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Review on: regenerative medicine, tissue engineering and stem cell therapy in diabetes mellitus

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ABSTRACT

Introduction. In view of the recent success in pancreatic islet transplantation, interest in treating diabetes by the delivery of insulin-producing β-cells has been renewed. Because differentiated pancreatic β-cells cannot be expanded significantly in vitro, β-cell stem or progenitor cells are seen as a potential source for the preparation of transplantable insulin-producing tissue. In addition to embryonic stem (ES) cells, several potential adult islet/β-cell progenitors, derived from pancreas, liver, and bone marrow, are being studied. To date, none of the candidate cells has been fully characterized or is clinically applicable, but pancreatic physiology makes the existence of one or more types of adult islet stem cells very likely. It also seems possible that pluripotential stem cells, derived from the bone marrow, contribute to adult islet neogenesis. Aim. In future studies, more stringent criteria should be met to clonally define adult islet/β-cell progenitor cells. If this can be achieved, the utilization of these cells for the generation of insulin-producing β-cells in vitro seems to be feasible in the near future. This review will focus on the potential of adult tissue-derived stem cells, in lieu of embryo-derived stem cells, for the treatment of diabetes. We discuss the role of adult islet stem/progenitor cells in normal physiology, highlight possible candidate cells isolated to date, and describe different approaches for stem cell-based therapy.

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INTRODUCTION

Diabetes is a syndrome characterized by an absolute or relative β-cell deficiency in terms of mass (type 1 diabetes mellitus, T1DM) [1]. In contrast, in type 2 diabetes (T2DM) insulin deficiency, while, due in part to loss of functional, responsive β-cells, is not absolute, but relative to the impaired insulin signalling present in this disorder [2], or pancreas is unable to produce insulin, whereas type 2 (adult onset diabetes) is caused due to insulin resistance of the cells [3].

Once insulin resistance develops in several tissues, insulin-stimulated glucose disposal is decreased and adipocytes release many free fatty acids (FFAs). Furthermore, increased FFAs inhibit the insulin action on liver, resulting in increased gluconeogenesis in the hyperglycemic state [4]. The International Diabetes Federation estimates that up to 95% of the ~380 million people worldwide who are suffer from type 2 diabetes [5]. It is harder to treat and typically occurs in adults as a result of excess weight or hormonal imbalances [6]. Type 2 diabetes mellitus has become an epidemic, and virtually no physician is without patients who have the disease [7].

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the pancreatic β-cells with consequent insulin deficiency to abnormalities that result in resistance to insulin action [8]. In the long term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease [9].

Over the years several sources of stem cells have been claimed to cater to the need of insulin producing cells. These include human embryonic stem cells, induced pluripotent stem cells, human perinatal tissues such as amnion, placenta, umbilical cord and postnatal tissues involving adipose tissue, bone marrow, blood monocytes, cord blood, dental pulp, endometrium, liver, labia minora dermis-derived fibroblasts and pancreas [10].

Treatment of Type 2 diabetes is complicated by several factors inherent to the disease process, typically, insulin resistance, hyperinsulinemia, impaired insulin secretion, reduced insulin-mediated glucose uptake and utilization [11]. It is well-known that exercise and diet control are helpful to manage glucose level at initial stage [12]. A novel therapeutic approach to reduce pancreatic β-cells are dysfunctional or altogether absent in diabetic patients, replacement of these cells has become the major target of stem cell research in diabetes [13]. There are a number of different sources of stem cells and the most investigated types of stem cells for DM treatment are: Embryonic stem cells [14], induced pluripotent stem cells of induced pluripotent stem cells [15], germ cell derived stem cells, and mesenchymal stem cells [16].

But, in addition to these therapeutic of DM either in-vivo or in-vitro approaches, the most important problem is choosing the best type of progenitor cell. Tissue Engineering is an interdisciplinary discipline addressed to create functional three-dimensional (3D) tissues combining scaffolds, cells and/or bioactive molecules. Tissue engineering/regenerative medicine strategies require interaction and integration with tissue and cells through incorporation of appropriate physical and cellular signals. Therefore, inclusion of modifying factors such as biologically active proteins and DNA are critical to success [17].

