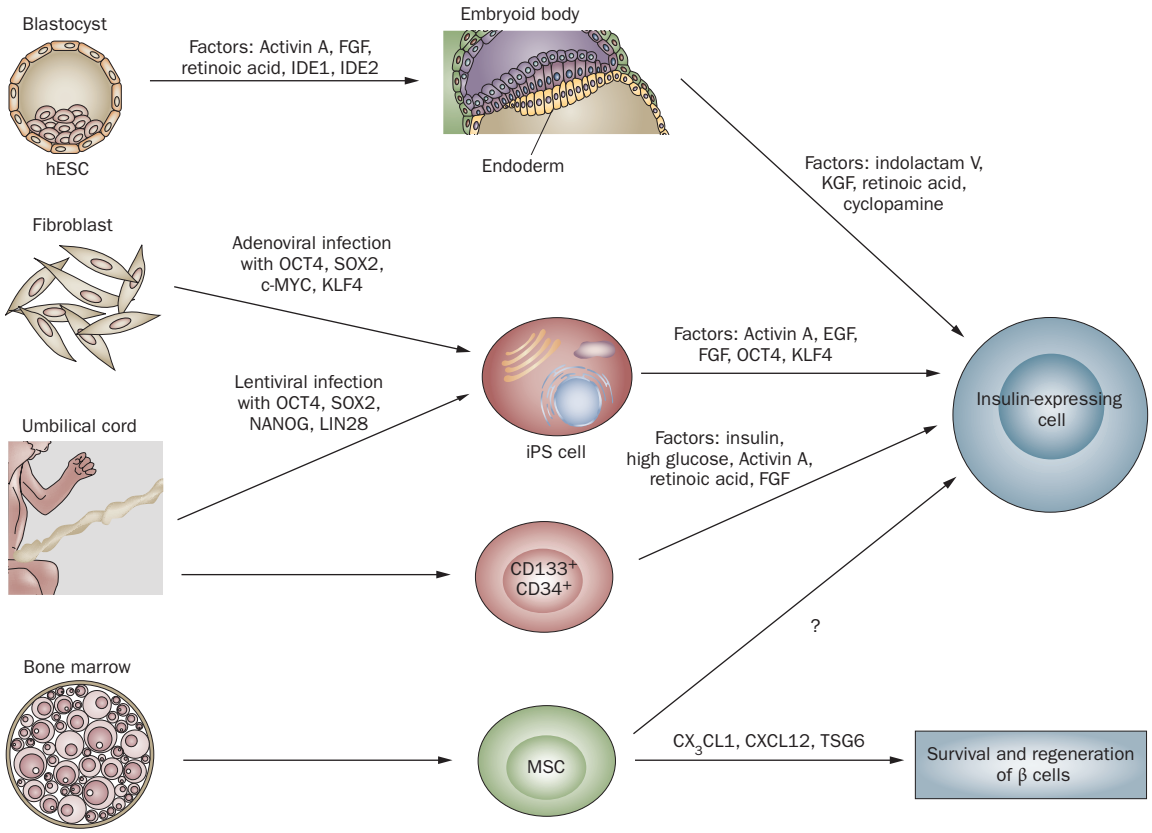

Figure 1 | Generation of insulin-expressing cells from pluripotent cells. Different differentiation protocols recapitulate the embryological development of β cells. Although robust insulin secretion in response to glucose has only been shown in hESC-derived insulin-positive cells, reprogramming of fibroblasts and umbilical cord cells into iPS cells could potentially represent an approach to obtain a renewable and available source of undifferentiated cells that can provide reliable models of disease *in vitro* and might eventually lead to a cell-derived therapy for T1DM. The generation of insulin-expressing cells from bone marrow-derived MSCs remains to be confirmed; however, these cells promote survival and regeneration of β cells. Abbreviations: c-MYC, Myc proto-oncogene protein; CX_3CL1, CX_3C-chemokine ligand 1; CXCL12, CXC-chemokine ligand 12; EGF, epidermal growth factor; FGF, fibroblast growth factor; hESC, human embryonic stem cell; IDE, induce definitive endoderm; iPS, induced pluripotent stem cells; KGF, keratinocyte growth factor; KLF4, Kruppel-like factor 4; LIN28, lin-28 homolog A; MSC, mesenchymal stromal cell; OCT4, Octamer-binding transcription factor 4; SOX2, transcription factor sex determining region Y box; T1DM, type 1 diabetes mellitus; TSG6, tumor necrosis factor-inducible gene 6 protein.

