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Focus on: The Endocrine Pancreas

Review

Lineage Reprogramming: A Promising Road for Pancreatic β Cell Regeneration

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Cell replacement therapy is a promising method to restore pancreatic β cell function and cure diabetes. Distantly related cells (fibroblasts, keratinocytes, and muscle cells) and developmentally related cells (hepatocytes, gastrointestinal, and pancreatic exocrine cells) have been successfully reprogrammed into β cells *in vitro* and *in vivo*. However, while some reprogrammed β cells bear similarities to *bona fide* β cells, others do not develop into fully functional β cells. Here we review various strategies currently used for β cell reprogramming, including ectopic expression of specific transcription factors associated with islet development, repression of maintenance factors of host cells, regulation of epigenetic modifications, and microenvironmental changes. Development of simple and efficient reprogramming methods is a key priority for developing fully functional β cells suitable for cell replacement therapy.

Cell Replacement Therapy in Diabetes Treatment

Diabetes is a chronic disease with global incidence. One of the most important strategies to treat diabetes is to recover functional pancreatic β cell mass, among which islet transplantation serves as an attractive method [1]. However, its clinical application is attenuated by the scarcity of human donor pancreas and the occurrence of immune rejection. Generating alternative sources of β cells is an appealing method for **cell replacement therapy** (see Glossary) for the treatment of type 1 diabetes, as well as severe, insulin-dependent type 2 diabetes. **Stem cells**, with their ability of self-renewal and differentiation, represent promising tools for cell-based therapies. Pluripotent stem cells (PSCs), including **embryonic stem cells (ESCs)** and **induced pluripotent stem cells (iPSCs)**, have the ability to differentiate into cells of all three germ layers, including islet β cells. The differentiated β-like cells could express specific transcription factors, exhibit glucose-stimulated insulin secretion (GSIS), form β cell-like secretory granules, and were able to attenuate or correct hyperglycemia when transplanted into diabetic mice [2]. However, in most differentiation strategies, it is difficult to obtain fully functional β cells, indicated by the presence of multihormonal cells and of immature secretory granules [2–4], and their differentiation efficiency is often low [2–4]. Recently, remarkable progress has been made in inducing differentiation of human PSCs into β cells with high efficiency and maturity [4,5]. Nevertheless, PSCs have the risk of teratoma formation. Thus, it is critical that differentiated β cells destined for transplantation are not contaminated with PSCs.

Somatic stem or progenitor cells are responsible for normal cell renewal and tissue repair, and have low potential for tumor formation [6]. For example, β cells can be generated from

Trends

Readily available cells, such as fibroblasts and blood cells, might be used for *in vitro* reprogramming into β cells in patient-specific transplantation.

In vivo β cell reprogramming will potentially be an important strategy for β cell regeneration. At present, the leading cell contenders for successful therapeutic transformation into β cells appear to be pancreatic endocrine α cells, exocrine acinar cells, and enteroendocrine cells.

For instructive strategies, complete small molecule-based reprogramming independent of gene manipulations will need to be extensively investigated with the goal to obtain fully functional β cells for clinical application.

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endogenous pancreatic progenitors in injured adult mouse pancreas [7]. Somatic stem cells also have the ability to differentiate into other cell lineages. For instance, mesenchymal stem cells could be induced to secrete insulin [8]. However, somatic stem cells often have limited ability for proliferation, thus a large amount of cells are needed to acquire enough target cells for transplantation [6]. In addition, the presence and origin of some tissue-specific progenitors remain unverified and hotly debated, and absence of accurate markers makes their isolation difficult [9,10]. Differentiation protocols for somatic stem cells vary between different laboratories, efficiencies are usually low, and the acquired β cells are immature [10]. Notably, β cells have the ability to dedifferentiate and redifferentiate [11] (Box 1), which protects β cells from apoptosis during stress, and retains β cell properties and optimal β cell mass once stress is removed [12]. Nevertheless, the redifferentiation strategies failed to restore β cell phenotype *in vitro* [13]. While both stem cell differentiation and β cell plasticity might provide attainable β-like cells for cell therapy in diabetes, lineage reprogramming of non-β cells opens new possibilities to develop potentially fully functional β cells to be used for transplantation.

