Mini-review

Stem cells therapy for type 1 diabetes

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Abstract

In this article, we have reviewed the developments of studies of stem cells therapy for type 1 diabetes since this century. Review of the literature was based on computer searches (PubMed) and our studies. Type 1 diabetes can now be ameliorated by islet transplantation, but this treatment is restricted by the scarcity of islet tissue. Hopes for a limitless supply of a substitute for primary islets of Langerhans and progress in stem cell biology have led to research into the feasibility of stem/progenitor cells to generate insulin-producing cells to use in replacement therapies for diabetes. An increasing body of evidence indicated that, in addition to embryonic stem cells, several potential adult stem/progenitor cells, derived from pancreas, liver, spleen, and bone marrow could differentiate into insulin-producing cells in vitro or in vivo. However, significant controversy currently exists in this field. Moreover, safe suppression of autoimmunity or specific tolerance to auto-antigens for patients with type 1 diabetes must be achieved before this promising new technology can lead to a great progress in clinical practice. To prevent type 1 diabetes through genetic engineering of hematopoietic stem cells represents another new strategy. Much basic research is still required.

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1. Introduction

Type 1 diabetes (T1D) is the result of an autoimmune attack against the insulin-producing beta (β) cells of the endocrine pancreas. Although routine insulin injections can provide diabetic patients with their daily insulin requirements, blood glucose excursions are common and result in hyperglycemic or hypoglycemic episodes.
a normal β cell. The recent success of the Edmonton Protocol for pancreatic islet transplantation [1,2] and the development of non-invasive imaging of islet grafts using positron-emission tomography [3] has sparked new interest in transplantation of insulin-producing cells. However, the amount of donor islet tissue is severely limited and will allow for the treatment of only a small fraction of patients with insulin-dependent diabetes. Moreover, differentiated β cells cannot be expanded efficiently in vitro [4]. In order for β-cell replacement therapy to really have a major clinical impact, new sources of transplantable insulin-producing cells need to be developed.

In this review, we focus on the stem-cell-based possibilities for the generation of insulin-producing cells for transplantation. We shall also mention new strategy to prevent T1D through genetic engineering of hematopoietic stem cells (HSCs).

1.1. Pancreatic adult stem/progenitor cells

Significant controversy currently exists within the area of pancreatic adult stem/progenitor cells and no adult pancreatic stem cell has been fully characterized. However, several candidate cells have been identified, isolated, and partially characterized. It is important to note that differentiation protocols at present allow for only small amounts of insulin production compared with pancreatic islets.

1.1.1. Pancreatic duct-derived stem/progenitor cells

There is some evidence to suggest that pancreatic stem/progenitor cells reside within pancreatic ductal cells, where they can differentiate and migrate to form new islets during both organogenesis and regeneration [5,6]. Ramiya et al. first described the generation of new islets from pancreatic ductal epithelial cells in vitro [7]. The authors grew pancreatic ductal epithelial cells manually isolated from prediabetic adult non-obese diabetic (NOD) mice in long-term cultures, where they were induced to produce functioning islets containing alpha (α), β and delta (δ) cells. These in vitro-generated islets showed temporal changes in mRNA transcripts for islet cell-associated differentiation markers, responded in vitro to glucose challenge, and reversed insulin-independent diabetes after being implanted into diabetic NOD mice. Furthermore, a recent study demonstrated that fetal pancreatic ductal cells could differentiate into insulin-producing cells [8]. Transplantation of the pseudo-islets consisting of β cells derived from ductal cells reversed streptozotocin-induced diabetes in nude mice. Our study also indicated that adult porcine pancreatic duct epithelial cells could differentiate into insulin-producing cells in vitro and these derived pseudo-islets were positive for expression of β-cells marker and could secrete insulin [9].

Our study demonstrated that transcriptional factor pancreas duodenum homebox-1 (PDX-1) might play an important role in differentiation of pancreatic stem cells into pseudo-islets cells [10]. More recently, a study indicated that, with the capacity of hepatocyte growth factor (HGF), neonatal pig pancreatic duct-derived cell monolayers could be induced to form three-dimensional islet-like cells that synthesize and release proinsulin and subsequently insulin [11]. These data suggest that duct cells are a source of pancreatic progenitor cells. However, the specific cells in the pancreatic ducts that are the progenitors giving rise to the insulin-producing cells were not identified or characterized.