two factors, OCT4 and SOX2, thus making therapeutic use of reprogrammed cells potentially safer and more practical.[35] The initial use of retroviruses or lentiviruses to deliver the transcription factor genes raised concerns about viral integration into the host genome increasing tumorigenicity risk. Novel protocols use repeated transfection of expression plasmids that resulted in iPS cells without evidence of plasmid integration.[36] Nevertheless, although the protocols for this reprogramming are evolving rapidly and no longer require the use of oncogenes and viral vectors, whether iPS cells are truly equivalent to hESCs with respect to pluripotency is not yet fully elucidated.

iPS cells have also been generated from umbilical cord blood by lentiviral overexpression of the reprogramming factors OCT4, SOX2, NANOG and LIN28.[37,38] The efficiency of reprogramming was similar to that of keratinocytes and fibroblasts. Since umbilical cord blood is a juvenescent cell source, its use addresses the concerns that arise with the use of adult somatic cells, which can accumulate mutations over the lifetime of an organism.

The first studies to use iPS cells for the generation of insulin-positive cells are only just emerging. In one study hESC-like iPS cells, derived from skin cells by retroviral expression of OCT4, SOX2, c-MYC and KLF4, underwent a serum-free *in vitro* culture protocol that resulted in islet-like cell clusters that expressed insulin.[39] Two of the four cell lines derived in this study could differentiate, one of which released low amounts of C-peptide (0.15 ng/μg DNA) in response to 40 mM glucose. Although the researchers verified that the rate of apoptosis was not elevated at this extremely high glucose level, no osmotic controls were presented and the C-peptide levels were extremely low (equivalent of 2–3 pg C-peptide per 100 islets). The investigators' conclusion that these cells were glucose-responsive, therefore, is still questionable. In a different study,[40] iPS cells derived from skin biopsies of patients with T1DM were differentiated into cells which express insulin, C-peptide, glucagon and somatostatin. Three transcription factors (OCT4, SOX2 and KLF4) were used to reprogram these adult fibroblasts into iPS