This review will be included to establish a novel tissue engineering approach for diabetes mellitus (DM) by fabricating a tissue sheet composed of pancreatic islet cells for *in vivo* transplantation [18]. One alternative to organ or tissue transplantation is to use a renewable source of cells. Stem cells are clonogenic cells capable of both self-renewal and multiline age differentiation [19]. This review will discuss the current evidence and strategy behind these stem cell sources, as well as the advantages and disadvantages of each [13]. Therefore, treatment strategies for DM should be aimed at restoring beta cell mass and/or function, in addition to improving insulin sensitivity. The aim of this review is to give an overview of the existing knowledge of current experimental strategies in the treatment of DM covered by tissue engineering and regenerative medicines [20].

Current and future cell-based therapies of DM

The methods for generating pancreatic beta-cells include a method of creating pancreatic beta-cells in vitro and implanting them into the body and a method of regenerating pancreatic beta-cells in the body via gene introduction or the administration of differential proliferation factors to the body. Moreover, the number of pancreatic beta-cells is also low in type 2 diabetes, caused by the compounding factors of insulin secretory failure and insulin resistance; therefore, if pancreatic beta-cells can be regenerated in a living body, then a further amelioration of the pathology can be expected. The development of pancreatic beta-cell-targeting regenerative medicine can lead to the next generation of diabetes treatment [21].

Curative therapy for diabetes mellitus mainly implies replacement of functional insulin producing pancreatic β cells, with pancreas or islet-cell transplants. However, shortage of donor organs spurs research into alternative means of generating β cells from islet expansion, encapsulated islet xenografts, human islet cell-lines, and stem cells. Stem-cell therapy here implies the replacement of diseased or lost cells from progeny of pluripotent or multipotent cells. Both embryonic stem cells (derived from the inner cell mass of a blastocyst) and adult stem cells (found in the postnatal organism) have been used to generate surrogate β cells or otherwise restore β-cell functioning [22]. Cell therapies with human embryonic and adult stem cells have emerged as an alternative management for various diseases. These cells were able to proliferate and differentiate into various cell types including those bearing a phenotype of insulin-secreting β-cells [23].

Stem cells

Stem cells possess an exceptional quality to replenish itself and to produce any specialized cell types under appropriate microenvironment. A rapidly dividing stem cell produces two new cells, each having two choices depending upon the requirement of the organism. Thus, a newly produced cell either may remain as a stem cell or it may undergo further differentiation to become a more specialized cell with specific function [24]. The stem cells have the potential to become any type of specialized cell such as a myocyte, blood cell, hepatocyte and brain cell (Figure 1).

Embryonic stem cells

Many cell signaling and epigenetic factors involved in the differentiation process are still unknown, although the presence of markers such as *PDX1*, *Isl1*, and *Foxa2* are indicative of pancreatic β-cells. The exact composite and temporal progression of transcription factors present in pancreatic cells is important for

identification, as many of these factors are seen in different combinations in other cell lineages. The differentiation process is meant to mimic the embryological development of the pancreas [13].

Pancreatic and duodenal homeobox 1 (Pdx1) is a transcription factor that regulates the embryonic development of the pancreas and the differentiation toward β cells. Previously, we have shown that exposure of mouse embryonic stem cells (mESCs) to high concentrations of diethylenetriamine nitric oxide adduct (DETA-NO) triggers differentiation events and promotes the expression of Pdx1. Here we report evidence that Pdx1 expression is associated with release of polycomb repressive complex 2 (PRC2) and P300 from its promoter region [25].

