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Regeneration of pancreatic beta cells

Hee-Sook Jun[1]

[1]*Lee Gil Ya Cancer and Diabetes Institute, Gachon University of Medicine and Science, 7-45 Songdo-dong, Yeonsu-ku, Incheon 406-840 Korea*

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1. ABSTRACT

Diabetes mellitus results from inadequate mass of insulin-producing pancreatic beta cells. Type 1 diabetes is characterized by absolute loss of beta cells due to autoimmune-mediated destruction. Type 2 diabetes is characterized by relative deficiency of beta cells due to lack of compensation for insulin resistance. Restoration of deficient beta cell mass by transplantation from exogenous sources or by endogenous regeneration of insulin-producing cells would be therapeutic options. Mature beta cells have an ability to proliferate; however, it has been shown to be difficult to expand adult beta cells *in vitro*. Alternatively, regeneration of beta cells from embryonic and adult stem cells and pancreatic progenitor cells is an attractive method to restore islet cell mass. With information obtained from the biology of pancreatic development, direct differentiation of stem and progenitor cells toward a pancreatic beta cell phenotype has been tried using various strategies, including forced expression of beta cell-specific transcription factors. Further research is required to understand how endogenous beta cells differentiate and to develop methods to regenerate beta cells for clinically applicable therapies for diabetes.

2. INTRODUCTION

Blood glucose concentration is normally controlled by hormones produced in the islets of Langerhans in the pancreas. Insulin, produced by pancreatic beta cells, is released when the concentration of glucose in the blood rises and induces glucose uptake from the blood to muscle and fat tissues, restoring normal blood concentrations. Diabetes mellitus is a devastating disease characterized by uncontrolled hyperglycemia, which causes serious clinical problems including blindness, kidney failure, stroke, heart disease, and vascular disease. It is expected that the number of people with diabetes will increase to 300 million by 2025.

There are two major forms of diabetes: type 1 and type 2. In type 1 diabetes, beta cells are lost by autoimmune-mediated destruction (1, 2), resulting in absolute deficiency of insulin. In type 2 diabetes, both insulin resistance and reduction of the beta cell mass occur (3, 4) (Figure 1). Therefore, beta cell replacement by transplantation from exogenous sources or pancreatic islet regeneration is an attractive strategy for diabetes therapy. Exercise, diet, and treatment with anti-diabetic drugs that

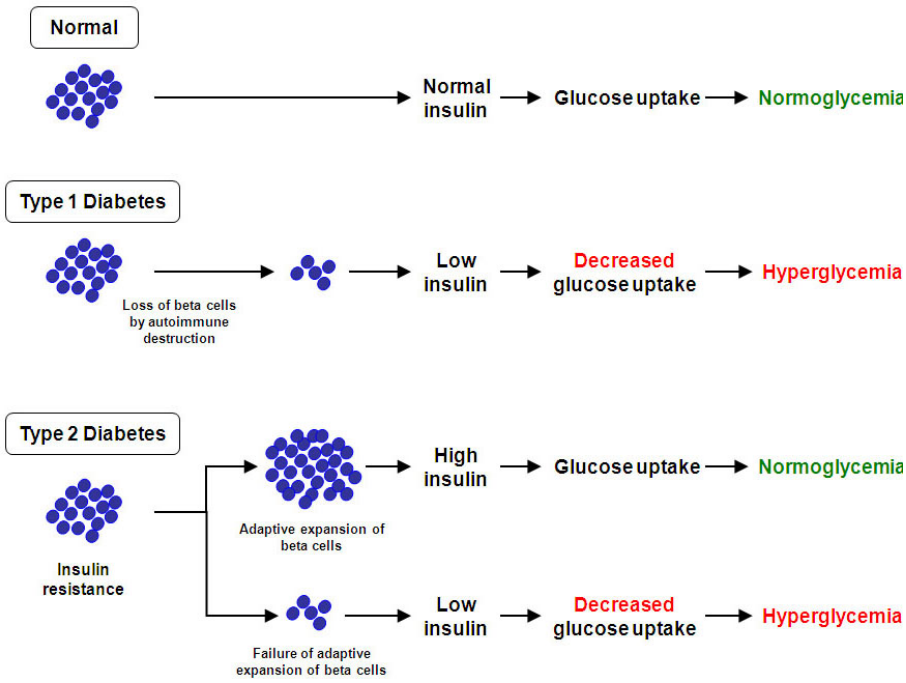

Figure 1. Inadequate beta cell mass causes type 1 and type 2 diabetes. In the normal situation, insulin is secreted in response to blood glucose levels and promotes glucose uptake, resulting in normoglycemia. In type 1 diabetes, beta cells are destroyed by autoimmune processes, leading to insulin deficiency and hyperglycemia. In type 2 diabetes, insulin resistance leads to adaptive expansion of the beta cell mass, resulting in hyperinsulinemia and normoglycemia. If the adaptive expansion fails, then hypoinsulinemia and hyperglycemia result.

either increase insulin secretion, improve insulin sensitivity, or reduce hepatic glucose production are the first choice for treatment of type 2 diabetes, but exogenous insulin is eventually required for about half of type 2 diabetic patients.

