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Stem Cells Therapy in Diabetes Mellitus

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Abstract

Diabetes is one of the top 10 leading causes of morbidity and mortality, affecting nearly 350 million people worldwide. β-cell replacement represents an attractive prospect for diabetes therapy but treatment options remain quite limited. There is increasing hope placed on insulin producing cells derived from human pluripotent stem cells, even as the approach faces continued challenges. The most effective protocols thus far have produced cells that express insulin, and have molecular characteristics that closely resemble genuine insulin-secreting cells. However, these cells demonstrate little sensitivity to glucose – an issue that will hopefully be resolved in coming years. This review summarizes recent progress in obtaining cells that express insulin from different progenitor sources, and highlights the major pathways and genes involved in diabetic patients.

Keywords: Stem cells; Diabetes mellitus; Cell therapy; Insulin

Abbreviations: BMSCs: Bone Marrow-derived Stem Cells; c-MYC: Myc Proto-oncogene Protein; CXCr: CXC-Chemokine Receptor; DE: Definitive Endoderm; DPP4: Dipeptidyl Peptidase 4; EB: Embryoid Body; EGF: Epidermal Growth Factor; ES: Embryonic Stem; ESCs: Embryonic Stem Cells; FACS: Fluorescence-activated Cell Sorting; FGF: Fibroblast Growth Factor; FoXa2: Forkhead Box Protein A2; GLP1: Glucagon-like Peptide 1; hESCs: Human Embryonic Stem Cells; De1: Definitive Endoderm 1; IPCs: International Programme on Chemical Safety; iPS: Induced Pluripotent Stem; KlF4: Kruppel-like Factor 4; LIF: Leukemia Inhibitory Factor; LIN28: Lin-28 Homolog; MAFA: Transcription Factor MAFA; MSCs: Mesenchymal Stem Cell; Mtpn: Myotrophin; NeuroD: Neurogenic Differentiation Factor; Ngn3: Neurogenin-3; OCT: Octamer-binding Transcription Factor; Pax4: Paired Box Protein 4; Pdx1: Pancreatic and Duodenal Homeobox1; PKU: Phenylketonuria; SGLT2: Sodium-dependent Glucose Cotransporter 2; SOX2: Sry related HMG box-2; T1DM: Type 1 Diabetes Mellitus; TGFβ: Transforming Growth Factorβ; TZDs: Thiazolidinediones

Introduction

Diabetes mellitus is a devastating and complex metabolic disease, expected to affect over 500 million people worldwide by the year 2030; up from 350 million in 2010 [1]. Approximately 95% of patients suffer from type 2 diabetes, and its prevalence is expected to increase in the future [2]. Furthermore, the age of onset for type 2 diabetic patients is trending toward earlier onset in adulthood [3]. Diabetes is associated with severe long-term micro- and macrovascular complications, and carries a high rate of morbidity and mortality. Indeed, both type 1 and 2 diabetes are a significant public health concern with numerous debilitating complications, leading to a constant increase in treatment costs.

Currently, both type 1 and type 2 diabetes can be treated with insulin analogues and Pramlintide. Pramlintide or Amylin is a 37-residue peptide hormone that delays gastric emptying, and endorses satiety and inhibits glucagon secretion; averting post-prandial prickles in blood glucose levels. Recombinant modifications of insulin can act faster and longer, similar to endogenous insulin [4]. The following drugs are used to treat type 2 diabetes: 1) Metformin, augments insulin release, 2) Sulphonylureas (Thiazolidinediones (TZDs) and Meglitinides), increase insulin sensitivity, 3) Bromocriptine, antagonizes dopamine D_2 and serotonin receptors, 4) Glucagon-like peptide 1 (GLP1) analogues, 5) Alpha-glucosidase inhibitors, 6) Dipeptidyl peptidase 4 (DPP4) inhibitors, and 7) Sodium-dependent glucose cotransporter 2 (SGLT2) inhibitors [5-11].