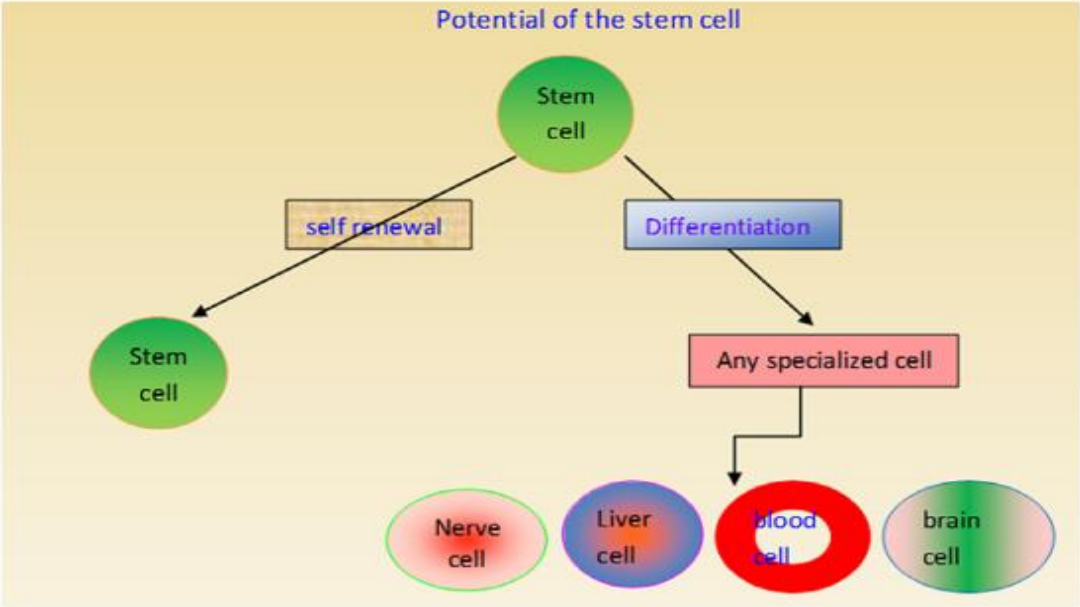

Figure 1. Self-renewal and differentiation potential of the stem cells [26].

TISSUE ENGINEERED PANCREATIC SUBSTITUTES

Tissue restoration is the process whereby multiple damaged cell types are replaced to restore the histoarchitecture and function to the tissue. Several theories, have been proposed to explain the phenomenon of tissue restoration in amphibians and in animals belonging to higher order [27].

A profound knowledge of the development and differentiation of pancreatic tissues, especially islets of Langerhans, is necessary for developing regenerative therapy for severe diabetes mellitus. A recent developmental study showed that PTF-1a is expressed in almost all parts of pancreatic tissues, in addition to PDX- 1 *PDXI*, a well-known transcription factor that is essential for pancreas development [28]. Tissue engineering may use one of three basic strategies: isolated cells or cell substitutes, tissue inducing substances, or cells placed within matrices. For the purposes of IDD, the first approach is already being applied in islet transplantation. Since β-cells do not significantly expand in cell number *in vivo* the second approach of a tissue inducing substitute is considerably more challenging. Alternatively, it has been reported that exocrine pancreas tissue can be induced to take on a β-cell phenotype through metaplasia so a similar approach could be envisioned to target those cells [29].

Mesenchymal stem cells

Curative therapy for diabetes mellitus mainly implies replacement of functional insulin-producing pancreatic β cells, with pancreas or islet-cell transplants. However, shortage of donor organs spurs research into alternative means of generating β cells from islet expansion, encapsulated islet xenografts, human islet cell-lines, and stem cells. Stem-cell therapy here implies the replacement of diseased or lost cells from progeny of pluripotent or multipotent cells. Both embryonic stem cells (derived from the inner cell mass of a blastocyst) and adult stem cells (found in the postnatal organism) have been used to generate surrogate β cells or otherwise restore β-cell functioning [22].