Intensive exogenous insulin therapy is used for treatment of type 1 diabetes, but the tight regulation of insulin in response to physiological change is not possible, resulting in episodes of hyperglycemia and hypoglycemia. Recent clinical advances have made it possible to safely graft allogenic human islets into diabetic patients using a minimal, non-steroidal immunosuppressive drug regime (5). In conjunction with improvements in islet isolation techniques, this has increased the success rate for independence from exogenous insulin for type 1 diabetic patients (6, 7). However, a serious limitation is lack of sufficient islets to meet the patient demand. Various methods are being investigated to provide an alternative source of insulin-producing cells. In this review, we will discuss different approaches to produce and generate insulin-producing cells from existing beta cells, embryonic stem cells, and adult stem cells (Figure 2).

3. TRANSCRIPTION FACTORS INVOLVED IN DEVELOPMENT OF PANCREATIC BETA CELLS

Understanding the molecular mechanisms for pancreatic development can be important in developing a method for efficient differentiation of pancreatic beta cells. So far, many transcription factors and transcriptional regulators involved in pancreatic development have been studied (8-10).

During vertebrate development, gastrulation results in three principal germ layers: ectoderm, mesoderm, and endoderm. The endoderm germ layer forms the foregut, which then gives rise to the thyroid, lungs, liver, stomach, and pancreas. Foregut formation depends on the proper anterior-posterior patterning of the endoderm (11), and transforming growth factor, fibroblast growth factor (FGF) and wingless-type MMTV integration site family (Wnt) signaling pathways are important for posterior endoderm development (12, 13). FGF, retinoic acid, and hedgehog signaling pathways are required for establishing the pancreatic organ within the developing gut tube, and transcription factors such as SRY (sex determining region Y)-box (Sox)17, homeobox gene HB9 (Hlxb9), hepatocyte nuclear factor (HNF)-6, HNF-3beta (also known as foxhead box A2, Foxa2), and pancreatic and duodenal homeobox 1 (Pdx-1) are required for proper pancreatic development (9, 14). Sox17, expressed throughout the endoderm after gastrulation, is the earliest specific marker of definitive endoderm (15) and is required for pancreas formation. Hlxb9 is a critical factor for pancreatic endoderm development, as Hlxb9-deficient mice show impairment of dorsal pancreas development, and the expression of Pdx-1, which is a key transcription factor for pancreatic endocrine development and growth, was not detected in these mice (16). Pancreas-specific transcription factor-1a (Ptf-1a) is

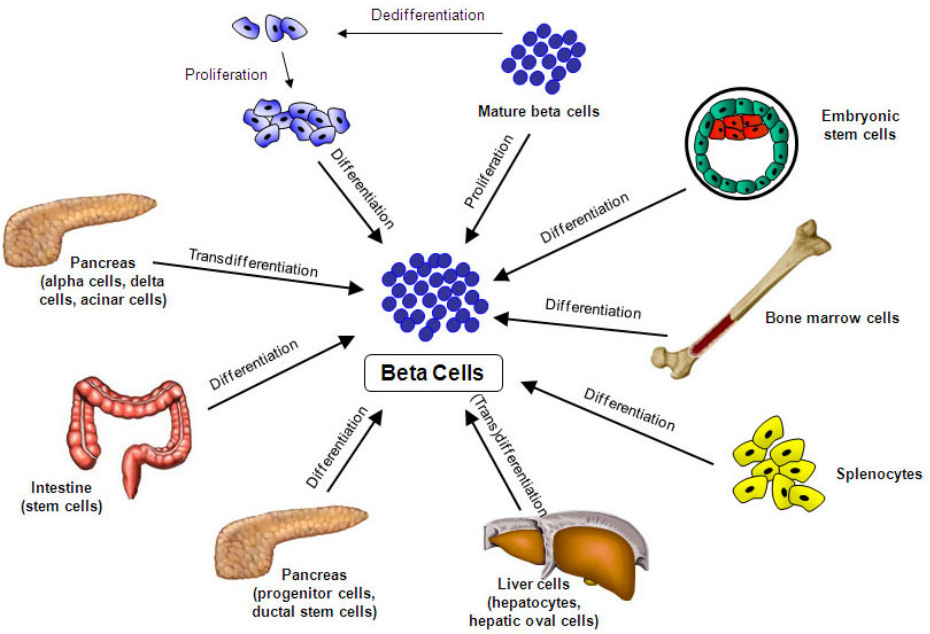

Figure 2. Potential sources for generation of new beta cells. In principle, beta cells can be generated by the proliferation of existing beta cells; differentiation from embryonic stem cells; differentiation from adult stem cells found in various tissues such as the bone marrow, splenocytes, liver, pancreas, and intestine; transdifferentiation of pancreatic alpha, delta, or acinar cells; or dedifferentiation of beta cells followed by proliferation and subsequent differentiation.

