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Current Cell-based Approaches for the Treatment of Diabetes Mellitus

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Introduction

Currently, the incidence of diabetes has reached epidemic proportions. It has been estimated that diabetes affects at least 200 million people in the world and it is predicted that by 2025 this number may double (Halban, 2004). Type 1 diabetes represents a minority (less that 10%), however, this type tends to be more aggressive and difficult to manage. The onset of type 1 diabetes peaks in early adolescence, resulting from the autoimmune destruction of insulin-secreting β cells in the pancreatic islets**.** The survival of these patients depends on the administration of multiple daily insulin injections. As a result, β-cell replacement therapy could, in principle, provide a potential cure for type 1 diabetes (Halban, 2004; Lechner and Habener, 2003).

Islet transplantation has rendered some patients insulin independent for a number of years, but requiring a possibly toxic immunosuppression (Halban, 2004; Ryan *et al*., 2005). The use of this therapy has been, however, restricted due to a shortage of transplantable material covering only a small fraction of patients (about <0.5% of needy recipients) (Halban, 2004; Lechner and Habener, 2003).

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Abbreviations: Pdx1; Pancreas-duodenum homeobox 1, Hlxb9; Homeobox transcription factor, Shh; Sonic hedgehog, Ptf1a-p48; Pancreas specific transcription factor 1a, Hes1; Hairy and enhancer of split homolog, Ngn3; Neurogenin-3, FGF; Fibroblast Growth Factors, Isl1; Islet-1, HNF1b; Hepatocyte nuclear factor 1 beta, Pax4; Paired homeobox gene 4, ITS; Insulin-Transferrin-Sodium Selenium, STZ; Streptozotocin, MAPC; Multipotent adult progenitor cells, NIPs; Nestin-positive islet-derived cells, PDSs; pancreatic duct-derived stem cells, BAP; bio-artificial pancreas.

In addition, type 2 diabetes is typically associated with obesity and it is now becoming manifest in younger individuals. It is induced by insulin resistance and inadequate insulin secretion, and it is initially treated with oral hypoglycemic drugs with such patients eventually requiring insulin therapy in the later stages of the disease. In fact in type 2 diabetes, a reduction of the β-cell mass of up to 50% has been recognized. As a result, the β-cell replacement of these missing cells may improve the insulin delivery malfunction of this form of the disease (Halban, 2004).

In this review paper we focus our attention on the potential cell sources for the treatment of diabetes. We also discuss the physiological β-cell function and regulation, which may provide useful clues to help us generate the functional β-cells using the currently available cell culture and gene transfer technology.

Pancreatic embryonic development

From its earliest stage of development, it is clear that the pancreas is dually integrated within a single organ**.** The dorsal and ventral buds from the foregut/midgut which appear during embryonic development become fused together (Wells and Melton, 1999). This early event strongly induces and commits the tissues by extracellular signals from neighboring cells, thus resulting in the expression of tissue- and cell type-specific patterns of transcription factors among the intercellular soluble factors released within the tissue: these are the signals and soluble factors from the notochord and the cardiogenic primordium that regulate the dual initial formation of the primitive pancreas (Soria, 2001; Wells and Melton, 1999; Wells and Melton, 2000)**.** This event determines the dominant and localized expression of Pdx1 (pancreas-duodenum homeobox 1) and Hlxb9 (homeobox transcription factor) expressed along the pancreas while Shh ("sonic hedgehog") is repressed (Edlund, 2001; Edlund, 2002; Soria, 2001; Wells and Melton, 1999). Thereafter, these primitive pancreatic tissues differentiate into the two cell populations which are later recognized in the adult tissues as endocrine and exocrine derived cells.

The early cell-fate differentiation process involves the expression of the several well-coordinated transcriptional factors and signals known as the Delta-Notch signaling system (Apelqvist *et al*., 1999; Edlund, 2001; Edlund, 2002; Jensen, 2004). The embryonic exocrine-committed cells express both Ptf1a-p48 (pancreas specific transcription factor 1a) and also Hes1 (hairy and enhancer of split homolog), the latter which serves as a transient repressor allowing the cells to proliferate partially undifferentiated, whilst their progeny only express Ptf1a-p48 (Apelqvist *et al*., 1999; Edlund, 2001; Jensen, 2004). In contrast, early endocrine progenitors initially express Ngn-3 (Neurogenin-3) and then later co-express Beta2/Neuro D. The further proliferation of these progenitor cells is mainly stimulated by fibroblast growth factors (FGFs) (Cras-Meneur *et al*., 2001; Elghazi *et al*., 2002; Hart *et al*., 2003).