Originally identified by Friedenstein et al. in 1976 [30] as a fibroblast-like cell population capable of generating osteogenic precursors, the mesenchymal stromal cells derived from the bone marrow (BM) are a

rare, heterogeneous, stromal population of multipotent non-haematopoietic progenitor cells with the capacity to differentiate into multiple mesenchymal lineages, including bone, fat and cartilage. Due to this characteristic, Caplan [31] dubbed them "mesenchymal stem cells" (MSCs), which has been recently changed by a consensus statement recommendation to "multipotent mesenchymal stromal cells" [32]. Other studies have identified pluripotent cells capable of differentiation along endodermal and neurectodermal lineages, including neurons, hepatocytes and endothelial cells [33], [34]. Such stem cells, isolated from BM, have been referred to as "multipotent adult progenitor cells" (MAPCs), "marrow-isolated adult multilineage inducible cells" (MIAMIs) [35] and "very small embryonic-like stem cells" (VSELs) . However, even if the transdifferentiation capacities of these primitive cell types is of major interest, obtaining them requires highly specific culture conditions and, so far, it has not been possible to isolate these cells from fresh BM. Whether or not they represent a culture phenomenon remains an unanswered question [36].

MSCs administration can prevent and treat diabetic nephropathy, which is a complication of DM and is defined as progressive kidney disease caused by angiopathy of the capillaries supplying the kidney glomeruli. MSCs have been used for the treatment of diabetic nephropathy in nonobese diabetic/severely compromised immunodeficient (NOD/SCID) and C57 black 6 (C57/BL6) mice, which succumb to DM after application of multiple low doses of STZ. About 30–60 days after STZ injection, kidneys of treated mice showed the presence of abnormal glomeruli characterized by increased deposits of ECM protein in the mesangium, hyalinosis, and increased number of macrophages in the glomeruli [37].

Induced pluripotent stem cells

The use of iPSCs untangles regenerative therapy in diabetes from ethical constraints, but also poses its own unique challenges. The production of iPSCs from human fibroblasts was first demonstrated by Yamanaka and colleagues through retroviral transduction of four transcription factors (*Oct-3/4, Sox-2, Klf-4*, and *c-Myc*) in a process termed direct reprogramming. In lieu of the high tumorigenic potential of direct reprogramming resulting from genome integration and activation of oncogenic *c-Myc*, additional research proved iPSCs could be produced from somatic cells in the absence of *c-Myc*, but at the expense of efficiency [13]. Engraftment of mature insulin producing cells derived from induced pluripotent stem cells may represent the most promising treatment strategy for diabetic patients with impaired β-cell function [13].

β cells from direct reprogramming

One theme that has been explored extensively by researchers is to create new β cells from existing pancreatic cells. The rationale behind this approach is that because these cells are either β-cell precursors or developmentally related to β cells, the barrier to reprogramming them into functional β cells may be lower than in cells that are not as closely related developmentally.

In normal healthy conditions β-cells have a long life-span with a low proliferation rate [38]. In response to increased metabolic demand or after injury, however, the adult pancreas maintains or acquires the ability to produce new cells, particularly β-cells. The precise identification of the mechanisms involved in the maintenance of β-cell mass under different conditions could offer new hints to help generating new β-cells as a cell replacement therapy for treating diabetes [39].

Today, insulin-dependent patients rely on daily insulin injections. Transplantation of isolated islets from cadavers is problematic due to donor scarcity (about 6000 islets/kg of body weight are required [40], and is only applicable to certain forms of diabetes; in addition, transplantation has met with limited success due to restricted engraftment survival [41]. A promising approach relies on devising unlimited *in vitro* generation of insulin-producing cells derived from embryonic stem (ES) cells or, even more interestingly, from patient-derived induced pluripotent stem (iPS) cells [42]. Very recently, however, in view of new experimental evidence showing that adult differentiated pancreatic cells can reprogram and change their phenotype [43], exploration of the intrinsic spontaneous capacity of the adult pancreas to regenerate β-cells, in particular from heterologous origins, has acquired a new dimension as a route to the development of therapeutic treatments for diabetes [44].