known to be responsible for ventral pancreas specification (17).

Following pancreas specification and budding, the fate for proliferation or differentiation of progenitor cells, including endocrine and exocrine lineages, is determined. All pancreatic progenitor cells express Pdx-1, and inactivation of Pdx-1 after bud formation prevents both islet and acinar cell differentiation (18). The expansion and differentiation of pancreatic progenitor cells appear to be regulated by Notch signaling (19, 20). FGF-10 is the mesenchymal signal and is important for expansion of Pdx-1-positive pancreatic progenitor cells (21). Notch signaling regulates the expression of neurogenin-3 (Ngn-3), which is a key regulator of endocrine development and is expressed exclusively in endocrine precursor cells (22, 23). Inhibition of Notch signaling upregulates Ngn-3 and increases endocrine formation (14, 24), and activation of Notch1 prevents endocrine differentiation (19, 20). Recently, it was reported that Sox9 maintains the pancreatic progenitor population, and its proliferation by regulation of Notch pathway (25), and Sox9 activates Ngn-3 expression (26). In addition, Ptf-1a is reported to be required for the development of multiple pancreatic lineages and is involved in exocrine acinar cell development (27).

Many transcription factors such as Pdx-1, ISL LIM homeobox 1 (Isl-1), Ngn-3, NK2 homeobox 2 (Nkx2.2), NK6 homeobox 1 (Nkx6.1), neurogenic differentiation factor (NeuroD), Hlxb9, paired box gene (Pax)-4, and (Pax)-6 have been identified as islet differentiation factors. Ngn-3 is a key transcription factor for endocrine development and is absolutely required for islet cell development (28). Nkx2.2 is required for the final differentiation of beta cells and production of insulin (29, 30). Nkx6.1 and Pax-4 are downstream of Ngn-3 and appear to act as beta cell determining factors (31, 32). Pax-6 is required for islet cell proliferation, morphology, and beta cell function (33).

Much progress has been made on pancreas developmental biology including transcriptional regulation of pancreatic endocrine specification, growth, and lineage allocation, which contributes to our understanding of how endogeneous beta cells are made. Understanding pancreatic organogenesis will be a hint for translational research for beta cell regeneration.

4. REPLICATION OF BETA CELLS

The pancreas is composed of endocrine and exocrine tissues. The endocrine pancreas occupies less than 5% of the pancreatic tissue mass and is composed of cell clusters called the islets of Langerhans. The islets of Langerhans contain insulin-producing beta cells (about 80% of the cells in the islets), glucagon-producing alpha cells, somatostatin-producing delta cells, pancreatic polypeptide-producing cells, and ghrelin-producing epsilon cells. The exocrine pancreas occupies more than 95% of the pancreas and is composed of acinar and ductal cells, which produce digestive enzymes.

Mature beta cells can replicate throughout life, although at a low level (34), evidenced by incorporation of bromodeoxyuridine and expression of markers of cell cycle entry such as antigen identified by monoclonal antibody

Ki-67 (35, 36). Recent findings suggest that the beta cell population is maintained by replication of differentiated beta cells, all beta cells contribute equally to islet growth and maintenance (37), and the majority of the newly generated beta cells originate from pre-existing beta cells (38). The beta cell mass is dynamic and increases in response to environmental and physiological changes and insulin resistance (38, 39). In fact, beta cell replication can be stimulated by pregnancy (40), diabetogenic stimuli such as glucose and free fatty acids (41, 42), and growth factors such as hepatocyte growth factor (43), epidermal growth factor (EGF), gastrin (36, 44), betacellulin (45), glucagon-like peptide (GLP), or its long-lasting homolog, exendin-4 (46). In addition, members of the regenerating protein family such as Reg protein (47) and islet neogenesis gene associated protein (48) can stimulate proliferation of beta cells.

