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Breakthroughs and Views

Regeneration therapy of pancreatic β cells: towards a cure for diabetes?

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Abstract

Regeneration therapy is an approach which could potentially move us towards a cure for type 1 diabetes. It is classified into three categories: (1) In vitro regeneration therapy using transplanted cultured cells, including ES cells, pancreatic stem cells, and β-cell lines, in conjunction with immunosuppressive therapy or immunoisolation. (2) In ex vivo regeneration therapy, patients' own cells, such as bone marrow stem cells, are transiently removed and induced to differentiate into β cells in vitro. At present, however, insulin-producing cells cannot be generated from bone marrow stem cells. (3) In in vivo regeneration therapy, impaired tissues regenerate from patients' own cells in vivo. β-Cell neogenesis from non-β-cells and β-cell proliferation in vivo have been considered, particularly as regeneration therapies for type 2 diabetes. Regeneration therapy of pancreatic β cells can be combined with various other therapeutic strategies, including islet transplantation, cell-based therapy, gene therapy, and drug therapy to promote β-cell proliferation and neogenesis, and it is hoped that these strategies will, in the future, provide a cure for diabetes.

There are diverse therapeutic strategies for the cure of diabetes mellitus including: (1) the artificial pancreas (artificial insulin delivery systems), (2) pancreas transplantation, (3) islet transplantation, (4) cell-based therapy, (5) gene therapy, and (6) regeneration therapy. The usefulness of the artificial pancreas is limited by the short-term durability of the glucose sensor (about 1 week), and donor shortage and life-long immunosuppressive treatment are unsolved problems of pancreatic transplantation. The other therapeutic strategies will be discussed in detail in this paper.

Islet transplantation

Unlike pancreatic grafts with intact blood vessels, avascular islet transplants can be encapsulated for immunoisolation without immunosuppressive therapy. The capsule membrane is selectively permeable; low-molecular-weight substances such as nutrients, electrolytes, oxygen, and insulin are exchanged across the membrane, whereas immune cells and antibodies are excluded. However, it is difficult to achieve complete protection from immunological rejection because the insulin-permeable pores of the membrane also allow the efflux of small antigens and the influx of complement components and inflammatory cytokines [1]. Specifically, in type 1 diabetes mellitus, insulin itself, which is secreted from encapsulated islet grafts, is an autoantigen recognized by autoimmune cytotoxic T lymphocytes. Moreover, a functional oxygen supply is crucial for the proper operation and long-term survival of the islet grafts. However, attempts to achieve this by improving blood vessel formation around an immunobarrier membrane induce T cell recruitment, resulting in β-cell apoptosis by membrane-permeable cytokines. Around the capsules, fibrosis is induced by the capsule material, leading to a progressive loss of islet cells.

Islet transplantation into the portal vein is less invasive than pancreas transplantation as open surgical procedure is not required [2]. Instead, a catheter is inserted into the portal vein by a percutaneous transhepatic approach under ultrasound or angiographic guidance, and purified islet suspension is slowly infused while the intraportal hydrostatic pressure is continually monitored. Currently, the portal vein is considered the preferential site for islet graft implantation because the

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efficient blood and nutrient supply ensures a good clinical outcome. As insulin is first secreted from pancreatic β cells into the portal vein, the liver is exposed to the highest concentration of insulin of any organ, to control metabolic function. Therefore, intraportal islet implantation can achieve near-normal tissue distribution of insulin. Subcutaneous insulin injection or subcutaneous implantation of encapsulated islets, however, increases insulin concentration in the vena cava rather than in the portal vein, and therefore cannot attain strict glycemic control in diabetic patients with severe insulin deficiency because of the relative insufficiency of the insulin supply to the liver.

There have recently been substantial advances in immunosuppressive therapy. Previously, calcineurin inhibitors (cyclosporin or tacrolimus) and steroids were used for immunosuppression in islet transplantation. However, these drugs have diabetogenic effects due to their direct toxic effect on β cells and their induction of insulin resistance. An improved non-diabetogenic immunosuppressive protocol using low-dose tacrolimus, rapamycin (sirolimus), and anti-IL2-receptor monoclonal antibody brought about insulin independence for over a year in all seven of a group of patients with type 1 diabetes mellitus who underwent islet transplantation [3]. New monoclonal antibody therapies, including the anti-IL2-receptor antibody, anti-CD3 antibody, anti-CD154 antibody, and anti-TNFα antibody, affect T-cell signaling and may induce immune tolerance specific to islet grafts in the future. The progress of immunosuppressive therapy for islet transplantation and the excellent therapeutic results achieved thus far are accelerating research which may result in a solution to the shortage of human donor islets.