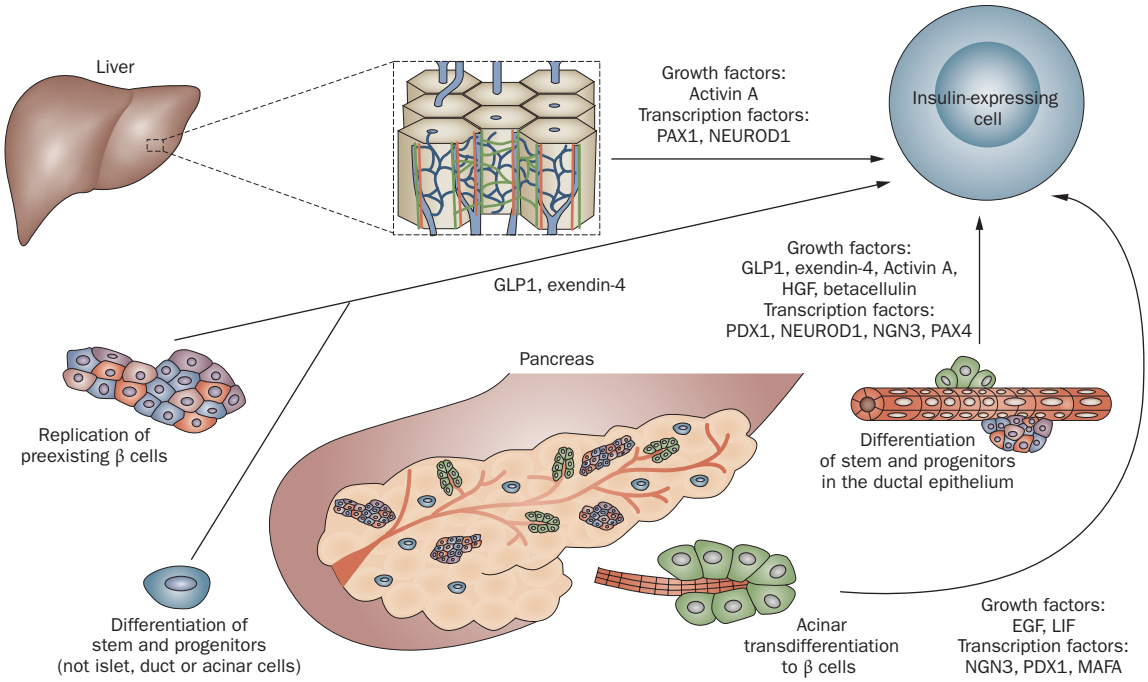

Figure 2 | Strategies to obtain β cells from organ-specific stem or progenitor cells. The pancreas itself can be a source of new insulin-expressing β cells either via replication of pre-existing β cells, transdifferentiation of acinar tissue into β cells, differentiation of progenitors within the ductal epithelium or differentiation of pancreatic stem or progenitor cells. The liver represents another source of embryologically related tissue that has been transdifferentiated into insulin-expressing cells. Abbreviations: EGF, epidermal growth factor; GLP1, glucagon-like peptide 1; HGF, hepatocyte growth factor; hESC, human embryonic stem cell; iPS, induced pluripotent stem cells; LIF, leukemia inhibitory factor; NEUROD1, neurogenic differentiation factor 1; NGN3, Neurogenin-3; MAFA, transcription factor MafA; PAX4, paired box protein; PDX1, pancreas and duodenum homeobox protein 1. Permission obtained from Macmillan Publishers Ltd © Bonner-Weir, S. & Weir, G. C. *Nature Biotechnol.* **23**, 857–861 (2005).

cells. These were then subjected to a directed differentiation protocol to produce cells that released human C-peptide and showed a fivefold increase in secretion of C-peptide in response to 20 mM glucose, which suggests that functional β cells can eventually be derived from iPS cells.

Nevertheless, iPS cells, if generated from patients with T1DM and transplanted back into the donor, would still be targeted by the immune system. Particular interest, therefore, lies in the generation of both immune cells and β cells from the same iPS cell to broaden our understanding of the autoimmune destruction of β cells.

Umbilical cord blood

In addition to its use for generating iPS cells, umbilical cords represent a potential and readily available source of mesenchymal stromal cells (MSCs) and blood stem cells that have been explored both as modulators of the immune response in T1DM and as potential sources of insulin-positive cells. Islet-like clusters derived from MSCs in the human umbilical cord released very low amounts of insulin *in vitro*.[41] The more selective approach of flow cytometry showed that cells from umbilical cord blood that express the human hematopoietic precursor markers CD133 and CD34 rapidly differentiated into cells that were positive for C-peptide and insulin by immunohistochemistry; however, insulin content and insulin secretion were not evaluated.[42] Our previous caveat about what defines a β cell must be remembered when interpreting these studies.

Bone marrow-derived mesenchymal stromal cells

Bone marrow-derived cells, including MSCs, have been shown to differentiate into various lineages and are readily available, which makes them an attractive source for regenerative cell-replacement strategies for T1DM. Transplantation of bone marrow-derived cells increased levels of serum insulin and reduced blood glucose levels in hyperglycemic mice with streptozotocin-induced pancreatic tissue damage, which improved the metabolic state and survival of recipients.[43] A careful histological analysis of the pancreas of these animals suggested that cells transplanted from the bone marrow homed to the site of pancreatic injury and promoted the formation of insulin-positive cells of recipient origin, probably by secreting products of the endothelial precursor cells. By contrast, several reports suggest that bone marrow-derived cells themselves differentiate into insulin-positive cells,[44,45] but this cell lineage switch remains to be confirmed.