Beta cell proliferation and increase in beta cell mass appears to be linked with cell cycle regulators, particularly cyclin D2 and cyclin-dependent kinase (CDK)4 (49, 50). The CDK inhibitor, p27, is expressed in adult mouse beta cells, and deletion of this gene results in islet hyperplasia (51). It was reported that the decline of beta cell proliferation with aging is correlated with the increased expression of $p16^{INK4a}$, a cyclin-D/CDK4 inhibitor, and that the absence of p16 expression increases proliferation of beta cells and beta cell survival after streptozotocin (STZ)-induced beta cell damage (52). Interestingly, a recent genetic study showed that type 2 diabetes is linked to the locus containing the p16 gene (53). In addition, recent studies showed that proliferation is reduced in beta cells deficient for the G protein alpha subunit, which is required for hormone-stimulated cAMP generation, and cyclin D2 expression was markedly reduced (54). Wnt signalling is also involved in regulating pancreatic beta cell proliferation by regulation of cyclin D2 expression (55). In addition, deletion of the gene encoding menin, a tumor suppressor that regulates the CDK inhibitor (56), enhances proliferation of pancreatic islet cells (57). Therefore, manipulation of cell cycle regulators in beta cells might be a useful strategy to expand the beta cell mass.

Although it is clear that mature beta cells can replicate, expansion of primary beta cells *in vitro* has not been successful, as mature beta cells have limited proliferative capacity in culture. Therefore, expression of oncogenes has been tried as a method to establish beta cell lines. Expression of simian virus (SV) 40 large T antigen in beta cells in transgenic mice results in a stable beta cell line, but these cells produce less insulin in the transformed state. When growth is arrested by cessation of T antigen expression, insulin production increases. Transplantation of these cells into STZ-induced diabetic mice restores normoglycemia (58).

Expansion of human primary pancreatic islet cells has also been tried. Primary adult islet cells can be stimulated to divide when grown on an extracellular matrix in the presence of hepatocyte growth factor/scatter factor, but growth is arrested after 10-15 cell divisions due to cellular senescence (59). Transformation of adult human pancreatic islets with a retroviral vector expressing SV40 large T antigen and v-Ha-ras Harvey rat sarcoma viral oncogene homolog $(H\text{-}ras)^{Val\ 12}$ oncogenes results in extended life-span, but eventually the cells enter a crisis phase followed by altered morphology, lack of proliferation, and cell death, suggesting that immortalization of human beta cells is more difficult than rodent beta cells. However, introduction of human telomerase reverse transcriptase results in successful immortalization (60), as human cells do not express telomerase. This immortalized cell line, beta-lox5, loses expression of key insulin gene transcription factors, and the introduction of Pdx-1, treatment with exendin-4, and cell-cell contact are required to recover beta cell differentiated function and glucose-responsive insulin production (61).

In another approach, human islets were propagated *in vitro* by transduction with retroviral vectors expressing SV40 T antigen and human telomerase reverse transcriptase flanked by loxP sites, and proliferation was stopped by excision of the immortalizing genes using an adenovirus expressing Cre recombinase. Removal of the immortalizing genes by Cre recombinase expression stops cell proliferation and increases the expression of beta cell-specific transcription factors, resulting in reversion of the cells. These cells are functionally similar to normal human islets with respect to insulin secretion in response to glucose and non-glucose secretagogues, although the insulin content and amount of secreted insulin is lower than human islets (62).

Establishment of insulin-producing beta cell lines by reversible immortalization of primary islets is a promising approach for replacing insulin injections, as a beta cell line can provide an abundant source of beta cells for transplantation. In addition, beta cell lines can be genetically manipulated to improve their function and survival. However, the functionality of the cell lines and safety issues remain to be further studied for therapeutic use.

5. GENERATION OF BETA CELLS FROM EMBRYONIC STEM CELLS

Embryonic stem (ES) cells are derived from the inner mass of a blastocyst. In principle, ES cells have the potential to generate unlimited quantities of insulin-producing cells, as they can be expanded indefinitely in the undifferentiated state and then be differentiated into functional beta cells. Therefore ES cells have been investigated as an alternative source of pancreatic beta cells. However, generation of fully differentiated beta cells from ES cells has been difficult and controversial. Particularly, beta cell differentiation from ES cells as determined on the basis of immunohistochemical evidence alone has been questioned, because insulin immunoreactivity can also result from insulin absorption from the medium, as well as from genuine beta cell differentiation (63, 64). Thus, C-peptide measurement is required to prove that these differentiated cells produce insulin. A variety of protocols have been tried to differentiate ES cells into pancreatic beta cells, and it was found that endoderm formation is required for successful differentiation of endocrine cells.

