Examining the therapeutic potential of various stem cell sources for differentiation into insulin-producing cells to treat diabetes

Évaluer le potentiel thérapeutique de différentes sources de cellules souches susceptibles de se différencier en cellules productrices d’insuline pour la prise en charge des diabètes

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Abstract

Novel strategies are being developed to generate stem-cell-derived insulin-producing cells (IPCs), which could reverse the growing incidence of diabetes worldwide. We reviewed studies of stem-cell-based therapies for pancreatic β-cell regeneration published between 1997 and 2017. Differentiation into IPCs can be achieved using various stem-cell sources: embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and several types of adult stem cells such as pancreatic, hepatic and mesenchymal stem cells. However, reliable cell replacement therapy for diabetes is still in its early stages, and safety and ethical concerns are pressing issues. It will be necessary to find means of identifying optimal stem-cell sources and of inducing β-cell differentiation without using genetic mutations. The present article examines the potential of various stem-cell candidates for IPC generation, and the current obstacles preventing emergence of a candidate source.

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Résumé

Le développement en cours de nouvelles stratégies pour générer des cellules productrices d’insuline (CPI) à partir de cellules souches pourrait inverser la courbe de l’incidence du diabète dans le monde. Nous avons analysé la littérature pour la période de 1997 à 2017 concernant les thérapies fondées sur la régénération des cellules β pancréatiques à partir de cellules souches. La différenciation en CPI peut partir de différentes sources de cellules souches: cellules souches embryonnaires, cellules souches pluripotentes induites, ou diverses cellules souches adultes pancréatiques, hépatiques ou mésenchymateuses, etc. Cependant, une approche fiable au développement des thérapies de substitution cellulaire dans le diabète reste encore balbutiante, et les questions de sécurité et d’éthique sont au premier plan. Il sera nécessaire de résoudre ces défis pour identifier les meilleures sources de cellules souches et pouvoir induire la différenciation en cellules β sans avoir recours aux mutations génétiques. Dans cet article, nous analysons le potentiel de diverses cellules souches comme candidats à la différenciation en CPI, ainsi que les obstacles qui empêchent actuellement l’identification de sources optimales.

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Keywords : Cell therapy ; Diabetes ; Insulin-producing cells ; Reprogramming ; Stem cells

Mots clés : Cellules productrices d’insuline ; Cellules souches ; Diabète ; Reprogrammation ; Thérapie cellulaire

1. Introduction

Type 1 diabetes occurs when β-cell mass is reduced to less than 20% of the normal level due to autoimmune destruction of cells, resulting in the inability to secrete insulin. In contrast, type 2 diabetes is characterized by insulin resistance and a decline in

β-cell function that progresses over time to an eventual loss of β-cells due to apoptosis [1,2]. Thus, preservation or replenishment of a functional β-cell mass has become a major therapeutic focus for both type 1 and 2 diabetes. Although pancreas or islet transplantation has demonstrated substantial potential for the treatment of diabetes, a critical obstacle is the shortage of organ donors. As a result, stem cells, which are a renewable source of multi- and pluripotent cells, could be employed to enrich the β-cell population and add the possibility of islet transplantation [3]. Novel strategies are currently being developed with the aim of generating stem cell-derived insulin-producing cells (IPCs), which may potentially reverse diabetes worldwide [4]. Thus, stem cell therapy focusing on the assistance of cellular reprogramming and β-cell regeneration will offer new therapeutic modalities for the treatment of diabetes [5]. Therefore, this article elaborates on the potential of various stem cells as candidates for the generation of IPCs as well as the current obstacles that exist for their therapeutic application.