Other studies, which used rat MSCs[46] or multipotent stromal cells from human bone marrow,[47] have shown similar results of enhanced insulin secretion and repair of

pancreatic islets after streptozotocin treatment in rodents. In the latter model, up to 3% of the infused human MSCs engrafted into the pancreas, and up to 11% of the infused cells engrafted into the kidney. This finding indicates that the highest levels of engraftment were seen in the two organs damaged in the diabetic rodent model, whereas no cells were detected in lung, liver or spleen. This selectivity of bone marrow-derived MSCs for damaged tissues could be partly explained by the release of factors from pancreatic islets, which promote migration of MSCs by a process mediated by CX_3C-chemokine ligand 1 and CXC-chemokine ligand 12.[48] In turn, cytokines secreted from MSCs are thought to create a tissue microenvironment that promotes β-cell activation and survival.

Preliminary data suggest that this approach might also be useful for the treatment of T2DM.[49] Patients with T2DM received an intrapancreatic autologous bone marrow stem cell infusion; after 1 year, these patients showed improved metabolic control and reduced insulin requirements. Although this study provides a proof of principle regarding the capacity of MSCs to improve glycemic control, its application as a standard T2DM therapy seems unlikely owing to the costs and risks involved in this procedure.

Engraftment, however, might not be necessary for human MSCs to enhance tissue repair as shown by a thought-provoking study,[50] in which intravenous MSCs improved myocardial infarction in mice even though cells embolized in the lung. The embolized cells were activated and secreted the anti-inflammatory protein Tumor necrosis factor-inducible gene 6 protein (TSG6), which mediates anti-inflammatory effects through the inhibition of neutrophil migration.[51–54]

Organ-specific stem or progenitor cells

The advantage of organ-specific stem or progenitor cells (Figure 2) is their consignment to a specific differentiation pathway, which, hypothetically, would require less *in vitro* manipulation to obtain fully functional β cells than the use of less committed pluripotent stem cells. Putative pancreatic stem cells with clonogenic potential and multipotency,[55] have been isolated from adult rodent pancreas. Cells derived from these precursor cells have been shown to express low amounts of insulin mRNA *in vitro*;[56–58] however, their contribution to the β-cell mass has not yet been established. Cells that resemble the oval cells of the liver have been reported in the pancreatic ducts, but their true identity and function has yet to be clarified.[59] The identification and study of tissue progenitor cells has been facilitated by the generation of transgenic mouse models such as the mouse telomerase reverse transcriptase (mTert)–green fluorescent protein (GFP) mouse.[60] GFP-positive cells which expressed mTert—a ribonucleoprotein complex that catalyzes the extension of chromosome ends—were found in the nonislet pancreatic tissue after treatment for 3 days with the glucagon-like peptide 1 (GLP1) agonist exendin-4.[61] This finding suggests the presence of activated stem cells. With the emerging complexity of multiple hierarchies of stem cells in liver and intestine,[62,63] the possibility of similar, rare populations of true stem cells residing within the pancreas must be considered.

Box 2 | Pathways involved in stem cell to β cell differentiation

- TGFβ signal transduction has been implicated in the induction of definitive endoderm, a process that proceeds by first establishing common mesendoderm progenitor cells.[119–122] Both Nodal and Activin A are TGFβ superfamily members and are essential for endoderm formation.[123]
- Wnt signaling is positively regulated by Activin A[124] and has been suggested to participate in pancreas development, given the expression of several Wnt genes, receptors and antagonists in the developing adult pancreas.[125,126] *In vitro,* Wnts influence endoderm formation[17] and have been effectively activated in current protocols to obtain β cells from hESC.
- Hedgehog signals are inhibitory for the early stages of pancreas formation from the gut endoderm.[127,128] Cyclopamine, a plant alkaloid that potently inhibits Hedgehog signaling, is used in current β-cell differentiation protocols to promote pancreatic development.[128]
- Notch signaling should be inactivated after the initial steps of differentiation, since its inhibition accelerates endocrine differentiation, while constitutively active Notch signaling represses the development of both endocrine and exocrine tissue.[129–132]

Box 3 | Transcription factors required to generate insulin-secreting cells

Although the transcription factors expressed during embryonic development of the pancreas[133] are many, the following factors are indispensable ones, without which cells are unable to produce and secrete insulin in an effective manner; however, many additional transcription factors are essential for specification, differentiation or maturation during the development of cells that secrete insulin in response to glucose stimulation.[133]