Since it was first reported that ES cells are capable of differentiating into insulin-producing cells (65), many different protocols have been tried to differentiate ES cells. Manipulation of culture conditions, multi-step protocols for selection of progenitor cells, and addition of growth factors and phosphoinositide kinase inhibitor can enhance differentiation into beta cells (66-68). Selected nestin-positive cells from embryoid bodies can be further differentiated in serum-free media with basic FGF and nicotinamide, resulting in generation of insulin-producing cells (66), but these cells do not reverse hyperglycemia. However, modification of this protocol by adding a phosphatidylinositol 3-kinase inhibitor increases insulin-production and reverses hyperglycemia (67). Another method is to drive beta cell differentiation by engineering the expression of genes for transcription factors such as Pax4, Pdx-1, Nkx2.2, and Nkx6.1. Overexpression of Pax4 in mouse ES cells promotes the differentiation of nestin-positive progenitor and insulin-producing cells, and these cells secrete insulin in response to glucose and normalize blood glucose when transplanted into diabetic mice (69). In the same study, the expression of Pdx-1 did not have a significant effect on the differentiation of insulin-producing cells from ES cells. However, another study demonstrated that the regulated expression of Pdx-1 in a murine ES cell line by the tet-off system enhances the expression of insulin and other beta cell transcription factors (70). Transfer of Nkx2.2 into mouse ES cells differentiates them into insulin-producing cells, and these cells secrete insulin in response to glucose (71).

To enrich insulin-producing cells from mouse ES cells, a cell-trapping selection method has been used. A neomycin-resistance gene regulated by the insulin promoter is transferred to ES cells, which drives differentiation of insulin-secreting cells, and transplantation of these cells restores normoglycemia in STZ-induced diabetic mice (65). In another study, mouse ES cells were transduced with a plasmid containing the Nkx6.1 promoter gene, followed by a neomycin-resistance gene to select the Nkx6.1-positive cells, and then the cells were differentiated in the presence of exogenous differentiating factors (72). The selected Nkx6.1-positive cells co-express insulin and Pdx-1 and beta cell-specific markers such as Nkx6.1, glucokinase, and sulfonylurea receptor, and transplantation of these cells into STZ-induced diabetic mice results in normoglycemia.

It was shown that human ES cells can spontaneously differentiate *in vitro* into insulin-producing beta cells, evidenced by the secretion of insulin and expression of other beta cell markers (73). Differentiation of insulin-expressing cells from human ES cells can be promoted by culture in conditioned medium in the presence of low glucose and FGF, followed by nicotinamide (74). Another report suggests that human ES cells differentiate into beta cell-like clusters when co-transplanted with mouse dorsal pancreas (75). Although several *in vitro* studies suggest the possibility of generating insulin-expressing cells from human ES cells, differentiation of truly functional beta cells from human ES cells has proven to be difficult.

Further advances in ES cell-derived differentiation of pancreatic beta cells involve a step-wise differentiation, which mimics endogenous pancreatic development – sequential stages of definitive endoderm, foregut, pancreatic precursor, endocrine pancreas, and then mature islet cells. Lessons learned from normal embryonic development have resulted in successful induction of definitive endoderm from ES cells *in vitro* (76-79). Culture of mouse ES cells in the presence of activin A under serum-free conditions results in visceral endoderm differentiation, evidenced by the expression of Sox17 and chemokine (CXC motif) receptor 4 (78). Similarly, production of human ES cell-derived definitive endoderm can be accomplished by low concentrations of serum and activin A. These cells express Sox17 and Foxa2 and can be enriched by chemokine (CXC motif) receptor 4 expression (79). Further studies by the same group reported differentiation of human ES cells to endocrine cells using a process which mimics *in vivo* pancreatic organogenesis. These cells produce pancreatic hormones; insulin, glucagon, somatostatin, pancreatic polypeptide, and ghrelin (80). In addition, recent studies reported successful generation of C-peptide-positive functional insulin-producing cells from human ES cells (81, 82) and mouse ES cells (83) by step-wise differentiation.

Because of their proliferative ability and capacity to differentiate in culture, ES cells have received much attention as a potential source of limitless quantities of beta cells for transplantation therapy to treat diabetes. Although *in vitro* studies suggest the possibility of generating insulin-producing cells from ES cells, production of truly functional beta cells from human ES cells for therapeutic use has not yet been reported, as the insulin content and insulin secretion are low as compared with normal islets and the yield is insufficient. Other limitations are ethical concerns and safety concerns with regard to tumor formation. In fact, transplantation of ES cell-derived insulin producing cells can reverse hyperglycemia for 3 weeks, but the rescue fails due to immature teratoma formation (84). Further studies are needed to develop an effective protocol for differentiating ES cells into sufficient amounts of fully functional pancreatic beta cells for therapeutic use.