However, the above view has been challenged by a recent study with a method of genetic lineage tracing, which clearly showed that pre-existing β cells, rather than pluripotent stem cells, are the major source of new β cells during adult life and after pancreatectomy in mice [12]. Although this study supports the idea that β-cell repopulation is the dominant mechanism for β-cell expansion in adult mice, it does not convincingly exclude that new islets are formed during neonatal life and after regeneration-inducing maneuvers such as partial pancreatectomy or duct ligation. Significant controversy still exists, more recently, Dor et al. found that β-cell regeneration occurs by neogenesis from stem or progenitor cells rather than by replication of pre-existing β cells, and that at least some cells within the non-endocrine pancreatic epithelial cells (NEPECs) population are capable of β-cell differentiation [13]. Non-endocrine-to-endocrine differentiation of NEPECs supports the existence of endocrine stem or progenitor cells within the epithelial compartment of the adult human pancreas.

Maybe more delicate research methods should be used to ulteriorly confirm or reconcile the above reports.

1.1.2. Nestin-positive islet-derived progenitor cells

Abraham et al. and Zulewski et al. [14,15] have reported that cells expressing the intermediate filament protein nestin, a marker of neural stem cells, can be isolated from human and rodent islets and expanded extensively in vitro. Insulin, glucagon, and PDX-1/IPF-1 expression, as well as low-level insulin secretion, can be detected in cultures of nestin-positive islet-derived stem/progenitor cells after addition of differentiating cytokines and growth factors. The authors concluded...
that nestin-positive islet-derived progenitor cells may participate in the neogenesis of islet endocrine cells. Also based on the studies of Maria-Engler et al. [16], nestin-positive cells, at least in vitro, may undergo the early stages of differentiation to an islet cell phenotype and that long-term cultures of nestin-positive human islet cells may be considered as a potential source of precursor cells to generate fully differentiated functional β cells. Recently, a study by Wang et al. [17] demonstrated that expanded nestin-expressing cells in vitro from islet-derived epithelial monolayers are heterogeneous, clonal analysis of nestin-positive cells reveals that a distinct subpopulation of nestin/PDX-1-expressing cells is capable of forming insulin-producing cells. More recently, using defined culture conditions, Eberhardt et al. isolated on a single cell basis nestin-producing cells from human pancreatic islets. These cells are positive for transcription factor islet-1 and nestin and have the potential to adopt a pancreatic endocrine phenotype with expression of critical transcription factors as well as the islet hormones insulin, glucagon, and somatostatin [18].

Controversial to these studies, a detailed analysis of early human pancreatic development showed that endocrine precursors do not express nestin [19]. Transgenic lineage tracing experiment in mice also conclusively showed that nestin-lineage cells contribute to the microvasculature but not endocrine cells of the islet [20]. The discrepancy may be due to the reality that tissue culture observations most likely represent a phenomenon specific for the in vitro conditions, which are much different from the normal development processes in vivo.

2. Other types of somatic stem cells

2.1. Liver stem cells

During embryogenesis, both the liver and ventral pancreas appear to arise from the same cell population located within the embryonic endoderm. It is assumed that the epithelial cell populations within the pancreas and liver might share common stem cell populations. So liver stem cells would be another attractive source for new β cells.

Yang et al. presented evidence [21] that highly purified adult rat hepatic oval “stem” cells could transdifferentiate into pancreatic endocrine hormone-producing cells when cultured in a high-glucose environment. These differentiated cells could self-assemble to form three-dimensional islet cell-like clusters that express pancreatic islet cell differentiation-related transcripts and islet-specific hormones. In addition, these oval cell-derived islet cell-like clusters displayed the ability to reverse hyperglycemia in a diabetic NOD-severe combined immunodeficiency (scid) mouse. With another approach, fetal human liver cells transduced with human telomerase (hTERT) and then pdx-1 produced cells with considerable amounts of stored and secreted insulin [22]. These cells not only secreted insulin in a regulated manner but also restored and maintained euglycemia for prolonged periods when transplanted into immunodeficient diabetic mice. Similarly promising results have been yielded with adult human liver cells. By using PDX-1 gene and soluble factors, Sapir et al. induced a comprehensive developmental shift of adult human liver cells into functional insulin-producing cells [23]. When transplanted under the renal capsule of diabetic, immunodeficient mice, these cells ameliorated hyperglycemia for prolonged periods of time. Recently, Tang et al. succeed in reprogramming liver stem WB cells into pancreatic endocrine precursor cells by persistent expression of Pdx1- and Pdx1-VP16 mediated by lentiviral vectors. Upon transplantation into diabetic mice, these cells become functional insulin-producing cells and restore euglycemia [24].