This review will focus on β-cell regeneration and its diverse mechanisms. In fact, exploiting the intrinsic capacity of the adult pancreas to produce new β-cells endogenously is probably the most promising way to develop cell replacement therapies to treat the forms of diabetes that result from massive β-cell loss. Nevertheless, a prerequisite for such an achievement will be to uncover the immunological basis of the pathogenesis of the disease. (Reference)

Antigen-presenting cells

So far, at least 15 distinct peptides derived from β-cells and their corresponding CD4+ T cells have been identified [45]. The presentation of β-cell antigens is a complex issue as β-cells themselves do not express MHC class II molecules. It can be surmised that presentation of β-cell-specific antigens is mediated by Antigen-Presening Cells (APCs) within islets of Langerhans. These professional dendritic cells (DCs) are able to load the peptide groove of their MHC class II complexes with peptides derived from β-cell granules [46]. In this context, local lymph nodes draining the pancreas are crucial to the selection and activation of diabetogenic T cells [47]. Here, the question arises, how the β-cell antigen presentation takes place. It is not clear yet, whether this occurs via migration of islet DCs to the lymph nodes or, instead, by drainage of β-cell products directly to the nodes and subsequent uptake by DCs in the draining lymph nodes. Based on our knowledge gathered from the NOD mouse, β-cell autoimmunity progresses in relatively well-defined "checkpoints". A first checkpoint is marked by DC infiltration of islets in 2- to 3-week-old NOD mice. Early detection of DCs and macrophages is followed by CD8+ and CD4+ T cells, NK cells, and B cells. During islet cell infiltration these cells encounter β-cell autoantigens such as GAD65 and islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP). The β-cell destruction resulting from inflammatory damage leads to release of cell contents including GAD65 and other autoantigens. Subsequently, these can be taken up by activated endothelial cells able to process and present disease-related epitopes of the GAD65 autoantigen [48].

Current and future cell-based therapies

Recently, Andreas Lechner and colleagues failed to see transdifferentiation into pancreatic β cells after transplantation of bone-marrow cells into mice [49]. Last year, Jayaraj Rajagopal and colleagues failed to derive β cells from embryonic stem cells [50]. However, others have seen such effects [51].

CONCLUSION

To date, no fully defined and clinically applicable stem cell, tissue engeenring and adult β-cell stem/progenitor has been isolated. Nevertheless, studies of the development and the physiology of the pancreas make the existence of pancreatic stem/progenitor cells highly likely. Additionally, several potential candidate cells are being studied, and although more rigid experimental criteria have yet to be met, the published results look highly promising. The utilization of adult stem/progenitor cells for the generation of insulin-producing β-cells in vitro and their use for the treatment of diabetes, therefore, seem to be feasible in the near future.

DECLARATIONS

Authors' contributions

MB conceived the review, coordinated the overall activity, and reviewed the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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REFERENCES

1. Peloso A et al., *Regenerative medicine and diabetes: targeting the extracellular matrix beyond the stem cell approach and encapsulation technology*. Frontiers in endocrinology, 2018. 9.
2. Matveyenko A and Vella A. *Regenerative medicine in diabetes*. in *Mayo Clinic Proceedings*. 2015. Elsevier.
3. Bose B, Katikireddy R and Shenoy S. *Regenerative medicine for diabetes: differentiation of human pluripotent stem cells into functional β-cells in vitro and their proposed journey to clinical translation*, in *Vitamins & Hormones*. 2014, Elsevier. p. 223-248.
4. Kalofoutis C et al., *Type II diabetes mellitus and cardiovascular risk factors: Current therapeutic approaches.* Experimental & Clinical Cardiology, 2007. 12(1): p. 17.