The surrounding mesenchyme plays a key role in providing most of the regulatory factors for the proper pancreatic development (Scharfmann, 2000). The expression of Isl1 (Islet-1) within the mesenchyme of the dorsal pancreas regulates the transitional differentiation of the dorsal pancreatic epithelium, thereby increasing the endocrine cell population (Ahlgren *et al*., 1997). Some endocrine cells have been reported to be derived from a subset of HNF1b (Hepatocyte nuclear factor 1beta)-expressing duct

cells, which transiently express Ngn-3 (Gu *et al*., 2002). Therefore, Ngn-3-expressing cells, while transiently co-expressing Pax4 (paired homeobox gene), differentiate into β cells expressing high levels of transcription factors Pdx-1, Nkx6.1, Nkx2.2, and Pax6 (Jensen, 2004). However, as soon as the cells start to express hormones, such as insulin and glucagons, then they stop their cellular division. Later during the fetal growth, the endocrine cells seem to be able to re-enter into the cell cycle (Jensen *et al*., 2000). It is clear that Ngn-3-positive cells play the main role in the embryonic development of the pancreas, thus giving rise to all the pancreatic endocrine lineages. Nevertheless, their contribution to the balance of adult tissue remains unclear. Recent evidence has indicated that their population dramatically declines at birth and later becomes undetectable in the adult tissues even during pancreatic regeneration after injury (Harmon *et al*., 2004; Lee *et al*., 2006). However, since the adult pancreas has common progenitor cells capable of giving rise to the whole pancreatic progeny (Seaberg *et al*., 2004; Zulewski *et al*., 2001), the differentiation of such cells mimics in part the normal pancreatic development. In addition, since embryonic stem (ES) cells have been successfully isolated and can be propagated *in vitro* (Thomson *et al*., 1998), they could thus serve as an attractive cell source which resembles the normal pancreatic development when they are properly differentiated (*Figure 1*).

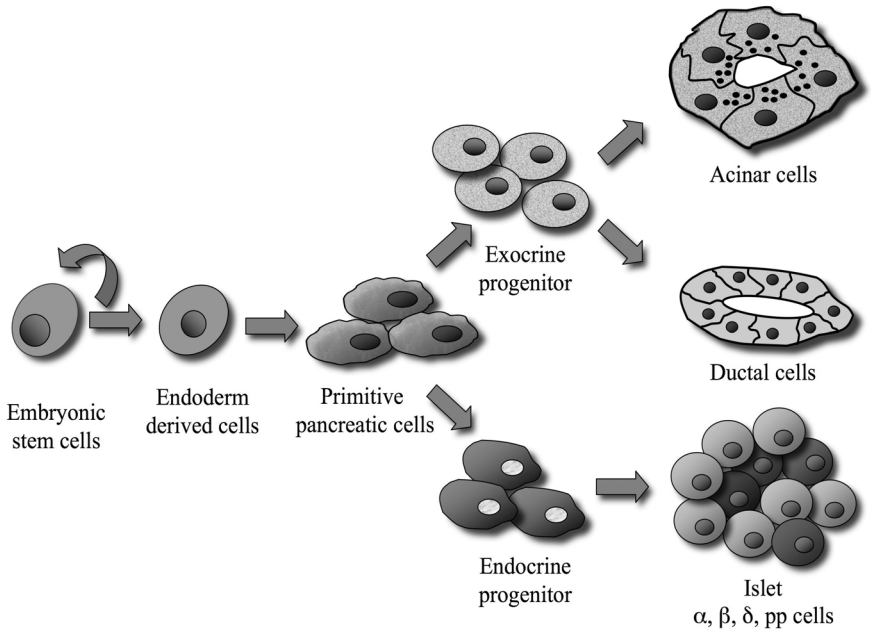

Figure 1. Pancreatic derived tissues. Undifferentiated ES cells (Oct 3/4-, Nanog-, and TERT-positive), endoderm derived cells (Foxa2-, Sox17-, and CXCR4-positive), primitive pancreatic cells (Shh- negative, HNF6-and PDX1-positive), endocrine progenitors (Ngn3-, PDX1-, and NeuroD-positive), exocrine progenitors (HES1-, Ptf1a-p48-, and CA-positive), exocrine cells (amylase-, and CA- positive), ductal cells (CK19-positive), α-cells (glucagon-positive), β-cells (insulin- positive), δ-cells (somatostatin-positive), pp-cells (pancreatic polypeptide- positive).