Liver cells could thus provide another clinically relevant source of precursors to be used for the generation of transplantable insulin-producing cells.

2.2. Spleen stem cells

Promising results of the studies by Faustman and co-workers electrified the diabetes community in 2001 and 2003 [25,26]. The protocol incorporated injection of a single dose of Freund’s complete adjuvant (FCA) into severely diabetic mice, coupled with repeated infusion of allogeneic splenocytes, resulting in restoration of normoglycemia and permanent disease extinction. The conclusion of this study was that the FCA had eliminated anti-islet autoimmunity and that the donor splenocytes had differentiated into insulin-producing (presumably β) cells, ultimately leading to islet regeneration. The work engendered special hope. Traditional islets, which must be culled from cadaver pancreases, are in short supply. Doctors would have the possibility of collecting spleens that people do not really need. Recently, three groups followed the Faustman protocol to repeat the above experiment [27–29]. Like Faustman and her colleagues, all the groups successfully treated a subset of animals, although their “cure” rates were lower. However, no group detected donor spleen cells in the blood or lymph nodes of mice or as new β cells.
in the pancreas, suggesting that the immune system had destroyed them.

2.3. Hematopoietic stem cells

Bone marrow (BM) is an important source of easily procurable adult stem cells. In addition to the ability of BM-derived stem cells to reconstitute the hematopoietic system [30], cells derived from the BM compartment can differentiate even towards ectodermal or endodermal directions [31]. Ianus et al. [32] reported that bone marrow harbors cells that have the capacity to differentiate into functionally competent pancreatic endocrine β cells. Within the time frame of their study, 1.7–3% of all β cells in the recipient mice were of donor origin 4–6 weeks after BM transplantation. Moriscot et al. also found that in vitro human bone marrow stem cells are able to differentiate into insulin-expressing cells by a mechanism involving several transcription factors of the β-cell developmental pathway when cultured in an appropriate microenvironment [33]. However, the discussion about the broad differentiation potential of BM-derived stem cells has become controversial as some of the original studies could not be confirmed by other laboratories [34]. Moreover, Wang et al. concluded in their study that hepatocytes derived form bone marrow arise from cell fusion and not by differentiation of hematopoietic stem cells [35]. Whether β cells can be derived from BM remains to be determined.

Hess et al. had an intriguing finding that transplantation of BM-derived stem cells initiated endogenous pancreatic regeneration [36]. It was found that engraftment of BM-derived cells to ductal and islet structures was accompanied by rapid proliferation of recipient pancreatic cells and neogenesis of insulin-positive cells of recipient origin. The authors speculated that transplanted BM-derived cells became vascular endothelial cells in the pancreas, which induced β-cell regeneration from host cells resident in the pancreas by secreting as yet unknown growth and differentiation factors. Several issues must be resolved before this approach might form the basis for a new treatment of diabetes [37]. Recently, the study of Izumida et al. indicated that the induction of the c-Met/HGF signaling pathway following BM transplantation promotes endogenous pancreatic regeneration in diabetic rats [38].

In addition to the above results, it is suggested that adult HSCs can re-introduce tolerance to autoantigens. Although BM or HSCs transplantation is a potential treatment for autoimmune disease, the clinical application of this approach is limited by the risks associated with allogeneic transplantation. In contrast, syngeneic transplantation would be safe and have wide clinical application. The study by Tian and colleagues demonstrated that transplantation of autologous HSCs modified to express diabetes-resistant MHC class II corrected a defect in central tolerance and protected the mice from the development of insulitis and diabetes [39]. Our research group also tries to prevent T1D by autologous HSCs transplantation. It is generally accepted that the context in which antigen presentation occurs controls the nature of T cell immunity [40]. Antigen presented by resting antigen-presenting cells induces T cell unresponsiveness [41,42] and inhibits antigen-specific antibody production [41]. Since antigen-presenting cells are derived from HSCs and gene expression could be effectively targeted to MHC class II-positive antigen-presenting cells in vivo by lentiviral vector transduction of human HSCs [43]. Further more, it is indicated that proinsulin is a key and primary autoantigen that initiates T1D and drives β-cell destruction [44,45]. We reason that targeting proinsulin gene expression to resting antigen-presenting cells derived from lentivirus-transduced engrafting HSCs could prevent T1D. This strategy might also be used to prevent the recurrence of autoimmunity after islet transplantation.

