Generation of pancreatic β cells from mesenchymal stem cells to treat type 1 diabetes

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Abstract

Introduction

Type 1 diabetes (T1D) is a chronic disease in which pancreatic β cells are destroyed by self-autoimmune attack. There are approximately 40 million T1D patients at 2011 in the world and this number will exceed 50 million by 2030. Patients who suffered T1D would have to receive insulin injections their whole life. Clinical studies show that transplantation of islets is a sufficient cure for aiding patient to relief from diabetes-related symptoms. However, the lack of applicable donor cells limits the treatment. Thus, a new source and approach to replace the loss of β cells for diabetes need to be investigated. Mesenchymal stem cells (MSCs) are a heterogeneous subset of stromal stem cells that can be isolated from adult tissues. Their multipotency and low immunogenicity are explored as a new treatment for generative medicine. Recent studies in stem cell therapy based on the generation of β cells from MSCs showed that MSCs may be a promising source of β cell in diabetes therapy. In this review, we summarize the studies on MSCs-based endogenous β cell regeneration or differentiation to insulin-producing cell (IPC) in vitro.

Conclusion

Transplantation of MSCs or IPCs differentiated from MSCs may be potential therapy to treat type 1 diabetes.

Introduction

Type 1 diabetes (T1D) is a chronic disorder due to the destruction of pancreatic β cells by autoimmune attack. Inadequate supply of insulin leads to hyperglycaemia and related serious complications¹. Islet cell transplantation has been explored as an effective treatment for type 1 diabetes by offering new β cells for controlling blood glucose. However, lack of sufficient donors limits this approach, because each patient needs two or four donor pancreases to obtain effective cells²,³,⁴. Thus, new alternative sources for transplantable islet β cells should be explored. Many studies have focused on using embryonic stem cells (ESCs) to produce replaceable β cells by specific culture in vitro and various experiments have successfully reversed hyperglycaemia in animal models by transplantation of the ESCs-derived β cells in the laboratory⁵,⁶,⁷.

However, ESCs as research materials cause controversy on ethics and their risk of tumorigenesis in vivo after transplantation limits their use clinically. Since Yamanaka et al.⁸,⁹ generated induced pluripotent stem (iPS) cells from skin cells, which can be redifferentiated to produce pancreatic β cells, iPS cells seemed a potential source for production of β cells. But some risks exist with the use of iPS. The use of viruses in stem cell reprogramming may cause cancer and the subsequent differentiation of iPS cells may increase genetic mutations. This may predispose differentiated cell types to malignant transformation.

In addition, if differentiated cells carried undifferentiated iPS cells they may also lead to teratoma formation. Thus, a possible source with low risks and ability to differentiate to pancreatic cells should be intensely investigated. Mesenchymal stem cells (MSCs) are a heterogeneous cell population with fibroblast-like morphology and potential to differentiate into a variety of mesenchymal lineages cells including bone, fat and cartilage, and they could proliferate on plastic culture flask in vitro¹⁰.

Although MSCs are mesoderm lineages they also have trans-differentiation capacities to differentiate along endodermal and ectodermal lineages, such as neurons, hepatocytes and endothelial cells¹¹,¹²,¹³. In addition, it has been proved that MSCs can be isolated from almost every type of connective tissue¹⁴ and have potential of differentiation into insulin-producing cells (IPCs) in vitro by specific culture¹⁵,¹⁶.

Wu et al. using rat bone marrow (BM)-derived MSCs to differentiate islet cells which could express insulin and transplantation of these cells was able to reduce glucose levels in non-obese (NOD) rats with diabetes¹⁷. Such abundant sources and multipotency of MSCs offered an unlimited supply for studies and applications of β cell regeneration. MSCs have also been shown their capacity to modulate the immunological activity by inhibiting proliferation of T cells and differentiation of dendritic cells (DC) in vitro experiments¹⁸,¹⁹,²⁰. Due to their immunomodulatory effect and multipotency, MSCs have already been used in some animal experiments as a treatment for type 1 diabetes²¹. In this review, we mainly discuss the methods of generating pancreatic β cells by human MSCs administration and summarise the recent studies on differentiating MSCs into IPCs in vitro.
**Discussion**

**Strategies to generate β cells from MSCs**

*MSCs: Identification, criteria and immunomodulatory effect*

MSCs were first isolated from bone marrow, called multipotent stromal cells and described by Friedenstein as spindle-shaped and clonogenic cells in monolayer cultures. They could differentiate into adipocytes, chondrocytes and osteocytes both in vitro and in vivo.