The pig has been identified as the most suitable animal donor for islet transplantation, and the antigenicity of pig organs can be reduced by transgenic approaches. However, in addition to graft rejection, the risk from xenobiotic viruses is a further problem of xenotransplantation. Porcine endogenous retroviruses (PERV) can infect human cells in vitro and mice in vivo after pig islet xenotransplantation, although PERV infection has not been detected by PCR analysis in any of the patients who have been treated with various living pig tissues.

Thus far the proliferation of islet cells from adult human donors is limited in vitro. If islet cells could proliferate in vitro in an unlimited fashion, however, this shortage could be easily overcome. Because fetal islets are rich in β-cell precursors, a sufficient islet supply would be ensured if these precursor cells could proliferate and differentiate into mature β cells. In general, however, as β cells proliferate in vitro, it has been demonstrated that their differentiated phenotypes showed crucial changes, including a gradual decline in insulin secretion. A better understanding of the mechanisms of β-cell proliferation and differentiation may enable the proliferation of highly differentiated β cells and the re-differentiation of low-differentiated β cells after proliferation in vitro, possibly through the addition of β-cell differentiation factors such as nicotinamide or by gene transfer.

Recently, pancreatic ductal epithelial cells isolated from adult mice were grown in long-term cultures and induced to produce a large number of functioning islets [4]. Cells expressing insulin and other pancreatic endocrine hormones were generated from mouse ES cells, and they self-assembled to form three-dimensional clusters similar in topology to normal pancreatic islets [5]. Furthermore, cells expressing a neural stem-cell marker, nestin, were observed in adult pancreatic islets and ducts, and considered to be pancreatic stem cells because of their proliferative capacity and ability to differentiate into various cell types with the phenotypes of pancreatic ductal epithelia, endocrine cells, exocrine cells, and hepatocytes [6]. These reports raise hope for regeneration therapy of pancreatic islets combined with islet transplantation techniques. However, more detailed characterization of the islets generated in vitro is necessary, including electron microscopic observation, as the in vitro differentiated endocrine cells are frequently deficient in secretory granules.

Cell-based therapy

In most cultured β-cell lines, oncogenes such as SV40 virus large T antigen and c-myc are highly expressed, and the resultant genomic instability leads to heteropliod mitoses, unlimited growth, apoptosis, and impaired insulin secretion after implantation of cultured β-cell lines. In contrast, ES cells maintain their genomic stability, and the utilization of β cells derived from mouse [7] and human ES cells [8] may circumvent tumor formation from β-cell grafts. However, contamination of undifferentiated cells in ES-derived β-cell grafts leads to the development of teratoma after their implantation.

Furthermore, the experimental results achieved using β-cell transplantation are inferior to those seen with islet transplantation. In rodents, the survival of transplanted β cells was prolonged by the presence of islet endocrine non-β-cells within the graft. In addition, glucagon secreted from α cells and cell–cell interactions amongst the four types of endocrine islet cells may achieve better glycemic control than implantation of β cells alone.

Gene therapy

Because β-cell-specific antigens are not expressed in non-β-cells, genetically engineered non-β-cells easily escape from autoimmune destruction in type 1 diabetic patients. Moreover, the utilization of insulin-secreting

non-β-cells could solve the problem of an insufficient supply of islets for transplantation. Hepatocytes appear to be the most promising candidate for insulin-producing non-β-cells in clinical applications [9]. The liver is the target organ for insulin, and it is exposed to the highest concentration of insulin, secreted from the pancreas into the portal vein. Therefore, insulin expression in hepatocytes in vivo may be able to reproduce near-physiological conditions. Because hepatocytes considerably outnumber β cells, insulin gene transfer into only a small percentage of hepatocytes could improve diabetes mellitus by inducing sufficient insulin secretion. As hepatocytes regulate the expression of metabolic enzyme genes in response to glucose and insulin, glucose-responsive promoters and insulin-sensitive promoters of these genes have been used to regulate transcription of the exogenous insulin gene in genetically engineered hepatocytes.

Although proliferation and apoptosis of β cells can be modulated by exogenous gene expression, an effective method for gene transfer into β cells in vivo has not as yet been developed. However, in vitro gene transfer into β-cell grafts and islet grafts before transplantation has been demonstrated to improve the therapeutic effects (Fig. 1).

Regeneration therapy

The techniques of regeneration therapy can be classified as: (1) in vitro regeneration therapy, (2) ex vivo regeneration therapy, or (3) in vivo regeneration therapy.