5. Bruin E et al., *Treating diet-induced diabetes and obesity with human embryonic stem cell-derived pancreatic progenitor cells and antidiabetic drugs.* Stem Cell Reports, 2015. 4(4): p. 605-620.
6. Luo G et al. *MedSearch: a specialized search engine for medical information retrieval*. in *Proceedings of the 17th ACM conference on Information and knowledge management*. 2008. ACM.
7. Stumvoll M, Goldstein J and Van Haeften W. *Type 2 diabetes: principles of pathogenesis and therapy.* The Lancet, 2005. 365(9467): p. 1333-1346.
8. Association D. *Diagnosis and classification of diabetes mellitus.* Diabetes care, 2014. 37(Supplement 1): p. S81-S90.
9. Organization H. *Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part 1, Diagnosis and classification of diabetes mellitus*, 1999, Geneva: World health organization.
10. Bhonde R et al., *Making surrogate β-cells from mesenchymal stromal cells: perspectives and future endeavors.* The international journal of biochemistry & cell biology, 2014. 46: p. 90-102.
11. Tiwari K and Rao M. *Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects.* Current science, 2002: p. 30-38.
12. Peng Y et al., *Addressing stem cell therapeutic approaches in pathobiology of diabetes and its complications.* Journal of diabetes research, 2018. 2018.
13. Lilly A et al., *Current stem cell based therapies in diabetes.* American journal of stem cells, 2016. 5(3): p. 87.
14. Yanes O et al., *Metabolic oxidation regulates embryonic stem cell differentiation.* Nature chemical biology, 2010. 6(6): p. 411.
15. Ge Q, Chen L and Chen K. *Treatment of diabetes mellitus using iPS cells and spice polyphenols.* Journal of diabetes research, 2017. 2017.
16. Si Y et al., *Infusion of mesenchymal stem cells ameliorates hyperglycemia in type 2 diabetic rats: identification of a novel role in improving insulin sensitivity.* Diabetes, 2012. 61(6): p. 1616-1625.
17. Howard D et al., *Tissue engineering: strategies, stem cells and scaffolds.* Journal of anatomy, 2008. 213(1): p. 66-72.
18. Shimizu H et al., *Bioengineering of a functional sheet of islet cells for the treatment of diabetes mellitus.* Biomaterials, 2009. 30(30): p. 5943-5949.
19. Soria A, Skoudy and Martín F. *From stem cells to beta cells: new strategies in cell therapy of diabetes mellitus.* Diabetologia, 2001. 44(4): p. 407-415.
20. Castells-Sala C et al., *Current applications of tissue engineering in biomedicine.* Journal of Biochips & Tissue Chips, 2013(S2): p. 1.
21. Kobayashi T, Yuasa and Okitsu T. *Regenerative medicine for diabetes mellitus.* Cell Transplant, 2009. 18(5): p. 491-6.
22. Hussain A and Theise D. *Stem-cell therapy for diabetes mellitus.* The Lancet, 2004. 364(9429): p. 203-205.
23. El-Ashmawy E et al., *Effect of human umbilical cord blood-derived mononuclear cells on diabetic nephropathy in rats.* Biomedicine & Pharmacotherapy, 2018. 97: p. 1040-1045.
24. Abdulazeez S. *Diabetes treatment: A rapid review of the current and future scope of stem cell research.* Saudi Pharmaceutical Journal, 2015. 23(4): p. 333-340.
25. Salguero-Aranda C et al., *Differentiation of mouse embryonic stem cells toward functional pancreatic β-cell surrogates through epigenetic regulation of Pdx1 by nitric oxide.* Cell transplantation, 2016. 25(10): p. 1879-1892.
26. Seita J and Weissman L. *Hematopoietic stem cell: self-renewal versus differentiation.* Wiley Interdisciplinary Reviews: Systems Biology and Medicine, 2010. 2(6): p. 640-653.
27. Young E et al., *Adult reserve stem cells and their potential for tissue engineering.* Cell Biochemistry and Biophysics, 2004. 40(1): p. 1-80.
28. Sumi S et al., *Stem cells and regenerative medicine for diabetes mellitus.* Pancreas, 2004. 29(3): p. e85-e89.
29. Bara L *Tissue engineering a pancreatic substitute based on recombinant intestinal endocrine cells*, 2008, Georgia Institute of Technology.
30. Friedenstein J, Gorskaja and Kulagina N. *Fibroblast precursors in normal and irradiated mouse hematopoietic organs.* Experimental hematology, 1976. 4(5): p. 267-274.
31. Caplan I and Bruder P. *Mesenchymal stem cells: building blocks for molecular medicine in the 21st century.* Trends in molecular medicine, 2001. 7(6): p. 259-264.
32. Horwitz E et al., *Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement.* Cytotherapy, 2005. 7(5): p. 393-395.