Further studies showed that the multipotent stromal cells are the collective precursors of mesenchymal tissues which not only are able to differentiate into mesoderm cells but also ectoderm or endoderm cells. As a consequence of their possible capacity for differentiation and self-renewal, these stromal cells, which existed in almost every type of connective tissue, were first defined as mesenchymal stem cells (MSC) by Caplan.

A recent report from the International Society for Cellular Therapy (ISCT) pointed that 'multipotent mesenchymal stromal cells' (MSC) is the present recommended name. While the defining characteristics of MSC are inconsistent among investigators, the isolated cells still have common characteristics. To offer specifications for pre-clinical and clinical studies, the Mesenchymal and Tissue Stem Cell Committee of the ISCT, based on the best currently available data, proposes a set of standards to define human MSC.

The criteria to define MSC have three standards: 1) adherence to plastic; 2) specific surface antigen (Ag) expression; and 3) multipotent differentiation potential. First, MSC should adhere to the plastic surface of tissue culture flasks when maintained in standard culture conditions.

Second, MSC must express CD105, CD73 and CD90, and the population of MSC with positive expression should be ≥ 95%. MSC is also a heterogeneous population with lack of expression of CD45, CD34, CD14, CD11b, CD79α, CD19 or HLA-DR (≤ 2%) and will not be confounded by other cells, such as hematopoietic cells, endothelial cells or macrophage and B-cells.

Third, MSC has a capacity for trilineage differentiation into mesenchymal tissues in vitro, including osteoblasts, adipocytes and chondroblasts. Each differentiation has commercial conditional culture and specific identification methods now; thus, investigators are convenient to evaluate differentiation property in their laboratories. Of course, this criteria is not constant, it will be adopted by the improvement of relevant studies.

In recent years, many studies have been carried out on the use of MSCs to treat T1D animal models. This is due to the fact that MSCS not only can differentiate into multiple mesodermal and non-mesodermal cell lineages but also can possess low immunogenicity and immunosuppression by modulating the immune function of the major cell populations, which are involved in alloantigen recognition and elimination. Some evidences in vitro demonstrated that MSCs have the potential of immunoregulatory effects. MSCs could inhibit monocytes maturation and the antigen-presenting function of dendritic cells (DCs), following decreasing MHC class II and co-stimulatory molecules, CD11c, CD83 and production of interleukin-12 (IL-12). When incubated with natural killer (NK) cells, MSCs can inhibit the cytolytic activity and proliferation of NK cells which have a key role in innate immunity. Another important immunoregulatory effect of MSCs is inhibition of T-cell proliferation.

Due to their immunomodulatory effects, MSCs have been used in clinical trials for graft-versus-host disease and other autoimmune diseases such as Crohn’s disease. Thus, MSCs as a promising source of cell therapy should be notable.

**MSCs initiates β cell regeneration**

MSCs have been used to treat diabetes and intensively investigated in animal models. Many results from the animal experiments showed that transplantation of MSCs could reduce blood glucose levels, gain weight and prolong lifespan of mice with diabetes. Grafted stem cells can migrate into the injured pancreas and exert their recovery effect. MSCs with the potential of homing to site of injury are related to several chemokines and their receptors. An in vitro study showed that bone marrow-MSCs (BM-MSCs) can express some chemokine receptors like CXCR4, CX3CR1, CXCR6, CCR1 and CCR7. These receptors were attempted to combine the chemokines such as CXCL12, CX3CL1, CXCL16, CCL19 and CCL3, which were released from pancreatic cells. The crosstalk mediated by these chemokines and receptors was considered as a response to MSCs migration into injury of the pancreas and this hypothesis was subsequently demonstrated by Sordi et al. They confirmed that the crosstalk between MSCs and pancreatic islets was motivated by CXCR4-CXCL12 and CX3CR1-CX3CL1 manner, and systemically administration of MSCs seemed a preferred way of MSCs homing to the injury site.

