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Cell Replacement and Regeneration Therapy for Diabetes

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Reduction of beta cell function and a beta cell mass is observed in both type 1 and type 2 diabetes. Therefore, restoration of this deficiency might be a therapeutic option for treatment of diabetes. Islet transplantation has benefits, such as reduced incidence of hypoglycemia and achievement of insulin independence. However, the major drawback is an insufficient supply of islet donors. Transplantation of cells differentiated *in vitro* or *in vivo* regeneration of insulin-producing cells are possible approaches for beta cell/islet regenerative therapy. Embryonic and adult stem cells, pancreatic ductal progenitor cells, acinar cells, and other endocrine cells have been shown to differentiate into pancreatic beta cells. Formation of fully functional beta cells and the safety of these cells are critical issues for successful clinical application.

Keywords: Beta cell; Diabetes mellitus; Differentiation; Islets of Langerhans; Regeneration; Stem cells

INTRODUCTION

Diabetes mellitus, a metabolic disorder, results from an inadequate mass of insulin-producing pancreatic beta cells [1-3]. Various formulations of short- and long-lasting insulin have been used to control blood glucose levels. However, the tight regulation of insulin in response to physiological change is not possible. This lack of regulation results in episodes of hyperglycemia and hypoglycemia. The development of a therapeutic method to regulate precisely the blood glucose levels will enable better management of diabetes. One logical therapeutic approach is the restoration of a functional beta cell mass.

Islet transplantation into diabetic patients is a promising method for restoring the functional beta cell mass. However, the limited supply of islets cannot meet the patient demand, and post-transplant immunosuppression can produce serious side-effects. To overcome these limitations, various methods providing an alternative source of insulin-producing cells are being investigated. Some of these methods include engineer-

ing non-beta cells to produce insulin, differentiation of insulin-producing cells from embryonic and adult stem/progenitor cells, and transdifferentiation of extra-pancreatic and pancreatic cells. In addition, *in vivo* regeneration of islet cells is being investigated.

In this review, we will provide recent advances, as well as research progress on beta cell replacement and regeneration therapy for treatment of diabetes using various strategies (Fig. 1).

ISLET TRANSPLANTATION

Islet or pancreas transplantation is a method to normalize metabolic control in a way that cannot be achieved with exogenous insulin. It was shown that whole pancreas transplantation ameliorated the complications associated with chronic hyperglycemia and eliminated the need for daily insulin injections. This procedure improved the quality of life. However, pancreas allografting caused immediate surgical risks and long-term complications [4].

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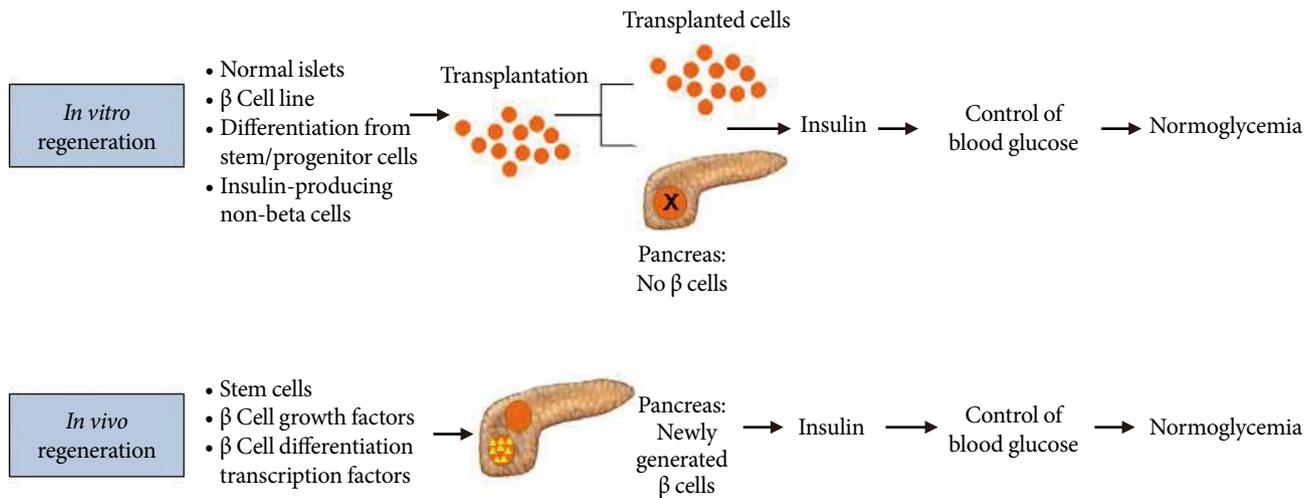E-mail: hsjeon@gachon.ac.kr