2. Stem cells as a source of IPCs

Stem cells, due to their potential for multiple differentiation and self-renewal can differentiate into all cell types of the human body, including insulin-producing β cells. Thus, stem cells are a promising source for the production of new β cells [6]. To date, using various approaches, researchers have tested the ability of different stem cells to differentiate into more specialized cell types. Differentiation to IPCs can be achieved from various stem cell sources, including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSC), and adult stem cells, such as pancreatic stem cells, hepatic stem cells, and mesenchymal stem cells (MSCs) (Fig. 1). Furthermore, the potential of a variety of stem cells to generate surrogate β cells or to restore β-cell function has been widely studied (Fig. 1). The advantages and disadvantages of these current stem cell-based methodologies for diabetes are summarized in Table 1.

3. Embryonic stem cells (ESCs)

From among the various types of stem cells, ESCs retain the highest plasticity and possess the potential to differentiate into all of the cell lineages of the body. This, coupled with their unlimited capacity for self-renewal, make ESCs a promising source of insulin-producing cells (IPCs), and also bring obvious safety and ethical concerns. Moreover, cell differentiation may cause changes in the secretome and phenotype that could induce an immune response by the host, thereby requiring immunosuppressive therapy. Advances in the development of efficient protocols for the production of ESC-derived pancreatic β-cells depend on the application of the extensive knowledge acquired about the common mechanisms of pancreas development. Initial in vitro studies reported the discovery of spontaneous differentiation of IPCs from human ESCs, but this occurred with low efficiency [6]. How this process occurs was elaborated on in a previous study where ESCs were found to differentiate into IPCs through five main stages: definitive endoderm, foregut, hindgut, endoderm of the pancreas, and then into endocrine cells, which is in accordance with the features of pancreas development [7]. Indeed, the differentiated cell clusters, composed of glucagon-, somatostatin-, and insulin-positive cells, were able to release insulin in response to glucose, which signifies their potential for use as a candidate stem cell source to treat diabetes.

An in vivo study demonstrated that pancreatic endocrine progenitor cells developed from ESCs can go on to differentiate into IPCs when transplanted in mice, and that they respond to changes in serum glucose concentrations [7]. In an earlier experiment using a differentiation protocol for neuronal progenitor cells, limited nestin-positive ESCs were observed to differentiate into β-like cells. Nevertheless, exceedingly few cells were positive for insulin gene expression and the cell aggregates obtained lacked expression of pancreatic endocrine transcription factor Pdx-1 (pancreas/duodenum homeobox protein 1). Moreover, a majority of the cells disappointedly possessed a neuronal phenotype [8]. Nonetheless, the differentiation and maturation of these cells continued following their in vivo transplantation in mice and further differentiation of these cells in vivo lead to their ability to secrete insulin. Consistent with the findings of Kahan et al., this study indicated that human ESCs have the potential to respond to environmental signals and can differentiate further under the direction of these environmental signals, in this case, giving rise to endocrine islet cells [9]. Some studies which also examine the relationship between the murine and human derived cells cultured in vivo, reveal that there is a conserved islet development mechanism in both mice and humans. In support of this, a previous study found that IPCs which were obtained through indirect co-culture with islets for 30 days could respond to glucose stimulation under physiological conditions. The results of immunocytochemistry and gene expression analyses revealed that indirect co-culture led to higher differentiation efficiency than chemical induction, mainly due to the presence of soluble factors and signals released from the mouse islets. Because of the lack of knowledge on the mechanisms of functional in vivo islet maturation, the method that is the most effective in the promotion of in vitro islet maturity has yet to be identified. To confirm the maturity and curative effect of differentiated cells, induction of ESCs into pancreatic progenitor cells equivalent to 6 to 9-week fetal pancreatic tissue was performed before undergoing transplantation into mice to undergo subsequent maturation [7]. On day 40
following transplantation, glucose stimulation was shown to cause the C-peptide to be released and was detectable in the recipient mice, indicating that human insulin was produced in mice through de novo synthesis. It is noteworthy that these cells had the ability to amend streptozotocin-induced hyperglycemia in these mice.