The physiological control of blood glucose levels can only be restored effectively by replacing the β-cell mass [12]. β-cells in the pancreatic islets of Langerhans are responsible for the production of insulin and much of the pathology of diabetes losses can be attributed to the loss of β-cell number and function [13,14]. In patients with type 1 diabetes, the onset of overt disease is assumed to occur when the β-cell mass falls below 20% of the normal range [15,16]; whereas in patients with type 2 diabetes, the β-cell mass is unable to meet the increased insulin demands of the body [17]. Eventually, the β-cell mass in type 2 diabetes also declines to 40–60% of the normal range. Indeed, in both type 1 and type 2 diabetes, restoration of a functional β-cell mass constitutes the central goal of diabetes therapy [18,19]. Exploring ways to protect or expand pancreatic β-cell mass and function could be an effective therapeutic approach, and β-cell replacement represents an attractive therapeutic prospect. Stem cells, particulary the pluripotent stem cells, demonstrate strong self-renewal abilities and potential to differentiate into all cell types of the body, making them a supreme cell source for regenerative medicine and tissue engineering [20-22]. In this review, we address some of the major advancements that could lead to regenerative therapy for diabetes mellitus.

Human Embryonic Stem Cells

Human embryonic stem cells (hESCs) have the ability to form cells derived from all three germ layers [23]. hESCs can be induced to differentiate into fetal-like pancreatic cells *in vitro* using a 33-day, 7-stage protocol [24]. The differentiation protocols for inducing hESCs

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into β-cells involves activation of Wnt and transforming growth factor β (TGFβ) signaling pathways [25-27]. Fibroblast growth factor (FGF) 10, retinoic acid and activin are used to induce the differentiation of hESCs into Pdx1 expressing cells [28-30]. Other markers used to identify definitive endoderm include SOX17, brachyury protein, FGF7, FoXa2, CXC-chemokine receptor (CXCr) 4 and Cerberus [31-35]. Definitive endoderm 1 and 2 (iDe1 and iDe2) have been shown to induce the construction of ultimate endoderm from mouse and human ESCs with about 70–80% efficiency, which is much higher than the differentiation induced by activin or nodal [36,37]. The next *in vitro* step is to reproduce the formation of the pancreatic dorsal anlage. This step is dependent on simultaneous retinoic acid signaling and inhibition of Hedgehog signaling, both of which have been effectively reproduced [38]. Activin a in conjuction with Wnt3a, as well as iDe1 and iDe2 in combination with FGF10 are capable of inducing development of endoderm cells into pancreatic progenitors *in vitro* [39]. Indolactam V activates protein kinase C signaling after treatment with Wnt3a, activin a, FGF10, cyclopamine and retinoic acid and results in induction of pancreatic progenitor cells expressing Pdx1 with close to 50% efficiency [40-42]. miR-375 has a critical role in early development since miR-375 is highly expressed in definitive endoderm and regulates expression of Mtpn and Pdk1 genes. Controlling the expression of miR-375 could also assist mature hESCs-derived β-cells [43,44].

hESCs have been differentitated into cells capable of synthesizing insulin, glucagon, somatostation, pancreatic polypeptide and ghrelin [45]. Therefore, they represent a novel alternative source for targeted therapies and regenerative medicine for diabetes. One approach is derived on the similarities of pancreatic β-cells and neuroepithelial development [46]. The other approach is based on reproducing the individual sequential steps that are known in normal β-cell ontogenesis during fetal pancreatic development. The hESC cell line, PKU1.1, can be induced to differentiate into insulin-producing cells (IPCs) using both protocols [47]. Although these hESC-derived cells containing insulin are similar to that of human islets, the cells lack the main function of glucose-stimulated insulin secretion *in vitro*. However, hESCs have been shown to secrete insulin in response to glucose after transplantation into immune deficient mice [48]. The final stages of differentiation to derive functionally mature β-cells from hESCs must occur *in vivo* [49].