Late fetal pancreatic growth and post-natal maturation

The embryonic pancreas has a small endocrine population, especially β cells, which are not able to support the metabolic demands of the fetus. Therefore, during the late phase of fetal gestation, a massive growth of the β-cell mass begins, thus resulting in the islet formation. The growth of fetal islets is mainly driven by a rapid proliferation and differentiation of progenitor cells (neogenesis), while β cell replication seems to play only a minor role in this process. The islet precursors have certain similar characteristics of the ductal cells in the adult pancreas, however they trend to differentiate into mature endocrine cells (Bouwens and Rooman, 2005). These facts could in part be related to the expression of both cytokeratin-19 and nestin, which are two of the characteristic markers of the putative pancreatic stem cells derived from adult pancreas (Seaberg *et al*., 2004; Zulewski *et al*., 2001). Along with all the other post-natal physiological changes in the body, the pancreas is not an exception to such events. The most recent evidence has shown that the pancreatic endocrine population can still grow meaningfully during the childhood, as most other tissues do (Bouwens and Rooman, 2005). However, the main mechanism responsible for the post-natal β cell growth seems to be cyclin D2-mediated β cell replication rather than the differentiation from the cell progenitors (Dor *et al*., 2004; Georgia and Bhushan, 2004). During this process, the β cell population is also under the control of several regulatory hormones (growth hormone, prolactin, insulin, glucagon, glucagon like peptide (GLP)-1, gastrin, cholecystokinine, acetylcholine, and leptin) and factors (IGF-I, IGF-II, EGF, FGFs, PDGF, HFG, Activin A, TGF-α, NGF, VEGF, Nicotinamide, IL-1, INF-γ, TGF-β, and TNF-α), which determine the compensatory balance (Bouwens and Rooman, 2005; Nielsen *et al*., 2001).

The adult pancreas

The adult pancreas has essentially two independent functions based on its tissue composition; the endocrine and the exocrine functions. Exocrine tissue represents more than 95% of the total mass. It is composed of a well-developed system of pancreatic ducts, which drains their enzymes from pancreatic acinar cells into the duodenum. Embedded within exocrine tissue are clusters of cells resembling islands in the sea, called the islets of Langerhans, which are a type of endocrine tissue. It is estimated, the human pancreas has about one million islets (Halban, 2004). The average size of one human islet is 200 μm and they represent about 1% of the total pancreatic tissue (Halban, 2004). These islets are formed mainly from four endocrine populations (Halban, 2004). The β-cell population represents about 80% of the cells within each islet, about 5% correspond to the δ-cell population, and the last 15% are either α-cells or pp cells depending on the location of the islets within the pancreas: islets derived from embryonic dorsal pancreas (tail and body in adult tissues) are predominantly populated with α-cells whilst islets derived from embryonic ventral pancreas (head and uncinate pancreatic process) are with pp-cells. The remnant pancreatic tissues are formed by connective, neural interactive, vascular, stem, and mesenchymal cells (Kulkarni, 2004). The mature β-cells can freely intake the glucose and then balance its concentration by secreting insulin into the bloodstream, thereby regulating the glucose uptake in the peripheral tissues. In contrast, α-cells secrete glucagon the functional antagonist of the insulin. Somatostatin which is secreted by δ-cells inhibits the secretion of both insulin and glucagon (Nir and Dor, 2005).

Therefore, a well-coordinated balance among all the pancreatic endocrine cells is required. Physiologically, β-cells have a turn over of every 40 -50 days: this is achieved by balancing apoptosis, a slow proliferation of the remaining β-cells and differentiation of the new islet cells (neogenesis) derived from progenitor epithelial cells located in the pancreatic ducts (Kulkarni, 2004; Zulewski *et al*., 2001). Meanwhile, after stimulation β-cell neogenesis takes place with 2-3 days, deriving from the intraislet progenitors (Lechner and Habener, 2003; Zulewski *et al*., 2001). This alternative mechanism guaranties the rapid restoration and maintenance of the total β cell mass.