In vitro regeneration therapy

In this therapy, patients' own cells are not used. Cultured cells, such as ES cells are induced to differentiate in vitro, and subsequently, the differentiated tissues are implanted into patients. For example, cultured artificial skin has widely been used for skin wound healing, protecting skin lesions, such as burns, until the graft is rejected. In vitro regenerating therapy for diabetes mellitus necessitates the life-long use of immunosuppressive therapy. Immunosuppressive drugs have various side effects, including immunosuppression itself, which opens the way for opportunistic infection and an increased incidence of malignancy, as well as direct drug toxicity. In particular nephrotoxicity and hypertension have been seen with cyclosporin and tacrolimus use. Despite the capacity for unlimited

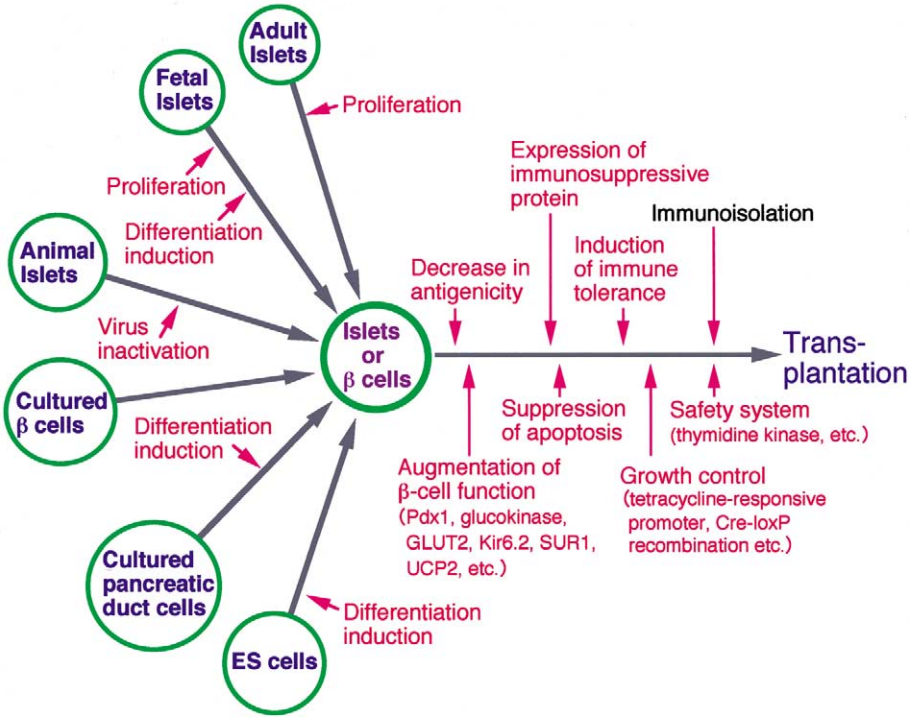

Fig. 1. The transplantation of genetically engineered β cells or islets. Various steps for gene transfer are shown in red [9]. "Augmentation of β-cell function" is a gene therapy strategy to restore rapid insulin secretion in response to glucose in low-differentiated β cells after long-term culture. "Growth control" of cultured β cells is performed by tetracycline-responsive oncogene expression or by the removal of oncogene via the Cre-loxP system before transplantation. "Safety system" functions by administration of gancyclovir, and eliminates uncontrolled transplants expressing thymidine kinase.

preparation of β-cells from cultured ES cells [5,7,8], pancreatic stem cells [6], or pancreatic duct epithelial cells containing islet progenitor cells [4], the serious side effects of immunosuppressive therapy have meant that the application of in vitro regeneration therapy and islet transplantation for diabetes mellitus has been limited to type 1 diabetic patients with severe clinical findings, such as those with reduced awareness of hypoglycemia, metabolic instability, or progressive diabetic complications.

Ex vivo regeneration therapy

Here the patients' own cells are transiently removed, undergo various treatments, and are subsequently reimplanted without immunosuppressive therapy. For example, skin autografts grown in culture are spontaneously accepted for life after transplantation. The patient's own ES cells can be generated by the nuclear transfer of his or her somatic cells into anuclearte oocytes obtained from another person. However as this difficult protocol is under strict legal regulation in many countries, it is unlikely that it will be widely used for the treatment of diabetes mellitus.

Mesenchymal stem cells derived from the patient's bone marrow can be used as a surrogate for the patient's own ES cells. Indeed, an artificial defect of the mandible (for example, bone tumor resection) is treated with bone regeneration from bone marrow cells in a surgical template of bioabsorbable lactate polymer implanted into the defect. Unfortunately mesenchymal stem cells from adult bone marrow are not thought to transdifferentiate into pancreatic β cells. When stem cells from adult mouse bone marrow were injected into an early blastocyst, they contributed to the formation of many tissues including brain, retina, lung, myocardium, skeletal muscle, liver, intestine, kidney, spleen, bone marrow, blood, and skin, but not pancreas [10].