The study by Kroon et al. showed that the stage of pancreatic progenitors can be determined in vitro, but to obtain further differentiation into bonafide endocrine islet cells, transplantation into mice was needed [7]. Although functional β-cells were produced in some studies, efficiency was low or there was a failure to generate highly selective β-cells. In more recent investigations, great efforts have been made to improve the effectiveness of ESC differentiation into pancreatic progenitor cells [10]. Moreover, when undifferentiated cells are transplanted, rigorous assessment of the oncogenic risk is needed [11].

However, it has been reported that tumourigenicity of ESCs potentially exists, which raises a concern not only to the graft but also to the health of the patient. The rate of teratoma formation is highly variable depending on cell maturation, purity, and the implantation techniques [7]. Cell purification techniques, such as fluorescence-activated cell sorting, magnetic-activated cell sorting, genetic selection, cell surface markers, and reporter ESC lines can help prevent the implantation of undifferentiated cells, thus decreasing the risk of oncogenesis [12]. Additionally, the ethical debate surrounding the harvest of hESCs has made the research on this topic controversial, and as a result, the majority of studies have focused on animal models.

4. Pluripotent stem cells (iPSCs)

Somatic cells that are transformed into pluripotent stem cells under specific conditions are known as iPSCs and are considered an appropriate source for the generation of large quantities of β-cells from an autologous non-embryonic origin [13]. The most commonly used approach for iPSC generation is the integration of genetic material into the host genome through the use of viral vectors. However, this approach incurs an intrinsic risk of tumor development due to the possibility of reactivation of viral transgenes. Many approaches to circumvent the utilization of viral vectors have been examined, including the use of non-integrating and excisable vector systems [14]. Nevertheless, even if these approaches are successful, it is possible that autologous iPSCs would still be subject to autoimmune responses after transplantation. However, recent advances have been introduced to improve the safety and to enhance the potential clinical utilization of iPSCs [15]. Melton et al. showed that functional human stem-cell-derived β cells (SC-β) can be directly generated from human pluripotent stem cells in vitro and these cells function similarly to primary human β cells both in vitro and in vivo posttransplantation. SC-β secrete quantities of insulin comparable to adult β cells in response to multiple sequential glucose challenges in vitro. Furthermore, these cells secrete human insulin into the serum of mice shortly after transplantation in a glucose-regulated manner, and transplantation of these cells obviously ameliorates hyperglycemia in diabetic mice [16].

A more recent study from this group showed that functional SC-β cells can also be generated from hiPSC derived from T1D patients in vitro. These SC-β cells respond to known anti-diabetic drugs that cause increased insulin secretion. The results from this study suggested that T1D SC-β cells can potentially be used for the treatment of diabetes, drug screening and the study of β-cell biology [17]. Furthermore, protocols have been developed to enhance the efficient differentiation of pluripotent cells toward development into functional islet cells. These employed strategies have utilized advances in the understanding of the underlying mechanisms that regulate pancreatic development in mice [18].

Table 1
Summary of regenerative methodologies.

