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Mini Review

Regeneration of Islet Beta-Cells from Stem Cells and Progenitors

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Abstract

The diabetes population sizes increasing rapidly all over the world. The transplant of insulin-producing β-cells into diabetic patients is considered to be a promising alternative to traditional insulin therapy for diabetes treatment. Pancreatic β-cell is one of the most important cell types in the islet of Langerhans in the pancreas, which is responsible for insulin secretion in leveraging blood glucose level. However, cell-based therapy for diabetes treatment is currently impeded due largely to the inability to produce large amounts of biologically functional β-cells through *in vitro* cultivation system. Human stem cells, including induced pluripotent stem cells, adult stem cells, and progenitor cells, have been considered to be promising cell sources for generating insulin secreting β-cells. Nevertheless, generating functional β-cells from these cell types remains a significant challenge. This article provides updated research results on the current status of the search for efficient and reproducible islet β-cell production approaches from stem cell and progenitor differentiation.

Keywords: Human stem cells; Progenitor; β-cells; Insulin; Differentiation; De-differentiation; Culture; Re-differentiation

Introduction

Diabetes mellitus has become a global epidemic in recent years. It has been predicted that over 300 million people worldwide will be diagnosed with type I or type II diabetes by the year 2025, and the most common form will be type 2 diabetes [1]. These diseases induce other diseases, including heart disease and stroke, high blood pressure, kidney disease, and blindness. Currently, the popular treatment for type I diabetes is limited to insulin supplement by either tablet or injection, while medical treatment for type II diabetes is mainly made up of closely monitoring blood glucose levels, diet, and exercise [2,3]. The transplant of islets into diabetic patients is a promising alternative to traditional insulin therapy. Unfortunately, there is a severe scarcity of donor islet tissues needed for β-cell transplant. This shortage has led to considerable efforts in searching for efficient methodologies for the effective treatment of both types of the disease. One of the major challenges that remain in β-cell therapy is the extremely low proliferation rate. Artificially elevating the proliferation rate can cause the β-cells to lose their original biological functions, including the ability to produce insulin [4]. Furthermore, the origin of islet cell neogenesis remains a mystery. Hence, the appropriate niches required for human β-cell *in vitro* generation and further expansion, as well as progenitors for producing β-cells, has been one of the major focuses of human β-cell regeneration research.

In the field of regenerative medicine, there are four potential types of cell sources that can produce β-cells suitable as cell sources for transplantation. They are stem cells, endocrine progenitors, other mature cells, and β-cell itself [5]. This article describes updated research results on the progress of seeking for efficient and reproducible islet β-cell mass production approach via the formation of new β-cells (neogenesis) or proliferation from these cell sources.

Pluripotent Stem Cell Differentiation into Insulin Secreting β-cells

The human embryonic stem cell (hESC) and its counterpart, named induced pluripotent stem cell (iPSC), are considered to be promising sources for β-cell generation *in vitro*. Generating insulin-producing β-cells from *in vitro* hESCs/iPSCs differentiation has been focused on creating tissue niches that mimic the *in vivo* microenvironments. It has been achieved through the stepwise differentiation media by utilizing knowledge accumulated from the last decade in studying mouse and other vertebrates' embryonic developments [6,7]. Four major steps are required to develop insulin-producing cells from human pluripotent stem cells (hPSCs), i.e. definitive endoderm formation, pancreatic progenitor formation, endocrine specification, and β-cell formation [8,9]. This step-wised approach has been widely adopted by many research groups with modifications to generate glucose-responsive, insulin-producing cells [10-14]. Critically, existing protocols are not straightforward. Consistent reproducibility is extremely poor between groups and experiments and no more than 10 % of cells in the hPSC-derived cells are insulin-positive. Thus, the differentiation of hESCs into insulin-secreting cells remains to be a big challenge due to 1) the lack of knowledge on the pathways involved in human pancreatic development; 2) the limited information about the signals essential for stimulating the development of pancreatic lineage. Hence, insulin-producing β-cell development from hPSC differentiation is still under improvement and investigation. The optimization of hPSC differentiation protocols, such as adjusting oxygen supply, using three dimensional culture platform to mimic physiological conditions in the body, and leveraging signaling pathways, have also been extensively explored with moderate progress [15-17]. Often, insulin secretion from these hPSC-derived cells could not respond

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to glucose levels. In addition, the risk of teratoma formation *in vitro* remains a major concern if therapeutic β-cells are produced from hESCs or iPSCs [18].

Endocrine Progenitors for Producing Islet β-cells

The pancreas is an endocrine organ with duct and three types of endocrine cells that release hormones into the bloodstream. They are α-cells which secrete glucagon, β-cells which secrete insulin, and δ-cells which inhibit the secretion of glucagon and insulin. Ductal cells are multipotent progenitors. The ductal epithelium has been believed to be one of the cell sources that can trans-differentiate into β-cells [19,20]. Ductal epithelial cells can differentiate into endocrine cells and release insulin in response to glucose [20]. Activin A and exendin-4 stimulate the trans-differentiation of human pancreatic ductal cells to β-cells [21]. A recent study reported that the vagal nerve is involved in the induction of endocrine progenitors and the neogenesis of β-cells [22]. Moreover, data suggested that β-cells can be generated from duct-lining acinar cells [23]. Signaling molecules such as growth factors and cytokines have been proven to be vital stimuli for β -cell regeneration. For instance, treatment of epidermal growth factor and ciliaryneurotrophic factor to diabetic mice promotes β-cell regeneration from acinar cells [24]. *In vivo* animal studies showed that human β-cells, along with acinar cells and ducts, can be generated after human embryonic pancreas is engrafted under the kidney capsule of *experimental* mice. In this model, β-cells come from duodenal homeobox 1 (PDX1)-expressing pancreatic progenitors [25]. However, factors that activate the proliferation of PDX1-expressing progenitor cells have not been identified. When human fetal pancreatic progenitor cells are isolated from the fetal pancreas; they are able to expand and differentiate into islet endocrine cells in the culture and generate insulin producing cells [26]. It should be noticed that the ductal origin for islet cells during neonatal growth remains controversial [27]. For instance, lineage tracing studies using genetic labeling confirmed that pancreatic ductal epithelium does not contribute to endocrine nor acinar cells during neonatal growth, indicating that the restricted plasticity of ductal epithelium and new β-cells must have originated from a source other than the ductal epithelium [28].