Induced Pluripotent Stem Cells

Embryonic stem (ES) and induced pluripotent stem (iPS) cells have potential applications in regenerative medicine for diabetes. Although iPS cells are a potential alternative to hESCs [50,51], their application is still limited in many countries. iPS cells have been engendered from mouse and human somatic cells by introducing SOX2 combinations of Kruppel-like factor 4 (KlF4), NANOG, octamer-binding transcription factor (OCT) 4, Myc protooncogene protein (c-MYC) and lin-28 homolog a (LIN28) [52]. Usage of the oncogenes, c-MYC and KLF4, raises the uncertainty of potential tumor formation [53,54]. This risk of using reprogrammed cells has been lowered by employing valproic acid, a histone deacetylase inhibitor that enables reassemble of primary human fibroblasts with only two factors, OCT4 and SOX2 [55]. The initial use of retroviruses or lentiviruses to deliver transcription factor genes raised the chance of viral integration into the host genome increasing the risk of tumorigenicity. Novel protocols have been developed that use repeated transfection of expression plasmids in iPS cells without any evidence of plasmid integration [56]. Although current protocols for this reprogramming are developing rapidly and no longer require the use of oncogenes and viral vectors, it is unclear whether iPS cells are truly equal to hESCs with respect to pluripotency [57].

Lentiviral overexpression of the reprogramming factors OCT4, SOX2, NANOG and LIN can induce the formation of iPS cells from umbilical cord blood [58,59]. Due of a juvenescent cell source, umbilical cord blood use addresses some of the concerns that arise from the use of adult somatic cells, such as accumulation of mutations over the lifetime of an organism [60]. Currently available differentiation protocols generate IPCs at a very low frequency. Furthermore, due to the lack of well-distinguished pancreatic beta cell-specific cell surface markers, it is difficult to purify IPCs from a mixed cell population. One of the main reasons for this limitation is insufficient PDX1 expression in the embryoid body (EB) or definitive endoderm (DE)-derived precursors [61]. However, ectopic expression of pancreatic and duodenal homeobox 1, an essential pancreatic transcription factor, in mouse ES cells results in improved differentiation into IPCs [62]. Unfortunately, iPS cells, if produced from a type 1 diabetic patient and transplanted back into the donor, would still be targeted by the immune system. Despite their limitations, the value of IPCs lies in their ability to generate both immune cells and β-cells and the potential to expand our understanding of autoimmune destruction of β-cells.

Although much progress has been made in this area, applications in clinic are still very limited. Transplanting encapsulated pancreatic progenitors derived from hESCs into diabetic recipients is a strategy that is now being explored in the Australian Diabetes Therapy Project [63]. There are still important issues to be addressed before this treatment is widely applicable, including difficulties in maintaining insulin independence, low success rates of islet isolation, multiple donor requirements, and side effects associated with the use of immunosuppressants.

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) have pro-angiogenic and immunomodulatory properties and the remarkable ability to expand, making them extremely attractive from a therapeutic perspective. Moreover, they are easily procurable from virtually every tissue [64]. One source of MSCs can be found in the bone marrow stroma. Bone marrow-derived stem cells (BMSCs) are an invaluable source of adult pluripotent stem cells. An early study reported that transplantation of c-kit expressing bone marrow-derived cells resulted in localization to ductal and islet structures and enhanced insulin secretion. Although occurring in low frequency, these results suggest that BMSCs have the ability to differentiate into β-cells [65]. Si found that autologous MSCs transplantation in a rat model of type 2 diabetes resulted in enhanced insulin secretion, increased islet numbers in pancreas and ameliorated insulin sensitivity, suggesting functional effects of autologous MSCs inoculum on insulin target tissues [66].

Preliminary studies have also tested the graft-promoting effects of BMSC transplantation in a cynomolgus monkey model. Allogenic BMSC transplantation significantly enhanced islet engraftment and function [67]. When human BMSCs were transplanted into NOD/scid mice with streptozotocin-induced pancreatic damage, enhanced insulin secretion and reduced hyperglycemia was observed. Interestingly, the human BMSCs mainly engrafted into the pancreas and the kidney, whereas no cells were detected in the spleen, lung or liver [68].

Since the effects of single MSC transplantation are relatively short (lasting for a period of four weeks), multiple intravenous

transplantations were performed and efficiently restored long-term blood glucose homeostasis in streptozotocin-induced diabetic mice. Despite the induced β-cell differentiation, about fifty percent of donor cells engrafted were surrounding the central veins in liver [69]. These data provide important clues about how to develop effective antirejection therapies and the results suggest that BMSC transplantation may be useful in ameliorating insulin secretion and improving tissue repair in patients with diabetes mellitus.