<table>
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<th>Advantages</th>
<th>Disadvantages</th>
<th>Reference</th>
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<tbody>
<tr>
<td>ESCs</td>
<td>Pluripotent differentiation capacity with unlimited multiplicative ability</td>
<td>Ethical constraints; highest risk for teratoma formation; elicits an autoimmune response</td>
<td>[16]</td>
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<tr>
<td>iPSCs</td>
<td>β-cell replicates can be generated without ethical controversy; easily accessible stem cell source of iPCs</td>
<td>Mutagenic potential of some reprogramming methods; barriers to long-term transplant viability and functionality; risk for teratoma formation, expression of more than one hormone and of an immature non-glucose-responsive phenotype</td>
<td>[63–65]</td>
</tr>
<tr>
<td>MSCs</td>
<td>Improved β-cell function through immunomodulation; low risk of autoimmune response due to lack of MHC II complexes; lower oncogenic risk than that of iPSCs/ESCs due to limited differentiation capacity</td>
<td>Incomplete and temporary therapeutic effect; requires chronic administration and adjunct therapy</td>
<td>[66]</td>
</tr>
<tr>
<td>Pancreatic adult stem cell</td>
<td>Differentiation to structures that resemble islets; multipotency and clonogenic potential</td>
<td>Specific markers for isolation and identification of these cell population are needed</td>
<td>[29,30,67]</td>
</tr>
<tr>
<td>Hepatic stem cells</td>
<td>Improved β-cell function using small molecules; the induced iPCs ameliorated hyperglycemia in diabetic rats</td>
<td>Low efficiency, nonselective effect</td>
<td>[54,68]</td>
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ESCs: embryonic stem cells; iPSCs: induced pluripotent stem cells; MSCs: mesenchymal stem cells.
A recent examination with substantial clinical significance, presented a novel strategy for transplanted iPSCs to produce transforming growth factor- and IL-10-secreting Tregs that were able to remarkably inhibit the immune responses of the host following their adoptive transfer into mice. Human iPSCs obtained by reprogramming human somatic cells, such as skin fibroblasts, may be a good alternative for human ESCs, thereby eliminating the ethical issues pertaining to ESCs. Stadtfeld et al. developed an adenovirus-based method for the production of iPSCs [19]. The successful conversion of stem cells into mature insulin-producing pancreatic cells is evaluated by the expression of various factors, but importantly, by PDX1 and NKX6-1 (NK-6 homebox 1) that are known to be essential determinants of mature β-cell function [20]. Retroviral delivery has traditionally been preferred for the generation of iPSCs due to its high efficiency. However, retroviral integration and tumorigenesis associated with proto-oncogene factors would severely limit its clinical application. However, other reagents, including the RNA-based Sendai virus (SeV), enable even greater genetic integrity [21]. Using this technique, no exogenous gene integration occurs because no exogenous genes are utilized in the conversion process. Another investigation revealed that human iPSCs have the potential to generate insulin-producing cells and that these differentiated cells can engraft and secrete insulin in vivo [22]. Taken together, these studies address the ethical and safety concerns surrounding iPSC therapy as well as its potential efficacy for application in the treatment of diabetes [23]. However, endocrine cells derived from iPSCs through in vitro differentiation frequently express non-selectively and display immature phenotype endocrine genes [24,25].

5. Adult pancreatic stem cells

Adult pancreatic stem cells are another potential source of β-cells because they possess the characteristics of multipotency and clonogenic potential. The pancreas is a source of the stemness potential of stem cells and shares identical embryological origin with β-cells, including duct cells, acinar cells, and stem cells, which can differentiate and be re-programmed to ensure the manifestation of a phenotype producing insulin [26]. However, advanced strategies are needed to isolate and grow adult pancreatic stem cells, and to differentiate them into β cells. Epithelial cells of the pancreatic duct were harvested and induced in vitro to become functional islets [27]. The resultant β-cells exhibit insulin secretion and glucose-dependent reactivity. Yet, future attempts to resolve the challenges in the harvest, purification, and proliferation of various pancreatic progenitor cell populations as well as induction of β-cell differentiation without genetic mutations need to improve the use of adult pancreatic stem cells in the treatment of diabetes.

Exocrine pancreatic cells, pancreatic ducts, and the islets of Langerhans have all been suggested as potential pancreatic stem/progenitor cell sources. Despite the initial controversies over their nature and even their existence [28], the successful differentiation of adult stem cells of the pancreas into islet-like cells has been observed. In previous studies, human pancreatic duct cells were able to proliferate in vitro and to differentiate into IPCs [29,30]. Furthermore, ductal progenitors were found to be able to generate into mature ductal epithelial cells after partial pancreatectomy in diabetic mice [31]. These results suggest that stem/progenitor cells exist within the pancreas and these cells might be a promising source of new islets. However, the identification of specific markers is definitely needed to improve the isolation of these cell populations.