Recent studies demonstrated that transplantation of adult c-kit+ bone marrow-derived cells can reduce hyperglycaemia & initiate endogenous pancreatic tissue regeneration by proliferation of recipient pancreatic cells, and grafted cells localized to ductal and islet structures. However, the mechanism of these c-kit+ bone marrow-derived cells to promote pancreatic cell proliferation is not investigated. Lin et al. transplanted BM-MSCs into STZ (streptozocin) induced Wistar rats, followed by intravenous administration of recombinant lentivirus encoding two different small hairpin RNAs (shRNAs) for interference with ngn3, and found that BM-MSCs could differentiate into insulin-producing cells (IPCs) and contribute to reversing hyperglycaemia. But differentiation of endogenous pancreatic stem cells also exists and plays a major role in the process of reversing hyperglycaemia in the studies of Lin et al. Subsequently, Koblas et al. transplanted human...
umbilical cord blood (HUCB) cells into immunocompromised diabetes mice and confirm their potential of differentiating into IPCs while the HUCB cells did not improve hyperglycaemia. Administration of higher dose of HUCB cells can significantly lower levels of blood glucose in non-obese (NOD) mice with an obvious reduction of insulin.

Nevertheless, studies of Beil et al. demonstrated that BM-MSCs can improve systemic hyperglycaemia and increase insulin production in NOD/SCID mice via stimulating subsequent β cell proliferation and islet vascularization, not through ngn3+ endocrine progenitors differentiation. Although islet repair mechanisms stimulated by MSCs is variant, using MSCs to promote β cell regeneration seems a feasible approach for diabetes therapy.

MSCs can secrete a variety of cytokines and growth factors, like vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF) and basic fibroblast growth factor (bFGF). It has been proved that these factors had anti-apoptotic, mitogenic and angiogenic effects. MSCs with therapeutic effects for diabetes may be through this paracrine mechanism to inhibit β cell apoptosis, promote cells survival and induce endogenous progenitor cell proliferation and differentiation.

Moreover, immunomodulatory effects of MSCs may also exert response to reverse hyperglycaemia. Mollie et al. obtained congeneric MSCs from NOR mice, and transplanted them into NOD mice with recent-onset type 1 diabetes. MSCs altered diabeticogenic cytokine profile, diminished T-cell effector frequency in the pancreatic lymph nodes, altered antigen-presenting cell frequencies and augmented the frequency of the plasmacytoid subset of DCs. All these results contributed to hyperglycaemia reversal in the long-term.

Generation of β cells by genetic manipulation of MSCs

Recent studies have found that given an adenovirus-borne with three transcription factors (Ngn3, Pdx1, and Mafa), acinar cells could shift into β-cells. This transition may explain that 1) Ngn3 stimulates acquisition of endocrine features; 2) Pdx1 aids deviation of β-cell fate; 3) Mafa promotes Pdx1 function, augments β-cell number and pushes the maturation of newly generated β-cells. Other researches have also demonstrated that the interconversion between endocrine cells such as α-to-β or the converse β-to-α fate-switch could be enforced via over expression of endocrine transcription factors. Overexpression of Arx in the Pax6+ endocrine precursors during embryonic stage heavily reduced β and δ-cells, but increased α- and PP-cells in adult pancreas. Furthermore, selective expression of Arx in adult β-cells induces them into α-cells.

When there is persistent Pax4 production in endocrine precursors or mature α-cells, it could convert α-cells into β-cell. In this situation, even gaining a large store of insulin, the mice would still develop diabetes; this may be due to an insulin resistance in the peripheral tissues. Overall, these genomic manipulations seem difficult to be applied in humans, but the methods will help us understand the mechanisms of plasticity and the epigenomic alterations that need to be induced on cell fate.