Recently, clinical application of autologous MSC transplantation in type 2 diabetes patients was reported to get prospective success. Estrada performed a phase 1 study in 25 patients with combined MSC transplantation and hyperbaric oxygen treatment and they found improved metabolic variables including fasting plasma glucose, C-peptide, HbA1c and calculation of C-peptide/glucose ratio, as well as reduced insulin requirements in these patients [70]. Another clinical study revealed that autologous MSC transplantation in 10 patients efficiently reduced insulin dependence, with three patients achieving insulin independence for some time. Importantly, no serious adverse effects were reported [71]. Although the underlying mechanisms are currently unclear, BMSCs may clearly become the ideal choice in therapeutic applications for diabetes.

Pancreatic Duct Cells

Several factors have been found to promote proliferation of β-cells. Brennand et al. in 2007 demonstrated that β-cells have the ability to sustain themselves through slow replication [72,73]. However, the mechanism remains unclear and the numbers of new β-cells are inadequate. Almost twenty years ago, pancreatic duct cells were found to possess the ability to form new islets with β-like cells [74]. In addition, adult exocrine pancreatic cells have been changed into functional β-cells through the combination of epidermal growth factor (EGF) and leukemia inhibitory factor (LIF) *in vitro* [75]. It is believed that the pancreatic duct, acinus and islet are derived from pancreatic duct epithelial cells after birth. Therefore, pancreatic duct epithelial cells are assumed to represent the main source of stem cells for pancreatic regeneration.

New β-cell formation from mouse ducts has been observed under suitable conditions [76,77]. Several strategies have been reported to produce insulin-expressing cells or β-like cells from rodent duct cells. Important growth factors including exendin-4, glucagon-like peptide 1 (GLP1), activin A, hepatocyte growth factor and betacellulin were reported to drive induction of insulin expression in pancreatic duct cell lines [78-83]. It has also been demonstrated that induction of insulin-producing cells from pancreatic duct cells can be achieved through adenoviral-mediated gene transfer technology by expressing Pdx1, neurogenin-3 (Ngn3), neurogenic differentiation factor (NeuroD) or paired box protein 4 (Pax4) [84]. However, these methods pose the risk of unexpected genetic modifications in target cells.

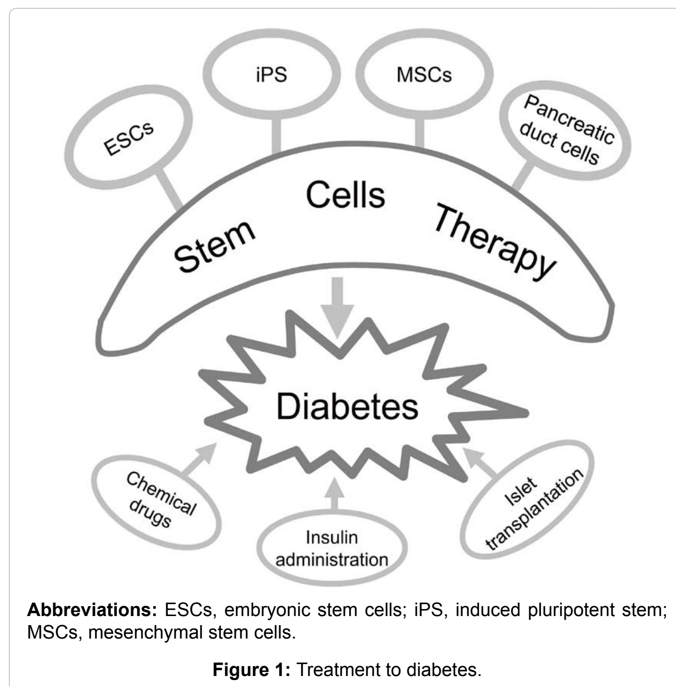
The delivery of recombinant protein into cells through protein transduction represents an effective method for the induction the β-like cells. Noguchi found that the Pdx1 protein, which plays crucial role in regulating pancreatic β-cell differentiation and insulin gene transcription, can permeate cells and perform similar functions as endogenous Pdx1 protein. Importantly, Pdx1 transduced pancreatic duct cells results in enhanced insulin gene transcription [85]. Recently, Kaitsuka found that protein transduction of three proteins, Pdx1, NeuroD, and transcription factor MafA (MAFA), can induce mouse ES and iPS cells into insulin-producing cells with glucose-response. When transplanted into diabetic mice, these induced insulin-producing cells have the ability to restore normoglycemia [86].