Despite the fact that the β-cells have a single function, namely to secrete insulin, the way that the cells secrete insulin while responding to blood glucose levels is quite a sophisticated and complex process. The transcription and translation of the insulin gene is tightly regulated by several transcriptional factors (PDX-1, NeuroD, Isl 1, hepatocyte nuclear factors (HNFs), etc), as shown in (*Figure 2*) (Ohneda *et al*., 2000; Sander and German, 1997). The timing of the expression of each of these transcriptional factors is perfectly synchronized to accomplish the transcription and translation of the insulin gene. The pre-pro-insulin should be cleavaged and matured during the post-translational processing stage (Ball and Barber, 2003). A single β-cell with a size of 15 μm can store about 10,000 insulin granules and a single insulin granule of the size of 300 nm contains approximately 200,000 molecules of the crystallized insulin (Halban, 2004). This is a well-orchestrated process, which is initially triggered by the glucose intake at the cell membrane and then eventually ends up with the glucose-responding insulin secretion (*Figure 3*) (Ball and Barber, 2003). Therefore, the generation of reliable pancreatic β-cells is quite a difficult task.

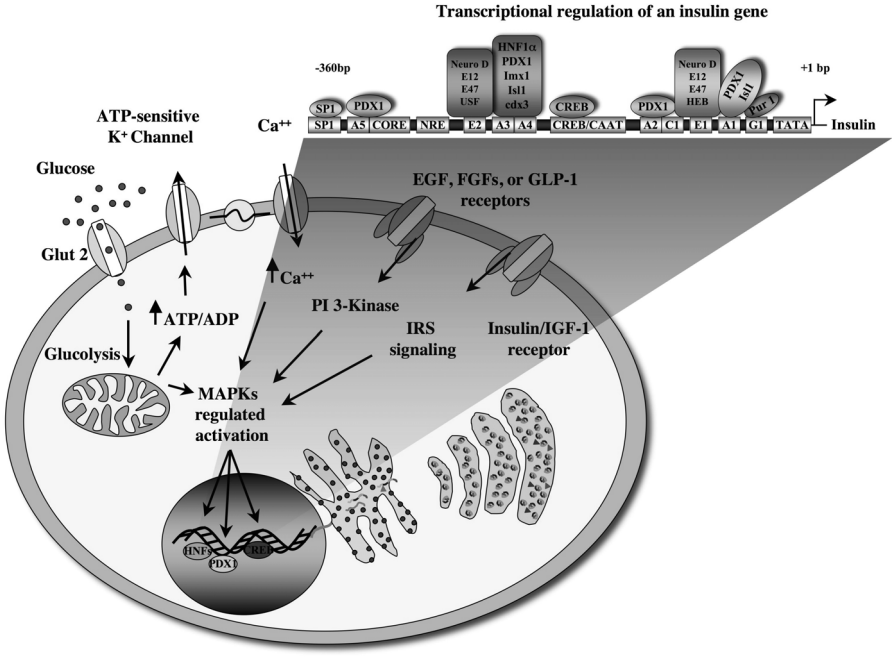

Figure 2. Transcriptional regulation of gene insulin in the β-cells**.** This schematic drawing shows a global view of the pathways and transcriptional regulation of the insulin gene after the stimulation of glucose or growth factors.