- Neurogenin-3 (NGN3) is essential for the development of the endocrine pancreas.[134] *In vitro*, overexpression of NGN3 in human ductal progenitor cells induced their differentiation into insulin-positive cells,[135] whereas its adenoviral delivery to exocrine cells induced transdifferentiation into β cells.[89]
- Pancreas and duodenum homeobox protein 1 (PDX1) is a crucial transcription factor in pancreas formation[136,137] and β-cell differentiation[138–140] and has been included in all protocols that introduce factors into cells with the use of viral vectors in order to reprogram them into β cells. In addition, PDX1 is also used as a marker to evaluate the degree of differentiation towards a pancreatic fate.
- Neurogenic differentiation factor 1 (NEUROD1) regulates insulin gene transcription[141] and is required for the terminal differentiation of cells which express insulin or glucagon.
- Paired box protein 4 (PAX4) is essential for proper β-cell development and becomes restricted to β and δ cell lineages.[142] Constitutive expression of PAX4 in combination with specific three-dimensional culture conditions led to the formation of islet-like spheroid structures from ESCs that produce increased levels of insulin.[143]
- Transcription factor MafA (MAFA) is a glucose-responsive transcription factor restricted to β cells in the pancreas. The switch from insulin-positive $MAFB^+MAFA^-$ cells to $MAFB^-MAFA^+$ cells is a late event in the β-cell differentiation pathway that suggests a function for MAFA as a maturation factor.[144] Overexpression of MAFA in immature β cells unresponsive to glucose can induce glucose-stimulated insulin secretion.[145]

Duct epithelial cells

Pancreatic duct epithelial cells have been suggested as the main source of progenitors for pancreatic growth and regeneration.[64] Genetic lineage tracing experiments have shown that cells that express carbonic anhydrase II within the ductal structures contribute substantially to the β-cell population after birth and after injury.[65] Another study,[66] showed activation of NGN3-expressing cells near or within the ducts that contributed to new β-cell formation after partial duct ligation in adult mice. These findings indicate

that, *in vivo*, cells within the ducts, whether all the epithelial cells or just a subpopulation, can act as pancreatic progenitors in adult rodents.

In vitro, rodent duct cells have been manipulated to form insulin-expressing cells. When GFP-negative pancreatic cell aggregates from transgenic mice which express GFP under the control of the mouse insulin promoter were cultured as monolayers for 1 week, 1–3% of the cells expressed GFP, and after differentiation, the cultures contained 1.9% of the amount of insulin mRNA found in purified GFP-positive β cells.[67] Important growth factors and strategies have been reported to drive induction of insulin expression in pancreatic duct cell lines, including GLP1 and exendin-4,[68–70] activin A and either hepatocyte growth factor or betacellulin,[71,72] as well as aggregation of tissue in serum-free medium.[73] Induction of the β-cell phenotype has also been shown by the use of protein transduction domains (small peptides, either intrinsic to the transcription factor, such as in PDX1 and NEUROD1,[74,75] or extrinsic) for the delivery of proteins into the cell from the external environment. Additionally, viral infection to introduce transcription factors required for islet development (for example, PDX1, NGN3, NEUROD1 or PAX4)[76] has been shown to induce the β-cell phenotype.

When human pancreatic tissue remaining after islet isolation was cultured with FGF7, islet-like structures formed and insulin and glucagon mRNAs increased 10-fold to 15-fold. Importantly, these new insulin-positive cells were glucose-responsive[77,78] and had the normal ultrastructure of islet cells.[77] Manipulation of these cells with growth factors such as GLP1, gastrin and epidermal growth factor (EGF) to obtain β cells was also shown by other studies,[78–81] which supports their potential to become functional β cells. Human ductal cells, purified using the carbohydrate antigen 19-9 antibody and immunomagnetic beads, then expanded and transplanted into immunocompromised mice, confirmed the ductal origin of these insulin-positive cells.[82] These data suggest that adult pancreatic duct cells could be a potential source for human β-cell replacement therapy, if new techniques to expand and differentiate them are developed.

Acinar cells

Acinar cells are the most abundant cell type in the pancreas and share a common progenitor with endocrine cells.[83] Via dedifferentiation, a process dependent on Notch signaling,[84] acinar cells could be a potential *in vitro* source for the differentiation into insulin-positive cells.