Intriguingly, trans-differentiation from acinar cells to β-cells occurs when micro environmental changes are present [29], even though the yield of trans-differentiation is usually very low [30]. Trans-differentiation can be significantly improved if the Notch signal pathway is inactivated [31]. β-cells cultured *in vitro* can dedifferentiate through epidermal-mesenchymal transition (EMT), a biological process that allows a polarized epithelial cell to go through multiple biochemical changes and convert to a mesenchymal cell phenotype [32]. These dedifferentiated cells can then be directed to re-differentiate into β-cells. This is indeed a reversible epithelial-to-mesenchymal transition. Utilizing this method, a two stage expansion protocol was reported for the mass proliferation of human insulin-producing cell. In this approach, a mixed islet cell population with up to 45% isolated cells was expanded for 16 doubling times. At the second expansion stage, the cells were re-differentiated into insulin secreting cells by using small molecules [33]. However, using this reversible EMT approach for β-cell production is debatable. Some groups reported failure to re-differentiate dedifferentiated cells into insulin-secreting β-cells [34]. Extensive reviews on β-cell neogenesis *in vivo* and proliferation *in vitro* can be found in previous reports [18,35]. In another approach, a model converting islet α-cells to β-cells has been developed by combining the pancreatic duct ligation, suggesting that α-cells can be β-cell progenitors [36]. Indeed, the regeneration of β-cells from α-cells occurs when β-cells are near totally ablated in experimental animals [37].

Other Potential Progenitor Cells for Islet β-Cell Production

Another focus in generating functional β-cells is to find cell types that can be converted into new β-cells for replacement therapy. When ttranscription factors PDX1, Ngn3, and MafA were over expressed in the livers of diabetic mice, the liver cells were reprogrammed into insulin-secretion cells and normal blood glucose levels were maintained for more than 14 weeks [38]. These three transcription factors were found to be the keys in reprogramming pancreatic exocrine cells into β-cells *in vivo* [39]. In another report, fetal human progenitor liver cells can differentiate into β-cells after having expressed the pancreatic PDX1 gene [40]. Upon transplantation of these genetically modified fetal progenitor liver cells to hyperglycemic immune deficient mice, they can replace β-cell functions in diabetic mice and maintain a stable blood glucose level in 70 days of experimental period [40]. This study indicates a different source of cells in patient transplantation for diabetes treatment. In addition, experimental mice and pigs were given a human insulin expression vector in the livers to investigate whether the vector can induce the livers into pancreatic trans-differentiation. As such, pancreatic trans-differentiation of liver tissues in mouse and pig occurred and the liver tissues expressed several β-cell transcription factors, such as PDX-1 and Neurod1, pancreatic hormones, glucagon, and somatostatin [41,42].

A very recent lineage-tracing study in mice discovered that β-cells dedifferentiate to neurogenin3-positive and insulin-negative cells when mice are in diabetes status. More importantly, these cells can later re-differentiate into mature neurogenin3-negative, insulin-positive β-cells after insulin therapy [43]. Additionally, this study clearly demonstrated that the main mechanism of insulin-positive cell loss in diabetes is β-cell dedifferentiation, rather than apoptosis [43]. Hence, β-cells can be directed to re-differentiate into insulin-secreting cells when they are in appropriate physiological conditions. A multistep protocol has been developed to generate insulin-producing islet-like cell clusters from human adipose tissue-derived stromal cells [44]. However, it requires further evidence and study on adipose tissue trans-differentiation into β-cells.

Conclusion

Seeking novel treatments to control the increasing rate of diabetes is still a significant challenge due to unavailable technology to generate sufficient biologically functional β-cells that can replace damaged or malfunctional islets. It remains unclear, however, which cell source will ultimately be proved to be successful in generating β-cells for clinical applications. Alternative strategy is to increase the number of the insulin-producing β-cells. Screening for enhancers of β-cell regeneration results in the discovery of the adenosine agonist

5-N-Ethylcarboxamidoadenosine (NECA), which shows promising in enhancing both β-cell proliferation and regeneration in diabetic mice [45]. Additionally, a class of compounds (adenosine kinase inhibitors (ADK-Is)) has been identified to be able to stimulate proliferation of primary β-cells in the species of mouse, rat, and pig [46]. Detailed reviews regarding a number of growth factors, transcription factors, as well as signaling pathways that facilitate and increase β-cell mass have been summarized elsewhere [18]. Continuous efforts should be made to restrain β-cell death and promote β-cell performance by improving niches and culture platforms. Alternatively, since the most common form of diabetes is type II diabetes, it is crucial to understand the mechanism of why β-cells become dysfunctional in order to optimize the treatment options. One hypothesis developed recently is that β-cells de-differentiate and return back to an immature state in the body. Mice study discovered that β-cells under physiologic stress, such as aging, can de-differentiate to a progenitor-like, multipotent development stage [47]. Thus, to develop a treatment that can stimulate the re-differentiation of β-cells is highly desired for β-cell regeneration. Knowledge on signaling pathways that promote re-differentiation would accelerate research in this direction.

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