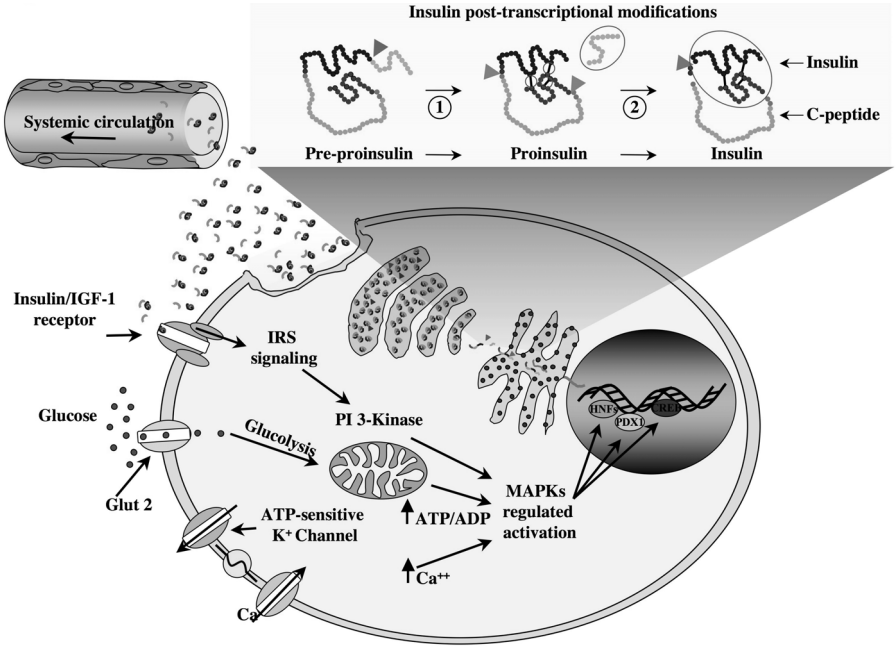

Figure 3. Post-transcriptional modifications and processing of the insulin in the β-cells. Pre-proinsulin, a polypeptide of 100 amino acids is rapidly translated (within a minute) after stimulation in the β-cells: 1) A major initial transition occurs in the cytoplasm after the removal of the signaling peptide and S-S bridge formation producing the proinsulin, which is exported to the apparatus of Golgi for storage and final maturation; 2) Proconvertases within the apparatus of Golgi cleavage the C-peptide producing the mature form of insulin and then releasing it upon glucose-mediated stimulation.

Differentiation of embryonic stem cells into pancreatic β-like-cells

The successful establishment of several embryonic stem (ES) cell lines has generated new prospects in the field of cell therapy. ES cells might constitute a potentially reliable cell source for pancreatic β cells, since the cells can be unlimitedly propagated and the cells produce tissues in three germinal layers (Thomson *et al*., 1998). Over the last five years, controversial but interesting results have been found in the attempt to differentiate ES cells into β-like-cells. Most of these protocols have described the generation of a small population of insulin-positive cells in differentiated ES cells (Kania *et al*., 2004; Sipione *et al*., 2004). Therefore, endocrine cells are considered to be a minority of the cells in the entire pancreas (Bouwens and Rooman, 2005).

Researchers should in the future perform selective evaluations of the pancreas-deriving tissues at the initial step instead of making spontaneous embryonic body (EB) formation. Pancreatic differentiation has been conducted through EB formation in most of the protocols, eventually resulting in an insufficient generation of the endocrine progenitors. It is well recognized that the selection of the cells with insulin-transferrin-sodium selenium (ITS) results in an overestimation of the generation of the endocrine progenitor population (Kania *et al*., 2004; Lumelsky *et al*., 2001; Sipione *et al*., 2004). In addition, we have to pay a close attention to the considerable

cytotoxicity induced by ITS selection, because apoptotic or dying cells uptake the insulin contained in the serum-enriched culture medium and either they appear falsely positive for insulin or otherwise they release such insulin into the culture medium again. Eventually such cells can be incorrectly identified as insulin positive-cells (Kania *et al*., 2004; Rajagopal *et al*., 2003; Sipione *et al*., 2004). Consequently, alternative methods instead of ITS selection must be explored to differentiate *bona fide* β-like-cells from the ES cells. Other researchers have included the introduction of reporter genes, such as drug resistant genes and/or fluorescent genes, regulated by the insulin promoter into the group of ES cells. Such a strategy allows for the selection of insulin promoter-positive cells in the whole cell population by positive drug selection and/or fluorescence-based cell sorting. Despite such sophisticated approaches, only a few insulin-expressing cells have been recovered so far (Soria, *et al*. 2000; Soria 2001). Since a meaningfully high degree of the promoter activity is required to drive the reporter gene, when this strategy works well, it guarantees the appearance of the β-cell phenotype in the cells and the generated cells are capable to restore euglycemia in diabetic STZ-induced mice (Soria, *et al* 2000; Soria, 2001). Of course, these cells reveal the high expression of all the β-specific transcriptional factors PDX1, Neuro D, Isl1, Pax4, Pax6, Nkx2.2, and Nkx6.1. (*Figure 2*). It is likely that differentiated β-like-cells produce a lower amount of insulin in comparison to normal β-cells of the adult pancreas, because such ES cell-derived β-like-cells may be immature in an *in vitro* environment without the contact of appropriate cell matrices and neighbors. It is also important to evaluate whether these cells have a property of physiological glucose-responding insulin secretion. Tightly regulated insulin secretion with glucose responsiveness is one the essential issues which must be clarified in order to effectively perform cell therapy for diabetes patients. Any cells that produce and release insulin in a constitutive manner cannot be candidates for cell transplantation in diabetes (Halban, 2004; Halban *et al*., 2001). Since some of key transcriptional factors, such as PDX-1 and Pax 4, play an important role in the physiological regulation of the insulin production and secretion, researchers have thus far used them as direct gene manipulation means to enhance the differentiation-inducing rate of the β-like-cells (Blyszczuk *et al*., 2003; Miyazaki *et al*., 2004). One of the most annoying issues associated with the use of the ES cells is the possible formation of teratomas after transplantation. Even though a highly optimized differentiation protocol allows us to generate functional β-like-cells from the ES cells, redundant safeguards should be provided to prevent any unexpected teratoma formation (Fujikawa *et al*., 2005).