6. GENERATION OF BETA CELLS FROM ADULT STEM CELLS

As with ES cells, adult stem cells have the potential to differentiate into other cell lineages, but do not have the ethical difficulties associated with ES cells. Beta cell neogenesis in adults has been reported in animal models of experimentally induced pancreatic damage, suggesting the presence of adult stem/progenitor cells (85). These adult stem/progenitor cells can be potential sources for the production of new insulin-producing cells (86-88). Bone marrow cells, umbilical cord blood cells, liver and intestinal cells, pancreatic ductal cells, and other cell sources have been investigated for their potential to differentiate into insulin-producing cells (Figure 2).

6.1. Bone marrow cells

Bone marrow cells are an abundant source of large quantities of stem cells. Evidence regarding the capability of bone marrow stem cells to differentiate into functional pancreatic beta cells is controversial. Bone marrow-derived stem cells have been shown to reduce blood glucose levels when transplanted into in diabetic mice, although no evidence was found for the differentiation of beta cells from these bone marrow cells (89, 90). *In vitro* differentiation of rat bone marrow cells induces expression of genes related to pancreatic beta cell development and function (91). These differentiated cells release insulin in response to glucose and reverse hyperglycemia when transplanted into diabetic rats (92). Ectopic expression of key transcription factors for the endocrine pancreatic development pathway such as insulin promoter factor, Hlxb9 and Foxa2 (93) or Pdx-1 (94) in human bone marrow mesenchymal cells generates insulin-producing cells. In addition, a mouse bone marrow cell line cultured in high glucose can express beta cell specific genes, but the insulin content is very low, less than 1% that of normal beta cells (95). Furthermore, it was reported that bone marrow stem cells differentiate into pancreatic beta cells *in vivo* without evidence of cell fusion (96). However, other studies could not reproduce these results (97, 98), fueling the controversy regarding the capability of bone marrow stem cells to differentiate into pancreatic beta cells. Some studies suggest that bone marrow-derived cells stimulate proliferation of endogenous progenitor cells in the pancreas, resulting in the increase of insulin-producing cells rather than transdifferentiation of bone marrow cells into pancreatic beta cells (89, 99).

6.2. Umbilical cord blood cells

As umbilical cord blood contains stem cells, is readily available in large amounts, and has a low risk for graft rejection, umbilical cord blood cells may be a source for the generation of insulin-producing cells. Human cord blood cells can be induced to express endocrine markers such as Isl-1, Pdx-1, Pax4, and Ngn-3 (100). In addition, transplantation of human cord blood cells to diabetic mice lowers blood glucose levels (101). Another study showed that stem cells isolated from human cord blood, which express stage-specific embryonic antigen-4 and the stem cell marker octamer-4, differentiate into insulin- and C-peptide-positive cells (102).

6.3. Liver and intestinal cells

Because the liver and intestinal epithelium are derived from gut endoderm, as is the pancreas (103), generation of islets from both developing and adult liver and intestinal cells has been tried as a source of insulin-producing cells. Hepatic oval stem cells can be differentiated into insulin-producing islet-like cells *in vitro* in the presence of high glucose. These cells express pancreatic beta cell markers such as Pdx-1, Pax4, Pax6, Nkx2.1, and Nkx6.1 and reverse diabetes when transplanted into diabetic mice (104). Similarly, a rat hepatic cell line, which stably expresses an active form of Pdx-1 along with a reporter gene, expresses endocrine genes and ameliorates hyperglycemia in diabetic mice. Exposure of these cells to high glucose can induce expression markers of mature islets (105). Another study showed that fetal human liver progenitor cells differentiate into insulin-producing cells when engineered to express Pdx-1 and transplantation of these cells reverses hyperglycemia in diabetic mice (106). Similarly, differentiation of adult hepatic progenitor cells by overexpression of Pdx-1 results in insulin secretion in response to glucose (107). It was also reported that adult human liver cells engineered to express Pdx-1 produce insulin and secrete it in a glucose-regulated manner. Transplantation of these engineered cells under the renal capsule of diabetic mice results in prolonged reduction of hyperglycemia (108). As well, ectopic islet neogenesis in the liver can be induced by gene therapy with Pdx-1 (109) or a combination of NeuroD, a transcription factor downstream of Pdx-1, and betacellulin, which reverses diabetes in STZ-treated diabetic mice (110).

Expression of Pdx-1 in a rat enterocyte cell line in combination with betacellulin treatment or coexpression of Isl-1 results in the expression of insulin (111, 112). Treatment of developing as well as adult mouse intestinal cells with GLP-1 induces insulin production mediated by the upregulation of Ngn-3, and transplantation of these cells into STZ-induced diabetic mice remits diabetes (113). Another study showed that neural progenitor cells can generate glucose-responsive, insulin-producing cells when exposed *in vitro* to a series of signals for pancreatic islet development (114). These results suggest that the controlled differentiation of liver or intestinal cells into insulin-producing cells may provide an alternative source of beta cells.