When human pancreatic duct cells were sorted using a carbohydrate antigen 19-9 antibody (CA19-9, a duct marker) and cultured under serum-free conditions, they could expand and enhance insulin transcription [87]. These observations suggested that human pancreatic duct cells could be a source of stem cells for β-cell differentiation; however, the induced cells had limited expansion and insulin secretion was not confirmed in a glucose responsive manner. Hoesli found that using a CD90 antibody to deplete fibroblast-like cells from the human pancreatic duct cells greatly improved the expansion of cells [88]. This finding suggests that obtaining pure pancreatic duct stem cells is crucial. Based on the FACS-sorted method, Lee purified human pancreatic duct cells using a CD133 antibody and found that these sorted cells could maintain ductal phenotypes by a self-renewing ability. When the transcription factors MafA, Pdx1, Pax6 and Neurog3 were co-expressed through an adenovirus-mediated transgenic system, CD133 plus cells cultured into spheres and had greatly enhanced insulin gene expression. Importantly, when transplanted into NOD scid gamma mice, the progeny of the CD133 plus cells showed insulin release in a glucose dependent manner and circulating human insulin was detected in the serum of host mice [89]. These data suggest that CD133 positive pancreatic duct cells could potentially be used in human β-cell replacement therapy if new strategies for safer expansion and differentiation are developed.

A significant amount of transdifferentiation takes place from intrahepatic biliary epithelial cells (IHBECs) through the use of certain transcriptional factors. The cells can express proteins characteristic of β-cells and secrete insulin [90,91]. Gordon Weir *et al.* [51] reported that the IHBECs were originally expanded using a novel collagen matrix protocol and these cells kept their biliary phenotype in culture. Ectopic expression of Pdx1, NeuroD or Pdx1-VP16, lead to β-cell genes (ins 1 and 2, PC 1 and 2) expressing the islet hormones glucagon and somatostatin. C-peptide expression was used as a biomarker to confirm β-like cells, and demonstrate the correct processing of insulin protein. β-cell functionality was proven by measuring insulin secretion in response to glucose. An interesting observation in this report was that the β-cell phenotype is only present in a sub-population of IHBECs, suggesting that not all IHBECs are able to transdifferentiate into a β-cell phenotype. This study strongly implies that it is possible to coerce the differentiation of IHBECs towards a β-cell phenotype, which supports the possibility that IHBECs have the potential to be useful for β-cell replacement therapy [92].

Conclusions

Both type 1 and type 2 diabetes are among the most amenable diseases for treatment. Functional restoration of existing β-cells, transplantation of stem cells or stem cell-derived β-like cells might provide new opportunities for treatment (Figure 1). However, the use of stem cells to generate a renewable source of β-cells for diabetes treatment remains challenging, largely due to safety concerns. Current differentiation protocols that use viral vectors to generate induced β-cells result in low numbers of functional β-cells, and possible unexpected genetic modifications. While BMSC transplantation could improve metabolic variables with no obvious side effects, Tang reported that long-term culture of human BMSCs raises the risk of malignant transformation post transplantation [93]. Safety issues, including sources of cells, must be carefully evaluated before clinical applications. The definition of stem cells depends on the cell surface



Abbreviations: ESCs, embryonic stem cells; iPS, induced pluripotent stem; MSCs, mesenchymal stem cells.

Figure 1: Treatment to diabetes.

markers, and their efficiency of differentiation greatly relies on the purity of cell source sorted by cell surface markers. Reports suggest that approximately 100,000 cells are needed for each recipient, but a low differentiation rate necessitate longer time in *in vitro* culture to develop adequate numbers of cells for transplantation. However, the longer culture time can increase the possibility of malignancy. Indeed, new technologies to improve differentiation efficiency are essential.

Monitoring clinical trials closely will be key. Development of a transplant registry in combination with assessment and optimization of clinical protocols will help identify optimal cell types and cell surface markers for characterization, and may ultimately lead to safe, effective treatments.

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