The adult stem cells as β-cell source for cell therapy

The discovery of multipotent stem cells (MAPCs) in adult tissue has resulted in a widespread belief in the possible effectiveness of cell therapy. MAPCs can differentiate into any type of adult tissue and they indeed contribute to the maintenance of the tissue homeostasis with circulation to the peripheral organs and then being tissue specific stem cells. Therefore, most of the tissues and organs possess the committed stem cells derived from MAPCs (Jiang *et al*., 2002). However, within the pancreas two main candidates for stem cells have been identified and partially characterized: the nestin-positive intra-islet progenitor cells (NIPs) and pancreatic duct-derived stem

cells (PDSs). The NIPs seem to have particularly more primitive characteristics than PDSs, since NIPs contain a side-population. In fact, PDSs can be propagated less favorably than NIPs due to the limited growth capacity. NIPs can differentiate into endocrine, exocrine and hepatic cells as well: by contrast, only endocrine and exocrine cells are derived from PDSs. PDSs can form aggregates with progressively increasing their insulin content, but the glucose responding capacity of such aggregated PDSs is lower than that of NIPs (Lechner and Habener, 2003).

These adult pancreatic stem cells (NIPs and PDSs) could thus possibly serve as a safer alternative cell source than derivates from the ES cells. NIPs and PDSs could be isolated from a biopsied pancreatic tissue, expanded, and then differentiated to β-cells *in vitro*. In particular, these newly differentiated β-cells may have some additional advantages over the native β-cells, such as, a lack of the expression the β-cell antigens that are the target for autoimmunity (Efrat, 2002; Lechner and Habener, 2003). This will prevent the recurrence of autoimmunity. Furthermore, these cells could also express higher levels of free-radical scavenging enzymes than the native β-cells, thus resulting in resistance to the apoptosis induced by inflammatory cytokines (IL-1β and TNF-α) after a transplant procedure (Tiedge *et al*., 1997). Finally, the utilization of those cells may be accomplished by autologous sampling, the removal of immunosuppression and any possible rejection (Lechner and Habener, 2003).

The role of the surrogates of β-cells in the treatment of diabetes

Developing surrogates of β-cells offers an alternative option for the treatment of diabetes. The considerable advances in trying to identify a surrogate for β-cells have been achieved in hepatocytes. Since hepatocytes naturally possess glucose-sensitive factors of Glut2, glucokinase, and L-pyruvate kinase, they possess far more advantages over other potential choices of muscle cells or fibroblasts to better mimic the normal β-cell function (Mitanchez *et al*., 1997; Okitsu *et al*., 2004; Thule and Liu, 2000). Since such generation of β-cell surrogates in hepatocytes requires gene transfer, it is preferred to perform such manipulation in the cells under a better-controlled *in vitro* environment (Okitsu *et al*., 2004). Because the hepatocytes are transduced with an insulin gene derived by glucose-sensitive promoters, they produce only pro-insulin in response of glucose stimulation. The hepatocytes lack of proconvertases which convert pro-insulin to mature insulin, they fail to mature the insulin from the pro-insulin stored *de novo* (Mitanchez *et al*., 1997). Therefore, researchers have attempted to transduce a furin-cleavable pro-insulin gene (FCPI) into the hepatocytes, thus leading to the maturation of pro-insulin to insulin by the cleavage of C-peptide (*Figure 3*) (Muzzin *et al*., 1997). In this case, the insulin produced and secreted by FCPI-expressing hepatocytes is constitutive and thus it is not exactly well coordinated with the glucose concentration. Towards this goal we have made an effort to establish an immortalized human hepatocyte cell line YOCK-13 which expresses a single insulin analogue (SIA) under the control of the promoter of an L-pyruvate kinase promoter. Since SIA does not require any post-translational modifications, SIA-transduced hepatoyctes can secrete insulin whilst responding to the glucose levels. The transplantation of YOCK-13 cells into the liver resulted in the control of the blood glucose levels in diabetic pigs (Okitsu *et al*., 2004). The generation of a functional surrogate of β-cell from cell lines or stem cells can survive relatively longer and lack

in the expression of β-cell antigens that induce the recurrence of an autoimmune disease (Efrat, 2002).