**Key factors involving in IPCs differentiation from hESCs**

Schuldiner et al. first demonstrated that human embryonic stem cells (hESCs) could spontaneously differentiate into insulin-producing cells (IPCs) when cultured in suspension. Assady et al. also reported adherent hESCs could express insulin under culture conditions. However, the efficiency of this spontaneous differentiation pattern was very low. Even though some researchers utilized gene manipulation to improve efficiency of islet-cell transduction by overexpression of Pdx1 or Ngn3, these methods had a limitation for future cell therapy because of contamination of exogenous DNA. Embryonic stem cells have been designed to differentiate into pancreatic β-cells by a step-wise manner in vitro. The progress aims to mimic embryonic development of β cells under conditional culture. There are three key developmental stages for generating islet β-cells: the first step inducing SCPs toward definitive endoder, the second step toward pancreatic progenitors or endocrine progenitors and the third step toward β cells.

Each step involved some key induction factors for differentiation. For example, activin A, which has been used for hES differentiation into endoderm lineages, is a key factor for definitive endoderm differentiation both in mouse and human ESCs. The second step is the specialization of pancreatic progenitors. In this step, many induction factors were used, such as RA, EGF, FGF10/indolactam V, et al. Deng et al. demonstrated that RA can induce pancreatic progenitor cells specification from hES-derived definitive endoderm cells. When EGF was added into induction system after RA treatment, it can improve PDX1 and Klf6 double-positive cells. A novel small molecule, indolactam V, can induce Pdx1 expressed cells from hES cells. The final step is the maturation of islet cells. bFGF, exendin-4 and BMP4 were used in induction media. Partial MSCs can express insulin and release insulin/C-peptide in response to glucose stimulus. The stepwise differentiation manner, which imitated the niche of islet cell formation by adding some key induction factors, raised the productivity of IPCs and avoided genome modifications. IPCs from different induction strategies may be a great source of functional β-cell for diabetes treatment. Therefore, the stepwise strategy with induction factors is an efficacy way to directly induce stem cell into differentiation of insulin-producing cells.

**Multiple sources of MSCs to generate IPCs**

Using different factor combinations to induce hESCs into IPCs has been investigated. Although different source of MSCs had different induction protocol, increasing quantity and maturity of IPCs was the common aim.
In vitro differentiation of human BM-MSCs into IPCs

IPCs can be induced from adult BM-MSCs by conditioned culturing. High glucose DMEM (25 mmol/L) containing β-mercaptoethanol could promote stem cell differentiation and mixed factors of EGF, bFGF, exendin-4, activin A could induce islet-cells to express insulin at both mRNA and protein levels.\(^{62,63}\) The differentiated IPCs by a 4-step procedure could secrete C-peptide and are able to normalize blood glucose levels of STZ induced hyperglycaemia mice for at least 9 weeks.\(^{64}\) Step 1: use a high-glucose culture to adapt cell environment. Step 2: promote BMSCs into nestin-positive cell combination of bFGF and EGF. Step 3: differentiate nestin-positive cells into IPCs with a mixture of EGF, HGF, nicotinamide and B27 in the culture medium. EGF is used to expand pancreatic progenitors. Nicotinamide and B27 induce nestin-positive cells to differentiate into IPCs, and HGF promote the pancreatic islet cells to differentiate and proliferate. Step 4: promote the maturation of endocrine cells with supplementation of EGF, exendin-4, betacellulin and zinc acetate. The stepwise induction of BMSCs is an effective way to produce insulin-producing cells, however obtaining BMSCs is invasive. Further study in this field is wanted to find more sources.