Fig. 1. Possible strategies for beta cell replacement and regeneration therapy. Insulin-producing cells can be restored by transplantation of cells derived from *in vitro* or *in vivo* regeneration. Transplantation of islets from normal subjects, insulin-producing cells differentiated from stem and/or progenitor cells *in vitro*, or non-β cells (e.g., hepatocytes) engineered to produce insulin can result in insulin production and control blood glucose levels. Introduction of stem cells, β cell growth factors or stimulation of β cell differentiation transcription factors can regenerate β cells *in vivo*, which then produce insulin and control blood glucose levels.

The first trial of islet transplantation was performed in rodent models and later in type 1 diabetic patients [5]. Short-term insulin independence was achieved in about 10% of the transplant recipients. Further advances in the islet isolation procedure and more effective immunosuppression strategies with a steroid-free regime called the "Edmonton protocol" [6] have been made. The success rate in achieving insulin-independence in islet-transplanted patients has been greatly improved. Recently it was reported that 70% of the transplant recipients showed insulin independence [7]. However, the lifelong immunosuppressive regimes to prevent transplant rejection and recurrence of autoimmune responses to beta cells are expensive and decrease the quality of life.

A variety of strategies to protect the transplanted islets from immune attack have been reported. One of these involves preventing the activation of antigen-presenting cells. Regulating the immune system by shifting the pathogenic effector cells to protective regulatory cells and blocking co-stimulatory pathways provides an alternative to immune attack. Another strategy is to engineer beta cells that are resistant to immune attack. Protective effects on transplanted islet cells can be gained by the transduction of genes for the expression of cytokines, antiapoptosis molecules, or growth factors [8].

Another strategy to protect islets from immune attack involves the microencapsulation of islets within synthetic polymers. There is easy passage of small molecules, such as glucose,

amino acids, and insulin. However, larger molecules, like antibodies and immune cells cannot gain entry. A major limitation of the microencapsulation strategy is its lack of biocompatibility. Oxygen deprivation presents another limit for the long-term survival of islets within a microcapsule [9].

A major hurdle for islet transplantation is a lack of an islet source to meet the needs of all type 1 diabetic patients. This paucity is secondary to the limited supply of cadaveric pancreas donors. Embryonic, fetal, neonatal, or adult porcine islets are possible sources for transplantation, since easily available pigs have many physiological similarities to humans [10,11]. However, there is a problem secondary to the hyperacute rejection of xenotransplants that carry the Gal-α-Gal antigen. Another possible source of beta cells for transplantation is primary beta cells that are immortalized by transduction with oncogenes for expansion. These also have a conditional expression system that can reverse oncogene expression [12,13]. However, this strategy carries the possibility of impaired insulin secretion and tumorigenicity.

Significant progress has been made in improving the immunosuppressive regimes. In particular, increasing the tolerance of islet transplants and reducing transplant rejection are necessary. There is also a need to find an unlimited source of insulin-producing cells that are required for the wider clinical application of islet transplantation.

INSULIN-PRODUCING NON-BETA CELLS

Genetically engineering non-beta cells to express insulin is an attractive strategy. The engineered cell will become an alternative insulin-producing cell that will have an advantage over intact islets. Non-beta cells may not be recognized by beta cell-specific autoimmune responses. A variety of cell types, including fibroblasts, hepatocytes, neuroendocrine cells, and muscle cells, have been engineered to produce insulin with varying degrees of success [8].

Since hepatocytes have a glucose-sensing system similar to that in pancreatic beta cells like glucose transporter 2 (GLUT2) and glucokinase, they draw much attention as target cells for insulin production. A preclinical model of diabetes was corrected by autologous transplantation of primary hepatocytes that were non-virally transduced with a glucose-responsive promoter-regulated insulin gene construct [14]. Intestinal K cells, possible surrogate beta cells, contain the necessary enzymes for processing proinsulin to insulin and have exocytotic mechanisms. A murine enteroendocrine cell line expressing insulin under the control of a glucose-dependent insulinotropic polypeptide not only reversed diabetes when transplanted, but also produced insulin in response to glucose [15]. Transplantation of bone marrow mesenchymal stromal cells expressing insulin under the glucose-responsive early growth response gene (EGR-1) promoter resulted in the remission of diabetes in mice [16].