Genetic engineered β-cell lines for the treatment of diabetes

The establishment of a highly differentiated immortalized human pancreatic β-cell line represents a highly attractive cell source. Despite these great efforts, an extremely low success rate has so far been achieved. Most of the generated cell lines are difficult to be propagated *in vitro*, especially after 15 passages, and such cell lines gradually lose their differentiated function over the time *in vitro* (Itkin-Ansari *et al*., 2003). Among all of the engineered human β-cell lines, only two cell lines β-lox5 and NAKT-15 continue to possess the normal β-cell characteristics and functions to at least some degree.

Both β-lox5 and NAKT-15 cells have been established using adult human β-cells recovered from isolated normal islets (Itkin-Ansari *et al*., 2003; Narushima *et al*., 2005). Although we formerly worked on immortalizing several human liver cells of hepatocytes, endothelial cells, stellate cells, and cholangiocytes, we encountered difficulty in immortalizing β-cells (Kobayashi *et al*., 2000a; Kobayashi *et al*., 2000b; Maruyama *et al*., 2004; Matsumura *et al*., 2004; Narushima *et al*., 2005; Shibata *et al*., 2003). This may be explained in part by the fact that mature β-cells have a short half-life (about 50 days) and they also tend to be fragile when used in an *in vitro* culture (Zulewski *et al*., 2001). Most of mature β-cells rapidly undergo apoptosis after islet isolation procedure itself (Ryan *et al*., 2005). Next, the loss of the cell-to matrix interaction and the stress induced by the tissue culture render β-cells undergo apoptosis and become un-differentiated.

A single transduction with simian virus 40 large T antigen (SV40T) is sometimes sufficinet to immortalize human cells, however, the transduction of SV40T in the mature β-cells increases their lifespan by approximately 10-folds (de la Tour *et al*., 2001; Itkin-Ansari *et al*., 2003). In our experience of immortalizing human β-cells, we first subjected the human islets to the monolayer formation in order to expose the β-cells for retroviral SV40T transduction (*Figure 4*). Secondly, the SV40T-transduced cells were stained with a Newport green dye, which is well known to be a zinc indicator, so as to recover the β-cell population. Thirdly, we retrovirally introduced hTERT, which was in conjunction with GFP, into such Newport green-positive cells to completely immortalize the cells. Fourthly, hTERT-and SV40T-transudced β-cells were subjected to a single cell cloning using an automated cloner. Clonal screening included the gene expression assays, tumorigenic evaluation, and glucose-responding insulin secreting capacity. Eventually, we were able to establish a highly differentiated human pancreatic β-cell clone NAKT-15.

As shown in *Figure 4*, both SV40T and hTERT are flanked by a pair of LoxP. The expression of Cre recomibinase can remove LoxP-flanked SV40T and hTERT in NAKT-15 cells. Such reverted NAKT-15 cells were more functional than parental NAKT-15 cells (Narushima *et al*., 2005). The establishment of a highly differentiated human β-cell line can constitute a model for basic research and cell therapy under pertinent safety conditions. Despite the observation that reverted NAKT-15 cells were not tumorigenic in immunodeficient mice, additional safeguards should nonetheless be provided for the clinical application. One of the particularly attractive approaches