In vitro differentiation of human umbilical cord MSCs into IPCs

Human umbilical cord MSCs are able to differentiate into IPCs under differentiated medium. Wu et al.\(^{65}\) used a one-step method to form islet-like cell clusters which could highly express the pancreatic-specific transcription factor PDX-1 and pancreatic-specific marker C-peptide. The differentiation medium contained a kind of hormone pentagastrin except commonly used factors such as activin-A, exendin-4, HGF. Under the same differentiation medium, BM-MSCs formed smaller islet-like cell clusters and expressed lower pdx1 and C-peptide. A specific three-step induction of Wharton’s jelly MSCs could obtain insulin-producing cells without formation of islet-like cells clusters. Retro-orbital transplantation of differentiated IPCs could alleviate hyperglycaemia in STZ-induced mice.\(^{66}\) Thus, human MSCs from the umbilical cord could differentiate into IPCs in vitro and had a therapeutic effect in vivo.

In vitro differentiation of human adipose tissue-derived MSCs into IPCs

Human adipose tissue-derived mesenchymal stem cells (ADSCs) also have the potential to differentiate in insulin producing cells. Under a similar differentiation medium, Jiyeon et al.\(^{67}\) used human adipose tissue-derived MSCs and were able to obtain a pancreatic endocrine phenotype. Okura et al.\(^{68}\) used low-attachment culture dishes to obtain resembled embryoid bodies from ADSCs. By following the step-wise methods established by Segev et al.\(^{69}\) in which IPCs were regenerated from embryoid bodies, they acquired insulin-producing cells. After 21-days of condition culture, cells derived from ADSCs had functional similarities with pancreatic islet cells, i.e., the presence of insulin- and C-peptide-coexpressing cells in clusters and glucagon expression on the cell surface.

In vitro differentiation of human endometrial stromal stem cells (ESSC) into IPCs

Studies demonstrated that mesenchymal stem cells can be isolated from human endometrium and have the ability to differentiate into multipolineligues, such as chondrogenic, adipogenic, osteogenic, neurogenic and cardiogenic cells. Thus, the potential of ESSCs to differentiate into IPCs was also investigated. Culture of ESSCs in DMEM-LG supplemented with bFGF, EGF and platelet-derived growth factor-BB could obtain insulin-secreting cells which had related expression profiles of islet tissues by cDNA microarrays.\(^{70}\) Xenotransplantation of differentiated ESSCs into mice with diabetes could restore blood insulin levels. Recently, Taylor et al.\(^{71}\) used an efficient protocol based on expression of β-cell markers, in promoting production of IPCs. There were 3 main steps for differentiation. In step one, they used retinoic acid to adjust MSC to endodermal lineages; in step two, they used FGF-10 and (-)-indolactam V to induce ESSCs to islet cells; in step three, exendin-4 and Activin A were added in L-DMEM to obtain more insulin-expressing cells. The differentiated cells injected into kidney capsules of hyperglycaemic mice could reduce blood glucose and prevent complications.

Moreover, ESSCs exhibited an easily accessible, renewable and immunologically identity. Thus, ESSCs may be a promising source of stem cells with potential therapeutic applications in diabetes.

Conclusion

Research of use of MSCs to treat diabetes in animal models had many progresses. Direct transplantation of MSCs into hyperglycaemic mice could reduce blood glucose and prolong lifespan by improving β cell loss and islet structure.

However, hypoglycaemic effect was limited and the blood glucose was still at a higher level relative to normal control. Hypoglycaemic effect and the survival time of mice should be analysed over a longer period of time to find the best therapeutic effect on diabetes models. Dose and frequency of MSC administration also need to be investigated. Moreover, the mechanism of promoting β cell regeneration by MSC transplantation need further confirmation and this result can provide a better direction for MSC based diabetes therapy. In vitro direct differentiation of MSC to IPCs had many available methods. Induced IPCs could functionally release insulin and C-peptide. Implantation of differentiated MSC into diabetes models could efficiently reduce blood glucose to the normal level. The IPCs from MSC induction seems to be an ideal source of β cell for cell replacement therapy in diabetes. Nonetheless, transformation efficacy and maturity in vitro differentiation are very low. How to...
search a simple method that can obtain a large of mature islet-like β cell seems to be important.

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References


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