3. Embryonic stem cells

An alternative source of highly proliferative, pluripotent cells which has received much more attention is embryonic stem (ES) cells. Derived from the inner cell mass of the blastocyst, these cells have the capacity to differentiate into all three embryonic germ layers in vitro. The first report of successful generation of insulin-producing cells from mouse ES cells was published in 2000 [46]. Using a cell-trapping system, Soria et al. had obtained an insulin-secreting cell clone from undifferentiated ES cells. Clusters obtained from this clone were implanted in the spleen of streptozotocin-induced diabetic animals. Transplanted animals correct hyperglycemia and restore body weight. However, the number of insulin-positive cells was very low. An interesting study by Lumelsky et al. described the generation of insulin-secreting structures similar to pancreatic islets from mouse ES cells through a five-step protocol [47]. Glucose triggers insulin release from these cell clusters by mechanisms similar to those employed in vivo. When injected into diabetic mice, the insulin-producing cells undergo rapid vascularization and maintain a clustered, islet-like organization.
Recently, Blyszczuk et al. described an efficient strategy for the in vitro differentiation of ES cells into the pancreatic lineage. The protocol includes the spontaneous generation of multilineage progenitor cells and their differentiation induction by growth and extracellular matrix factors into C-peptide/insulin-positive islet-like clusters. The differentiated cells release insulin in response to high-glucose concentrations [48]. Moreover, the study of Marenah et al. demonstrated that the stable glucose-dependent insulinotropic polypeptide analogue could enhance differentiation of mouse ES cells towards a phenotype expressing specific β-cell genes and releasing insulin in response to secretagogues and glucose [49]. Human ES cell research is still at an early stage. However, it has become apparent that many principles established in mouse ES cells cannot be directly applied in human ES cells. Brolen et al. [50] presented a method for inducing differentiation of human ES cells into insulin-producing β-cell-like cells by signals from the embryonic mouse pancreas. These human ES cells-derived insulin (+) cells share important features with normal β-cells, such as synthesis (proinsulin) and processing (C-peptide) of insulin and nuclear localization of key β-cell transcription factors. Further improvement of this method may lead to the formation of an unlimited source of cells suitable for transplantation.

However, research in this field is surrounded by controversies. Recently, Milne et al. demonstrated that mouse ES cells readily differentiate into extra-embryonic endoderm in vitro. They suggested that the insulin-expressing cells generated in this and other studies are not authentic pancreatic β cells, but may be of extra-embryonic entodermal origin [51]. It was also shown that many of the insulin-positive cells generated from ES cells were not synthesizing insulin de novo, but that the insulin was uptaken from the culture medium and released by apoptotic cells [52,53]. In spite of these reports, researchers are still optimistic that ES cells provide a potential source for β-cell replacement.

4. Similarities and differences between stem-cell-based therapy and traditional islet cells transplantation

Insulin-producing cells derived from stem cells and islet cells extracted from donor pancreas have some similarities in transplantation for T1D therapy. They all provide the hope of sufficiently tight control of blood glucose to avoid diabetic late complications that current diabetes drug therapies have been almost impossible to avoid. They can be transplanted by the same approach, e.g. injected into the portal vein of the liver. They are also faced with the same problems, such as immunological barrier and/or recurrence of autoimmunity. However, stem-cell-based therapy is much different from traditional islet cells transplantation therapy. Stem cells provide a theoretically unlimited supply of insulin-producing cells for transplantation. Transplantation of insulin-producing cells derived from recipient’s own stem cells will have no immunological barrier. Even the insulin-producing cells may be induced from the remained stem cells in vivo directly. Moreover, stem cells represent a perfect target for gene therapy. New gene therapy strategies based on stem cells will be developed to prevent T1D.

5. Conclusion

Despite the conflicts and difficulties encountered in the field of stem cell research, the possibility that insulin-producing cells can be induced in vitro or in vivo inspires us to deepen our understanding of basic stem cell biology. Further studies in this area will improve our understanding of stem/progenitor cell differentiation. Maybe in the near future, it should be possible to generate sufficient β cells in vitro to solve the current shortage of insulin-producing cells from donor islets. However, safe suppression of autoimmunity or specific tolerance to auto-antigens for patients with T1D must be achieved before this promising new technology can lead to a great progress in clinical practice. Two studies indicated that CD3-specific monoclonal antibody treatment restored self-tolerance in patients with new-onset T1D. The mechanism of action of the anti-CD3 monoclonal antibody may involve direct effects on pathogenic T cells, the induction of populations of regulatory cells, or both [54,55]. This and other new immunoregulation strategies might prove useful as components in regimens of stem-cell-based therapy for T1D.

Conflict of interest

There are no conflicts of interest.

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