The regulation of insulin production by glucose-responsive promoters in non-beta cells exhibits slow kinetics, which may cause hypoglycemia. This situation is due to the requirement for insulin transcription and translation, as compared with the rapid release of insulin from beta cells by exocytosis. Pancreatic beta cells have unique characteristics specific to the production of insulin, such as specific peptidases, glucose-sensing systems, and secretory granules that can release insulin promptly by exocytosis in response to extracellular glucose levels. Thus, it is very difficult to mimic this tight regulation of insulin production in response to physiological levels of glucose in non-beta cells.

STEM CELL-DERIVED BETA CELLS

Multiple studies have shown that it is possible to direct *in vitro* differentiation of stem and progenitor cells toward insulin-producing cells. Embryonic stem (ES) cells have the potential to generate unlimited quantities of insulin-producing cells. Theoretically, ES cells could be expanded indefinitely in the undifferentiated state and then differentiated into functional beta cells [17]. Two major strategies are used for the differentiation of ES cells into insulin-producing cells: the embryoid body formation and the definitive endoderm formation. A recent report showed that the stepwise differentiation of ES cells, which mimics endogenous pancreatic development, could generate functional beta cells [18]. However, the yield is still very low and the differentiated beta cells are much less functional as compared with primary islets. More efficient protocols need to be developed for the differentiation of ES cells into mature functional beta cells.

Adult stem cells are the most important source for cell therapy for various disease models, as they are free from the ethical problems of ES cells and could provide an unlimited resource. Many studies reported that insulin-producing cells can be generated from adult stem/progenitor cells that are present in bone marrow, adipose tissue, liver, intestine, spleen, salivary glands, neuronal tissues, and umbilical cord blood [19].

Adult stem/progenitor cells from liver tissue are a good source for making insulin-producing cells, as the liver and pancreas share common bipotential precursor cells within the embryonic endoderm. Isolated neurogenein 3 (Ngn3)-positive cells from the injured adult mouse pancreas [20] or clonally identified cells from adult pancreatic islets and ductal populations [21] have the ability to differentiate into cells with beta cell function. Umbilical cord blood (UCB)-derived mesenchymal stem cells (MSCs) may be an ideal source of stem cells that can be obtained without pain or risk of viral contamination to the donor. UCB-derived MSCs have a multi-lineage differentiation capacity under specific manipulation of the culture conditions. Several studies have shown that UCB stem cells can be differentiated into insulin-producing cells.

Adipose tissue-derived stem cells (ADSCs) are located within the stromal vascular fraction of adipose tissue. ADSCs can be isolated in high numbers from human adipose tissue at low risk to the patient. An interesting study reported the successful differentiation of functional insulin-producing cells from the stem cells of human eyelid adipose tissue using a two-step culture condition [22]. Similar to other stem cells, Pdx-1-transduced ADSCs derived from human or murine tissue could differentiate into insulin-expressing cells under specific culture conditions [23]. Thus, ADSCs have the potential as a useful source for cell replacement therapy in diabetes.

Despite the success of the differentiation protocols, as de-

scribed in this review, none of the protocols are yet able to produce fully functional mature beta cells. Further research is required to understand how endogenous beta cells differentiate and to develop methods for generation of sufficient functional beta cells for clinically applicable therapies for diabetes.

IN VIVO REGENERATION OF BETA CELLS

Approaches for *in vivo* regeneration are to stimulate replication of beta cells and induce neogenesis. The beta cell mass normally fluctuates in response to environmental and physiological changes. Beta cells can replicate throughout life, although at a low level. This replication can be stimulated by pregnancy and diabetogenic stimuli, such as glucose and free fatty acids, suggesting that beta cell growth can be artificially induced.

Growth factors, such as activin A and hepatocyte growth factor, have been used to induce beta cell replication. Other factors, like a combination of epidermal growth factor (EGF) and gastrin, keratinocyte growth factor, betacellulin (BTC), and glucagon-like peptide-1 (GLP-1) or its long-lasting homolog, exendin-4, have also been used to induce beta cell replication. In addition, members of the regenerating protein family, such as Reg protein and islet neogenesis gene associated protein (IN-GAP), can stimulate proliferation of beta cells and have been investigated as potential therapies for diabetes [24].

Both GLP-1 and EGF have been combined with gastrin as a therapy to restore the beta cell mass. These treatments restored normoglycemia in autoimmune non-obese diabetic (NOD) mice, both by restoring the beta cell mass and by downregulating the immune response [25,26]. In a similar study, combination therapy with EGF and gastrin induced neogenesis of human beta cells from pancreatic duct cells *in vitro*, as well as from human pancreatic cells implanted into immunodeficient NOD. scid mice [27].