33. Dezawa M et al., *Specific induction of neuronal cells from bone marrow stromal cells and application for autologous transplantation.* The Journal of clinical investigation, 2004. 113(12): p. 1701-1710.
34. Sanchez-Ramos J et al., *Adult bone marrow stromal cells differentiate into neural cells in vitro.* Experimental neurology, 2000. 164(2): p. 247-256.
35. D'Ippolito G et al., *Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential.* Journal of cell science, 2004. 117(14): p. 2971-2981.
36. Vija L et al., *Mesenchymal stem cells: stem cell therapy perspectives for type 1 diabetes.* Diabetes & metabolism, 2009. 35(2): p. 85-93.
37. Volarevic V et al., *Concise review: mesenchymal stem cell treatment of the complications of diabetes mellitus.* Stem cells, 2011. 29(1): p. 5-10.
38. Desgraz R and Herrera L, *Pancreatic neurogenin 3-expressing cells are unipotent islet precursors.* Development, 2009. 136(21): p. 3567-3574.
39. Teta M et al., *Very slow turnover of β-cells in aged adult mice.* Diabetes, 2005. 54(9): p. 2557-2567.
40. Robertson P. *Islet transplantation a decade later and strategies for filling a half-full glass.* Diabetes, 2010. 59(6): p. 1285-1291.
41. Deng S et al., *Islet alone vs. islet after kidney transplantation: metabolic outcomes and islet graft survival.* Transplantation, 2009. 88(6): p. 820.
42. Rood P et al., *Facilitating physiologic self-regeneration: a step beyond islet cell replacement.* Pharmaceutical research, 2006. 23(2): p. 227-242.
43. Thorel F et al., *Conversion of adult pancreatic α-cells to β-cells after extreme β-cell loss.* Nature, 2010. 464(7292): p. 1149.
44. Desgraz C, Bonal and Herrera L, *β-cell regeneration: the pancreatic intrinsic faculty.* Trends in Endocrinology & Metabolism, 2011. 22(1): p. 34-43.
45. Tisch R and McDevitt H. *Insulin-dependent diabetes mellitus.* Cell, 1996. 85(3): p. 291-297.
46. Calderon B et al., *Dendritic cells in islets of Langerhans constitutively present β cell-derived peptides bound to their class II MHC molecules.* Proceedings of the National Academy of Sciences, 2008. 105(16): p. 6121-6126.
47. Fändrich F et al., *Future strategies for tolerance induction:: A comparative study between hematopoietic stem cells and macrophages.* Human immunology, 2002. 63(10): p. 805-812.
48. Fändrich F and Ungefroren H. *Customized cell-based treatment options to combat autoimmunity and restore β-cell function in type 1 diabetes mellitus: current protocols and future perspectives*, in *The Islets of Langerhans*. 2010, Springer. p. 641-665.
49. Lechner A et al., *No evidence for significant transdifferentiation of bone marrow into pancreatic β-cells in vivo.* Diabetes, 2004. 53(3): p. 616-623.
50. Lobell B and Asner P, *Climate and management contributions to recent trends in US agricultural yields.* Science, 2003. 299(5609): p. 1032-1032.
51. Rajagopal J et al., *Insulin staining of ES cell progeny from insulin uptake.* Science, 2003. 299(5605): p. 363-363.