α cells may represent a reservoir of pre-β-cells in the adult [32] and presents reversible epigenetic suppression of β-cell genes. Treating human islets with the methyltransferase inhibitor results in partial α-to-β reprogramming [33]. Thorel et al. demonstrated that upon loss of β-cells, genetically marked α cells rapidly coexpress Nkx6.1, followed by expression of insulin and the adult β-cell markers Pdx1, Nkx6.1 and Glut2, subsequently forming the majority of regenerated β cells [34]. Nouha et al. reported that GABA induces the conversion of α cells into β-like cells through the downregulation of Arx expression. Long-term GABA exposure results in a significant increase in islet size and number due to a β-like cell hyperplasia and the treatment of transplanted human islets with GABA results in a loss of α cells and a concomitant increase in β-like cell counts [35].

β-cell mass restoration might be a promising diabetic treatment. It was reported that β-cell proliferation is the principal mechanism by which regeneration after pancreatic injury is achieved. GLP-1 and its analogs, such as exendin-4, can stimulate the regeneration of the pancreas and expansion of β-cell mass by the processes of both neogenesis and proliferation of β cells [36]. Rooman et al. showed that combined treatment with gastrin and epidermal growth factor can induce sufficient regeneration of a functional islet mass to restore glucose homeostasis in C57Bl/6J mice treated with alloxan [37].

6. Stem cells of the mesenchymal lineage

Mesenchymal stem cells (MSCs) can be obtained from various tissues such as bone marrow, the umbilical cord, the placenta, and adipose tissues. They are able to substantially expand in vitro and generate multiple cell lineages, although their exact nature and functions remain enigmatic [38]. However, advantageously, MSCs are considered to allow minimal risks of tumorigenesis [39–41].

MSCs that are isolated from the exocrine tissue of adult human pancreases have been found to express cell surface antigens identical to those derived from bone marrow, the umbilical cord blood, and adipose tissue. Moreover, MSCs from various sources have equivalent differentiation potential to endodermic and mesodermic cell lineages. The successful application of bone marrow-derived MSCs for the treatment of diabetes may result from the endocrine or paracrine mechanisms that assist existing cells, stimulate proliferation and insulin secretion, and effectively control blood glucose levels, rather than by direct differentiation into β cells. MSCs appear to preferentially home to sites of injury, facilitating the repair and the survival of neighboring cells [42–44]. Importantly, MSCs are able to secrete a number of factors, including chemokines, cytokines, and growth factors that improve the tissue microenvironment under injury conditions [45–48]. The trophic and paracrine effects of MSCs
are now considered of greater significance for tissue repair and regeneration than their ability of trans-differentiation when considered for application as a diabetes therapeutic. Several studies have reported that MSCs are a readily available source of insulin-producing cells [49–51]. Xie et al. utilized the rat injured pancreatic tissue extract to modulate rat bone marrow-derived MSCs differentiation into IPCs by traditional two-step induction. Though the insulin release in IPCs is only one-tenth of natural islet, when transplanted into the renal capsule, the IPCs were functional in vivo and capable of reversing hyperglycemia in diabetic rats [52].

Despite the existence of diverse, unclear mechanisms for their actions, bone marrow stem cells potentially promote the regeneration of injured pancreatic β-cells or can be used as an optional cell source for β-cells after induction. Undisputedly, IPCs derived from bone marrow stem cells have therapeutic effects against the treatment of diabetes. Ianus et al. reported that transplanted bone marrow cells could differentiate into functional pancreatic β-cells in vivo without cell fusion [53].