Lineage Reprogramming: An Innovative Technique in Diabetes Treatment

Studies on iPSCs have demonstrated that differentiated cells are not always permanently committed to their ultimate differentiation state but maintain the potential to convert into primitive stem cells under specific conditions [14]. Cell fate conversion between mature cells can also be achieved by lineage reprogramming, which is an innovative approach to generate cells with identical immunological profiles. Lineage reprogramming includes direct reprogramming, a process that triggers activation of pathways related to pluripotent cells, temporarily driving mature cells toward the pluripotent stage; these cells are then directed toward a lineage-specific path. Another approach is transdifferentiation, a process whereby a fully differentiated cell is reprogrammed into another cell type without reverting to the pluripotent stage. In this review, we focus on recent studies describing lineage reprogramming of different cell types, including distantly related cells (e.g., fibroblast and keratinocytes) and developmentally related cells (e.g., liver, gastrointestinal, and pancreatic exocrine cells). Using lineage reprogramming, different cell types can be converted into functional β cells, which can potentially be used for the treatment of both type 1 and type 2 diabetes (Figure 1, Tables 1 and 2).

Direct Reprogramming of Distantly Related Cells into Pancreatic β Cells

Skin cells, especially skin fibroblasts, are good candidates for direct reprogramming because they can be easily obtained in sufficient amounts. Katz *et al.* [15] established an *in vitro* method to transform adult human dermal fibroblasts into insulin$^+$ and glucagon$^+$ cells by using romidepsin (a histone deacetylase inhibitor) and 5-Azacytidine (a DNA methyltransferase inhibitor) to alter the epigenetic signature of cells. The reprogrammed cells exhibited expression of factors associated with islet development and function, including neurogenic differentiation factor (NeuroD), Isl1, glucose transporter 1 (Glut1) and Glut2. In another study, 5-Azacytidine treatment followed by a three-step protocol for the induction of pancreatic endocrine differentiation converted adult human fibroblasts into functional insulin secreting and glucose responsive islet-like cells that

Box 1. Islet β Cell Dedifferentiation and Redifferentiation

Mature β cells have unique characteristics, including expression of specific genes (such as *insulin*, *Pax4*, *GK*, *Glut2*, and *PC1*), structural elements (such as secretory vesicles), and specific function (such as glucose sensing and insulin secretion). Under the *in vivo* conditions of physiological and pathological stress or in *in vitro* long-term culture, β cells lose key components that are responsible for optimal performance. They regress back toward an earlier, more embryonic developmental stage or to a simpler unspecialized form [11,12,92]. Dedifferentiation may be a new mechanism of β cell failure in multiple forms of diabetes. Supplement with cytokines, which are used in stem cell differentiation *in vitro* [93], withdrawal of physiological and pathological stress *in vivo* [11], and application of some hypoglycemic strategies (e.g., insulin therapy) [12] are able to restore some characteristics of β cells. This reacquirement is called redifferentiation. The transcription factor, Foxo1, and the signaling pathways, Hedgehog/Gli and Notch, might be involved in the processes of dedifferentiation and redifferentiation [11,94,95]. However, the mechanism of these processes is still elusive.

Glossary

Cell fate determination: in embryogenesis, development depends on the accurate execution of differentiation programs through which a particular cell (or embryo) adopts specific cell fates. The cell fate determination can be divided into two states: the cell can be committed (specified) or determined. In the committed state, a certain fate can be reversed or transformed to another fate. If a cell is in a determined state, the cell is fixed in a specific fate and undergoes differentiation, which brings about actual changes in structure, function, and biochemistry. All these events result in the development of specific cell types.

Cell replacement therapy: cells are injected into a patient to replace the original cells; these cells are used in the treatment of degenerative diseases.

Embryonic stem cells (ESCs): cells that are derived from the inner cell mass of mammalian blastocysts. They have the ability to grow indefinitely while maintaining pluripotency. In addition, they are able to differentiate into cells of all three germ layers.

Epigenetic modifications: heritable alterations that do not involve changes in the DNA sequence but rather represent covalent modifications such as DNA methylation and histone modifications that alter DNA accessibility and chromatin structure, thereby resulting in selective gene expression or repression.

Induced pluripotent stem cells (iPSCs): first established by the Yamanaka group, they are pluripotent cells, similar to ESCs. iPSCs can be derived from differentiated cells by transfecting pluripotent factors or by adding cytokines, epigenetic regulators, and small molecules.

Stem cells: cells with the capacity to undergo self-renewal and lineage differentiation. According to their developmental potential, stem cells can be divided into different categories: totipotent, pluripotent, multipotent, and unipotent.

Streptozotocin (STZ): one of the most commonly used substances to induce diabetes in rodents. STZ can selectively destroy rodent islet β cells