Clinical trials are underway for beta cell regeneration in type 1 diabetes using synthetic exendin-4 (AC2993). Further clinical trials using immune suppressors and EGF and gastrin analogs (E1-INF™) for type 1 and type 2 diabetes have been reported [28]. In phase 2 clinical trials, INGAP peptide revealed no consistent treatment effects on fasting glucose, insulin, or C-peptide in type 1 and type 2 diabetic patients, although the effects on HbAlc and stimulated C-peptide were promising [29].

The expression of beta cell transcription factors either with or without a signaling molecule, such as a growth factor, has shown to be a successful method of generating new insulin-producing cells. Delivery of the pancreatic and duodenal homeobox-1 (Pdx-1) gene into the mouse pancreas or intraperitoneal injection of Pdx-1 protein induced beta cell neogenesis and ductal proliferation [30]. Delivery of the Pdx-1 gene along with the BTC gene into the pancreas of streptozotocin-induced diabetic rats via ultrasound-targeted microbubble destruction normalized blood insulin and C-peptide levels. Blood glucose levels were maintained below 200 mg/dL [31]. Intraperitoneal injection of recombinant Pdx-1 into streptozotocin-induced diabetic mice increased islet cell numbers and proliferation in pancreata [32].

An exciting report demonstrated that expressing a specific combination of three transcription factors, Pdx-1, Ngn3, and musculoaponeurotic fibrosarcoma oncogene homolog A (MafA), resulted in amelioration of hyperglycemia. The transcription factors were administered by an adenoviral mediated delivery reprogrammed pancreatic exocrine acinar cells into beta cells. These beta cells were indistinguishable from endogenous islet beta cells in size, shape, and ultrastructure [33].

Transcription factors, such as Pdx-1 and NeuroD, have been used not only to stimulate new insulin-producing cells in the pancreas, but also to confer beta cell-like characteristics to non-islet tissue, such as in the liver and the intestine.

Systemic injection of recombinant Pdx-1 resulted in expression of insulin and other genes related to pancreatic function not only in the pancreas, but also in the liver [32]. Many methods have been tried to differentiate liver cells into insulin-producing cells. Delivery of the Pdx-1 gene or NeuroD and BTC genes to the liver resulted in the expression of insulin and other islet-specific genes in liver. These genes ameliorated hyperglycemia in diabetic mice [34,35]. Systemic delivery of Pdx-1 carrying the VP16 transcriptional activation domain (Pdx-1/VP16) or Ngn-3 and BTC gene resulted in insulin production in liver [36,37].

In vivo regeneration in the intestine has also been investigated. Forced expression of Pdx-1 [38], MafA [39], or GLP-1 [40] in intestinal epithelia by adenovirus-mediated gene transfer induced the expression of insulin and lowered blood glucose levels in streptozotocin-induced diabetic animals.

The injection of stem/progenitor cells was shown to induce insulin-producing cells. It is unclear whether the injected stem/progenitor cells differentiate into insulin-producing cells or exert other effects that preserve or increase the beta cell mass. The injection of allogeneic splenocytes, in combination with complete Freund's adjuvant (to prevent anti-islet autoimmunity),

corrected diabetes in diabetic NOD mice [41]. However, it remains to be determined whether the injected splenocytes are the true source of insulin-producing cells. A successful clinical trial involving autologous hematopoietic stem cell transplantation and immune suppressor in diabetic patients was reported [42]. Although beta cell function was shown to be increased, the mechanism of action was unclear. *In vivo* islet cell regeneration therapy is very challenging. Most methods are still in the early stages and are not yet ready for clinical application.

CONCLUSION

Significant progress has been made in cell-based therapies to treat diabetes. Islet transplantation is a promising strategy for the reconstitution of a functional beta cell mass. This method provides stable glycemic control without hypoglycemic episodes, as well as independence from exogenous insulin. However, a shortage of islet donors is a major limiting factor for cell replacement therapy in type 1 diabetes. The differentiation of pluripotent stem/progenitor cells into insulin-producing cells creates a possible source for the generation of therapeutic insulin-producing cells. However, the clinical application of this technology is slow. Beta cell growth and differentiation factors, the expression of beta cell transcription factors, and the injection of stem/progenitor cells has been used to regenerate beta cells *in vivo*. Most results have been obtained from studies with animal models, but very little has been tried clinically. The development of beta cells and their maintenance postnatally needs to be fully understood. The underlying mechanisms for the normal renewal process in adults will accelerate the clinical application of islet cell replacement and regeneration therapy for diabetic patients.

ACKNOWLEDGEMENT

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