7. Hepatic stem cells

Liver-to-endocrine pancreas transdifferentiation represents a promising approach for the generation of β-cell surrogates, owing to the shared common bipotential precursors within the embryonic endoderm that exists between the liver and pancreas [41,48]. The majority of the documented methods for induction necessitated gene manipulations; thus, the cells obtained after the induction were non-selective for β-cell phenotypes and function, Yang et al. demonstrated that the pancreatic endocrine hormone producing cells derived from adult hepatic stem cells express markers of endocrine pancreas, including PDX-1, PAX-4, PAX-6, Nkx2.2 and Nkx6.1, as well as the endocrine hormones insulin, glucagon, and somatostatin [54]. Jin et al., recently reported that immortalized liver epithelial progenitor cells that were derived from regenerative liver could differentiate into insulin-positive cells through stable transduction with PDX-1 and subsequent treatment with various growth factors. These cells secreted insulin in response to glucose and transplantation of these cells could ameliorate streptozotocin-induced diabetes in severe combined immune deficiency (SCID) mice [55].

Chen et al., found that without the use of genetic or epigenetic manipulations, cultured mature hepatocytes were able to dedifferentiate into adult liver progenitors. In their study, highly-purified mature hepatocytes, collected from the liver of a healthy rat were labeled with a fluorescent dye PKH2. The obtained liver-derived progenitor cells were identified as PKH2-positive and shared phenotypic similarities with oval cells, which were reported earlier to have the potential to generate into mature hepatocytes both in vivo and in vitro [56]. More recently, Liu et al. demonstrated that small molecules can be used to differentiate rat hepatic stem-like cells into IPCs that expressed distinct pancreatic β-cell marker genes. Furthermore, insulin is released in response to certain glucose concentrations and the induced IPCs can ameliorate hyperglycemia in diabetic rats [57]. The identification of a chemical strategy implemented for the induction of hepatocyte-derived IPCs is an important approach for diabetic cell therapy in the near future.

8. Conclusions

Recent advances in the generation of cells for therapeutic purposes have enhanced expectations of producing substantial amounts of glucose-responsive β-cells that can be used for the treatment of diabetes. However, the establishment of a trustworthy cellular replacement approach for diabetes treatment is still in its beginning stages. Recent studies have highlighted the need for future studies worldwide to overcome two main challenges of cell-based therapy: the efficiency and safety of transplanted cells [56,58–61]. Regretfully, implantation-related unfavorable effects, such as tumorigenesis or immune rejection, may greatly hinder the implementation of these cell-based therapy strategies. Despite certain obstacles existing in size, porosity, and biocompatibility of the materials, encouraging results have been obtained from the combined application of some novel techniques such as encapsulation and the administration of moderate immunosuppressants, which improve the survival and functionality of the encapsulated transplanted cells. Adult stem cells are cell sources with a stemness ability lower than that of ESCs, but are considerably safer and exempt from ethical considerations. Nevertheless, there are a number of impediments for extensive clinical application of these cells, including stem cell shortage, invasiveness of the procedures for collection, and difficulties in the isolation and in vitro expansion of these cells. MSC investigations have received much attention due to their easy isolation and extensive capacity for in vitro expansion. The encouraging outcomes from studies revealed that MSCs have significant potential for differentiation into IPCs. Yet, MSC-derived IPCs barely improve the long-term outcomes of streptozotocin-induced diabetic mice [62]. There has been a continuous search for stem cell sources with optimal properties, such as differentiation efficiency, post-transplantation safety, broad accessibility, and low complexity of procedures involved. Each of the IPC strategies to date has their merits and drawbacks. Thus, it is not possible to determine exactly which one of them will be successful in various induced methods; however, even under the best circumstances, extensive testing of stem cell/somatic cell-derived candidates from the human pancreas could not be performed to show that these induced cells that fully replicate all functions of the endogenous cells. Although we have taken enormous steps toward generating such cells, but owing to not completely functional cells and relatively low efficiency induce, more significant work remains to be done before cell transplantation of stem cell-derived cells becomes a reality.

Disclosure of interest

The authors declare that they have no competing interest.
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