6.4. Pancreatic stem cells

A large body of evidence suggests that adult pancreatic ducts are the main site of beta cell progenitors. Throughout life, the islets of Langerhans turn over slowly, and new small islets are continuously generated by differentiation of ductal progenitors (34). Islet cells are observed in close proximity to ducts in type 1 diabetic patients and partially pancreatectomized rodents (115, 116). It was found that islet-like aggregates are generated from mouse pancreatic ducts and ductal tissue-enriched human pancreatic islets, and these aggregates release insulin after glucose stimulation and express islet proteins (117, 118). Duct-derived cells from prediabetic nonobese diabetic (NOD) mice restores normoglycemia in diabetic NOD mice (117). Lineage-tracing studies showed that new beta cells originate from existing beta cells (38), questioning the contribution of ductal cells to islet regeneration *in vivo*. However, it was recently reported that 30% of new beta cells are not derived from replication of existing beta cells (119), suggesting that stem progenitor cells may have a contribution. In accord with this, a recent study reported that Ngn-3-positive cells in the injured adult mouse pancreas differentiated into functional beta cells (120).

Multipotent precursor cells clonally identified from adult pancreatic islets and ductal populations can differentiate into cells with beta cell function (121). The clonally identified cells proliferate *in vitro*, and the

expression of the Pdx-1 gene or treatment of ductal cells with Pdx-1 protein increases the number of insulin-positive cells or induces insulin expression (122, 123). Ectopic expression of Ngn-3, a critical factor for the development of the endocrine pancreas in humans, in pancreatic ductal cells results in their conversion into insulin-expressing cells (124). In addition, treatment of human islets containing both ductal and acinar cells with a combination of EGF and gastrin induces neogenesis of islet beta cells from the ducts and increases the functional beta cell mass (125).

In addition to ductal cells, exocrine acinar cells and other endocrine cells can generate beta cells. A lineage tracing study showed that amylase- and elastase-positive acinar cells can transdifferentiate into insulin-producing cells (126). An alpha cell line transfected with Pdx-1 could express insulin when treated with betacellulin. It was shown that treatment of rat exocrine pancreatic cells with EGF and leukemia inhibitory factor can induce differentiation into insulin-producing beta cells (127), which involves activation of Ngn-3 (128). Considerable evidence suggests that beta cells in the pancreatic islets can be dedifferentiated, expanded, and redifferentiated into beta cells by inducing the epithelial-mesenchymal transition process (129). Non-endocrine pancreatic epithelial cells also have been reported to differentiate into beta cells (130). These results suggest that pancreatic stem/progenitor cells are the source of new islets.

6.5. Other sources

Other sources of cells have also been investigated for their potential to differentiate into insulin-producing cells. Injection of allogeneic splenocytes in combination with complete Freund's adjuvant (to prevent anti-islet autoimmunity) corrected diabetes in diabetic NOD mice (131). This study suggested that the injected splenocytes are the source of new insulin-producing cells, but later studies failed to show evidence of donor splenocyte-derived differentiation of insulin-producing cells (132-134). In addition, stem cells isolated from the salivary gland (135), adipose tissue (136), amniotic epithelium (137), neurons (114, 138), and human peripheral blood (139) can be differentiated into insulin-producing cells.

The use of adult stem/progenitor cells for generating beta cells for transplantation therapy appears to be promising, although most studies have only been done in animal models. Further studies on the mechanisms for the differentiation of adult stem/progenitor cells into insulin-producing beta cells and characterization of the newly generated beta cells are required before these cells can be considered for clinical application.