Initial studies showed that acinar cells obtained from human exocrine pancreas were able to transdifferentiate into amylase-negative, cytokeratin 19-positive and mucin 1-positive duct cells.[85] Manipulations of cell culture conditions have yielded functional β cells from rat exocrine cells with a combination of EGF and leukemia inhibitory factor. *In vivo*, these exocrine-derived β cells were able to restore normoglycemia in diabetic mice.[86] Other studies on cultured, genetically marked, murine acinar cells[87,88] showed similar plasticity of acinar cells, which differentiated into ductal-like pancreatic precursors; however, these precursors were not shown to become functional β cells.

Another strategy involves direct reprogramming or *in vivo* transdifferentiation of acinar cells into β cells through adenoviral delivery of specific transcription factors (NGN3, PDX1 and MAFA) to the murine pancreatic parenchyma. Insulin-positive cells that resembled β cells in size and ultrastructural characteristics were increased by 20% after the triple adenoviral transduction but these cells did not form islets nor became incorporated into them. Nevertheless, these cells improved the fasting glucose levels of hyperglycemic animals.[89] Given the apparently direct shift from one differentiated phenotype to another, which was reported not to involve activation of the cell cycle or dedifferentiation, this approach might have a lower risk of tumor formation than one that involves a self-renewable, pluripotent cell type. The use of viruses, however, raises concerns about insertional mutagenesis and tumor initiation, which makes non-viral carriers for the delivery of molecules an alternative worth investigating.

Liver cells

Liver cells, which share expression of many specialized genes, such as transcription factors, glucose transporter type 2 and glucokinase, and develop from the same embryological origin as pancreatic cells—the primitive foregut—[90–92] are also considered an attractive source for transdifferentiation into functional β cells. A series of studies have virally introduced different pancreatic transcription factors into the liver, either *in vivo* or *in vitro*, and have shown some induction of the β-cell phenotype.[93–97]

Retrovirally immortalized human fetal liver progenitor cells have been successfully differentiated into insulin-expressing cells with a lentivirus vector that contains PDX1. These cells exhibited a β-cell-like phenotype, showed responsiveness to glucose and were able to restore euglycemia when transplanted in diabetic mice.[98,99]

Stem cells and autoimmunity

The challenge of a successful stem cell-therapy for the treatment of T1DM is not only to generate functional β cells but also to overcome the immune response both in terms of autoimmunity and rejection of allogeneic tissue. Safe and effective cell-based therapies for immunomodulation are under development, including approaches which use intravenous humanized antibodies against CD3 (part of the T-cell receptor complex),[100,101] subcutaneous heat-shock protein[102] or intravenous rabbit polyclonal anti-T-cell globulin.[103] Although many of these studies showed an effective preservation of β-cell function in the short term, very few patients no longer required insulin treatment. Probably, the safest and most effective immunomodulatory therapy will combine multiple modalities and approaches.

Bone marrow-derived MSCs and umbilical cord blood cells may benefit autoimmune diseases such as T1DM owing to their immune suppression and anti-inflammatory properties.[104,105] A clinical trial used autologous non-myeloablative hematopoietic stem cell transplantation in patients with newly diagnosed T1DM (<6 weeks since onset, glutamic acid decarboxylase antibody positive, no prior ketoacidosis) to directly modulate the

immune response.[106,107] In this trial, 12 of 20 patients who no longer required insulin after treatment remained insulin-independent for a mean of 31 months with HbA_{1c} levels of <7.0%. Although these results are striking, this study was controversial, as it was uncontrolled and very aggressive, owing to the use of the immunosupressive agents cyclophosphamide and antithymocyte globulin, with risks of acute drug toxicity, infections and sterility that might outweigh the benefits,[108] given the success of current insulin-based treatment.