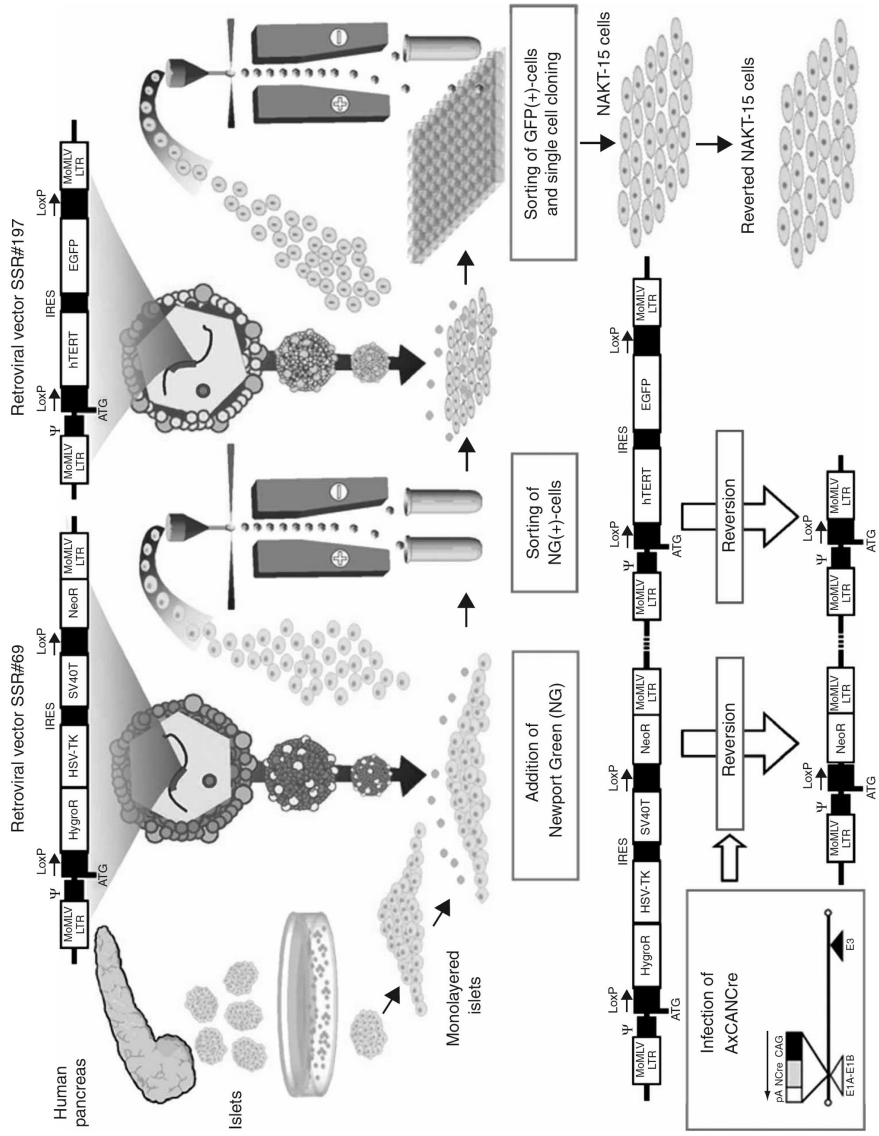

Figure 4. Strategy for immortalization for pancreatic ß-cells**.** A schematic drawing for establishment of reversibly immortalized human ß-cells is represented. Freshly isolated human islets were cultured in monolayers and transduced with the retroviral vector SSR#69. After hygromycin selection of SSR#69-transduced cells, sorting using Newport Green dye was performed. After transduction with SSR#197, the EGFP-positive cells were recovered and subjected to single-cell cloning, thus yielding the clone NAKT-15. The transient expression of Cre recombinase was induced by infecting the cells with a recombinant adenovirus expressing Cre recombinase (AxCANCre), which removed the SV40T and TERT genes that were flanked by LoxP sites. Neomycin (G418) selection and EGFP-negative cell sorting resulted in the collection of reverted NAKT-15 cells.

for the use of NAKT-15 cells is the development of the bio-artificial pancreas (BAP). The reverted NAKT-15 cells can be inoculated inside the BAP device and the cells have no chance to escape from the device as long as no unexpected device failure occurrs. The implementation of a successful design of BAP can perfectly allow the NAKT-15 cells to function without any fear of an immunological attack of the host immune system.

Conclusions

Although diabetes has recently reached epidemic proportions, a definitive cure for diabetes has still not been achieved. Despite this, islet transplantation has crossed over boundaries that formerly existed for the development of cell-based therapies for diabetes and offers considerable potential. Indeed, islet transplantation is now gaining attention as a potentially effective new type of diabetes treatment, although there are severe worldwide shortages of human pancreata. It is very unlikely that such donor organ scarcity will be resolved in the near future. Replacement of the missing β-cells represents an ideal treatment in type 1 diabetes and it also constitutes a major challenge to modern regenerative medicine. In this regard, two main approaches have been conducted to seek donor tissue supplies. One has steered towards β-cell differentiation of ES cells. Despite the controversial results that have so far been prematurely obtained, researchers still have high hopes with the ES route. Even though differentiated ES cells fail to properly resemble the β-cell phenotype, they can provide us with valuable information in the study of developmental biology. Whatever, once researchers have been able to successfully differentiate ES cells into functional β-cells, they will then have to establish a new system for dealing with immunotolerance in such an ES cell-based cell therapy.

Another approach has been aimed at β-cell line engineering using gene transfer technology. Such cell lines can be supplied for the appropriate cell therapy on a large scale and they would thus allow us to overcome the present human organ shortage. Although, genetically engineered β-cells can constitute a very attractive source, the cells are allogeneic enough to induce the host immune reaction. Therefore, such cells would have to be immuno-isolated inside the BAP device in order to achieve a long-term survival of such cells for a reliable and definitive cure of diabetes.

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