7. *IN VIVO* REGENERATION THERAPY

Generation of insulin-producing cells *in vivo* is also an attractive strategy for the treatment of type 1 diabetes. Various growth factors such as activin A, hepatocyte growth factor, keratinocyte growth factor, GLP-1, EGF, and betacellulin are known to stimulate pancreatic stem cells or progenitor cells and cause them to differentiate into an endocrine phenotype. Administration of recombinant human betacellulin improves glucose tolerance in alloxan- and STZ-induced diabetic mice, and in 90% of pancreatectomized rats by promoting beta cell regeneration (140-142). Treatment of alloxan-treated diabetic mice or diabetic NOD mice with EGF and gastrin reverses hyperglycemia by increasing the islet mass by neogenesis from ducts (125, 143). It was also reported that the combined treatment of activin A and betacellulin results in the regeneration of pancreatic beta cells in neonatal STZ-treated rats (144). In addition, treatment of type 1 diabetic NOD mice with a GLP-1 analog, exendin-4, and anti-lymphocyte serum as an immunosuppressor, resulted in rapid restoration of euglycemia after treatment, but intraperitoneal glucose tolerance tests were not completely normal at 80 days after treatment (145), probably due to insufficient numbers of regenerated beta cells resulting from the limited extension of the GLP-1 half-life afforded by this strategy. The production of GLP-1 or betacellulin *in vivo* by adenoviral delivery of these genes results in the restoration of euglycemia for a prolonged time and normal glucose tolerance by increase of beta cell mass (146, 147). The increase of beta cell mass might be due to the proliferation of existing beta cells and/or differentiation of stem/progenitors, which need to be identified by cell lineage studies. Recently, it was reported that the administration of keratinocyte growth factor into diabetic rats significantly increases ductal cell proliferation, resulting in the increase of beta cell mass (148). As well, treatment of STZ-induced diabetic mice with islet neogenesis associated protein can reverse hyperglycemia (48).

The expression of Pdx-1 (109) or a combination of NeuroD, a transcription factor downstream of Pdx-1, and betacellulin (110) in the liver remits hyperglycemia in STZ-induced diabetic mice. In addition, administration of an adenoviral vector encoding Pdx-1 or v-maf musculoaponeurotic fibrosarcoma oncogene homolog A (MafA) into the intestine was shown to convert intestinal cells into insulin-producing cells (149, 150).

Injection of mesenchymal splenocytes and complete Freund's adjuvant into diabetic NOD mice restores normoglycemia, and $CD45^-$ splenic mesenchymal cells were found to be responsible for the reversal of autoimmunity and the source of islet regenerating cells (131). A successful clinical trial involving autologous hematopoietic stem cell transplantation and immune suppressor in diabetic patients was reported (151). Although beta cell function was shown to be increased, the mechanism of action is not clear.

Clinical trials are now underway for beta cell regeneration in type 1 diabetes using exendin-4 and a combination of EGF, gastrin, and GLP-1 agonists along with immune regulators. Such treatments may not only provide a method to regenerate the beta cell mass from remaining beta cells in type 1 diabetic patients, but might be used in conjunction with islet transplantation therapies to expand and/or preserve the beta cell mass in transplanted islets.

8. SUMMARY AND PERSPECTIVES

Regeneration of pancreatic beta cells has been investigated as an attractive strategy for a cure for diabetes. Production of large amounts of beta cells by replication of beta cells *in vitro* has been tried using reversible immortalization, but the functionality of these engineered beta cells is still not optimal. Another option for the production of large amounts of beta cells is differentiation from stem cells. Information obtained from pancreas developmental biology has contributed to the design of strategies to differentiate stem cells into mature beta cells. Although a number of studies have reported successful differentiation of insulin-producing cells from embryonic or adult stem cells, yields are still too low for clinical needs and the differentiated beta cells are not as functional as endogenous beta cells. Step-wise differentiation of stem cells appears to be more effective than direct expression of pancreatic transcription factors. *In vivo* regeneration of beta cells using beta cell growth factors seems to be successful in animal models. Further studies are required to fully understand pancreatic islet development and to apply this knowledge to create safe and effective clinically applicable methods to regenerate pancreatic beta cells.

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Abbreviations: CDK: cyclin-dependent kinase; EGF: epidermal growth factor; ES: embryonic stem; FGF: fibroblast growth factor; Foxa2: forkhead box A2; GLP: glucagon-like peptide; Hlxb9: homeobox gene HB9; HNF: hepatocyte nuclear factor; H-ras: v-Ha-ras Harvey rat sarcoma viral oncogene homolog; Isl-1: ISL LIM homeobox 1; MAF: v-maf musculoaponeurotic fibrosarcoma oncogene homolog A; NeuroD: neurogenic differentiation factor; NOD: nonobese diabetic; Pax: paired box gene; Pbx1: pre-B-cell leukemia homeobox; Pdx-1: pancreatic and duodenal homeobox 1; Ptf-1a: pancreas-specific transcription factor-1a; Ngn-3: neurogenin-3; Nkx2.2: NK2 homeobox 2; Nkx6.1: NK6 homeobox 1; Sox: SRY (sex determining region Y)-box; STZ: streptozotocin; SV: simian virus; Wnt: wingless-type MMTV integration site family

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Send correspondence to: Hee-Sook Jun, Lee Gil Ya Cancer and Diabetes Institute, Gachon University of Medicine and Science, 7-45 Sondo-dong, Yeonsu-ku, Incheon 406-840 Korea, Tel: 11-82-32-858-9753, Fax: 11-82-32-858-8300, E-mail: hsjeon@gachon.ac.kr

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