A different cell-based therapy uses autologous umbilical cord blood as a source of immunomodulatory cells, as it contains a large population of immature, highly functional regulatory T lymphocytes with the potential to restore proper immune regulation.[109–111] This infusion could potentially decrease the inflammatory cytokine response and inactivate the effector T cells that play a part in cell-mediated autoimmunity.[112,113] An ongoing pilot study[110] on the safety and efficacy of autologous umbilical cord blood infusions has recruited patients with T1DM (average age 5 years) shortly after disease onset with stored autologous cord blood. No chemotherapy or other preparative therapy was given before the children underwent umbilical cord blood infusion. To date, 15 autologous infusions have been performed with no significant adverse effects.[105] Preliminary results of seven children after the first 6 months of treatment include lower HbA_{1c} levels and insulin requirements than children who received insulin therapy alone; however, no patient has achieved independence from insulin treatment.[110] Definitive results for all study participants will become available after the 2-year postinfusion period.

Stem cells and tumorigenesis

Major safety issues exist with the use of stem cells or cell lines. The many rounds of replication that these cells undergo before transplantation into the patient might lead to the accumulation of chromosomal abnormalities that could potentially be oncogenic.

Cell reprogramming may activate the p53 pathway as a stress response.[114] Efficient reprogramming, therefore, requires inhibition of the p53 pathway, but might then predispose the effectively reprogrammed cells to the formation of tumors.[115] The findings of a molecular connection between reprogramming and tumorigenesis underscore the need for strategies to avoid tumorigenesis before stem cells can be used in a clinical setting. Such strategies should eliminate undifferentiated cells from *in vitro* differentiated cultures either by negative selection approaches or by efficient differentiation protocols, since contamination of the differentiated cell population with even a few undifferentiated cells could lead to the formation of teratomas. Although flow cytometry cell sorting can be used to remove undifferentiated cells on the basis of cell surface markers in the research laboratory, this approach is highly unlikely to be viable for the production of the large numbers of cells needed for clinical use. Even the protocol for differentiation needs to be amenable for scale-up under good manufacturing production conditions for use in replacement therapy. Other approaches to remove undifferentiated stem cells could include the use of a 'kill gene' strategy, such as thymidine kinase, for undifferentiated cells; however, this approach would require genetic manipulation of the whole cell population and careful study to be certain of the efficacy of the removal of undifferentiated cells. Another strategy could involve the packaging of the resulting cells in either microcapsules or macrocapsules. Such encapsulation may also be effective to remove cells from direct contact with the host immune system.

Conclusions

T1DM is among the most amenable diseases for treatment with stem cells. Transplantation of whole pancreas or isolated islets has provided proof of principle for the replacement of β cells to achieve independence from the need of exogenous insulin treatment.[116–118] The use of stem cells in the generation of a renewable source of β cells remains a promising reality; clinicians and patients with T1DM eagerly await the successful use of stem cell-derived β cells in clinical practice. Current differentiation protocols take into account the reprogrammable cell type and the factors needed to modulate expression of a specific set of genes that give the β cell its unique identity. Even with an abundant supply of stem cell-derived β cells with robust glucose-responsiveness, however, many issues will still need to be addressed and resolved before this approach becomes a therapeutic option.

A major issue that needs to be addressed is the *in vitro* maturation of insulin-positive cells. Currently, robust glucose-induced insulin secretion, the main function of β cells, has only been possible in hESC-derived insulin-positive cells after *in vivo* transplantation for several months.[27,28] Effective functional maturation protocols need to be developed for *in vitro* use. Up to now, C-peptide levels secreted by *in vitro* differentiated islet-like cells from iPS cells and hESCs are very low compared with that of adult human β cells.

The use of viral vectors in the generation of iPS cell-derived β cells precludes their use in β-cell replacement therapy at this point. Low efficiency of successful differentiation to functional β cells[40] remains an issue, although the identification of highly efficient molecules that drive this process *in vitro* might provide an answer. To establish efficient differentiation procedures which increase the formation of insulin-secreting cells and their response to glucose is therefore essential.

Even though significant challenges remain, the constant advances in this field, as well as the rapid progress of science, make the possibility of an effective stem-cell therapy for T1DM a realistic goal for the foreseeable future.

Review criteria

A search for original articles published between 2004 and 2009 and focusing on the generation of β cells was performed in MEDLINE and PubMed. The search terms used were "clinical trials–diabetes–stem cells", "stem cells and type 1 diabetes", "human stem cells" and "insulin producing cells". All articles identified were English-language, full-text papers. We also searched the reference lists of identified articles for further papers.

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