In vivo regeneration therapy

Impaired tissues or cells can regenerate from the patients' own cells in vivo, and examples of successful in vivo regeneration therapy can be found in various diseases. In the treatment of chronic periodontitis, periodontal tissues destroyed by chronic inflammation are regenerated using guided tissue regeneration therapy that gives the periodontal cells wide space for regeneration, or using enamel matrix proteins that enhanced periodontal regeneration. For patients with renal anemia and granulocytopenia, erythropoietin and granulocyte-colony stimulating factor (G-CSF) are administered, respectively. Plasmid vectors for the expression of vascular endothelial growth factor (VEGF) or hepatocyte growth factor (HGF) have been directly injected into the muscles of ischemic lower limbs with occlusive peripheral artery disease to promote neovascularization from the remaining arteries. Recently, G-CSF administrated immediately after an acute myocardial infarction was reported to have induced stem cell mobilization from bone marrow to the peripheral blood supply. These cells transdifferentiated into myocardium at the infarct site, thus improving the clinical outcome. However, real transdifferentiation of bone marrow stem cells could have been confused here with the distinct mechanism of spontaneous cell fusion with the myocardium (false transdifferentiation) [11,12].

As has been demonstrated by these cases, in vivo regeneration therapy is, in general, more cost-effective, has fewer side effects, and is in addition more ethically and clinically acceptable than in vitro and ex vivo regeneration therapies. Therefore, in vivo regeneration therapy for diabetes mellitus could potentially be applied to all diabetic patients, if effective protocols or drugs for in vivo regeneration of β cells were to be developed. There are two major strategies for the in vivo regeneration therapy of pancreatic β cells: induction of β-cell differentiation and stimulation of β-cell growth.

Induction of β-cell differentiation in vivo

Recent developmental studies have revealed that the duodenum, liver, gall bladder, bile duct, and pancreas are derived from a common rudiment, i.e., the primitive duodenum, and thus share certain developmental characteristics. Furthermore, pluripotent somatic stem cells exist in the intestine, liver, and pancreas. Therefore, differentiation from these tissue stem cells into highly differentiated β cells is considered to be less complex to achieve than differentiation from ES cells. Recently, it was reported that gene transfer of pancreatic and duodenal homeobox gene 1 (Pdx1) into hepatocytes via adenoviral vectors induced transdifferentiation of hepatocytes into insulin-secreting cells, and that this ameliorated streptozotocin-induced hyperglycemia in mice [13]. Retrograde injection of a Pdx1-expressing adenoviral vector into mouse pancreatic ducts also promoted the differentiation of pancreatic duct epithelial cells into β cells (β-cell neogenesis). Retrograde injection into human pancreatic ducts can be performed using an endoscopic approach. As the detailed mechanisms underlying β-cell differentiation are clarified, better molecular targets for effective drugs or for gene therapy will emerge which will induce β-cell neogenesis or transdifferentiation from non-β-cells into highly differentiated β cells.

Stimulation of β-cell growth in vivo

The proliferation of β cells is stimulated by various secreted proteins, including insulin-like growth factors (IGFs)-I and II, platelet-derived growth factor, growth hormone (GH), prolactin (PRL), and placental lactogen. In rats bearing GH- and PRL-producing tumors, GH-expressing transgenic mice, and pregnant rats, β-cell mass increases and insulin secretion is enhanced, whereas in pituitary GH- and PRL-deficient dwarf mice, islet volume decreases by 2–5 times. Transgenic overexpression of parathyroid hormone-related protein or hepatocyte growth factor (HGF) in β cells has been reported to double or triple islet mass. Studies investigating β-cell signal transduction have indicated that insulin receptor substrate-2 (IRS-2), Akt1/protein kinase B (PKB), and cyclin-dependent kinase 4 (Cdk4) play important roles in β-cell proliferation. Both IRS-2 and Akt1/PKB mediate insulin/IGF signaling, and IRS-2-null mice show a decrease in islet mass. Cdk4-null mice develop insulin-deficient diabetes due to a reduction in β cells, whereas "knock-in" mice expressing a mutant Cdk4 that cannot bind its inhibitor protein display β-cell hyperplasia. These factors may provide molecular targets for β-cell proliferation by drugs or gene therapy in the future.

Strategic differences between type 1 and type 2 diabetes

In type 1 diabetes, the therapeutic effect of β-cell proliferation and neogenesis is limited, as long as the autoimmune destruction of β cells continues. Therefore, in vitro and ex vivo regeneration therapies with immunosuppressive drugs or immunological barriers continue to be considered potential therapies for type 1 diabetes.

In type 2 diabetes, proliferation of the patients' own β cells and β-cell neogenesis from patients' own non-β-cells are desirable strategies, because immunological problems can thus be circumvented. Therefore, ex vivo and in vivo regeneration therapies without immunological treatments would be most appropriate for type 2 diabetes. In vivo regeneration therapy for type 2 diabetes by drugs that promote β-cell proliferation or neogenesis, in addition to other regeneration therapies for type 1 diabetes, should be strongly considered due to its cost-effectiveness, safety, and potential world-wide application in the treatment of a large number of diabetic patients.

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References

[1] D.W.R. Gray, An overview of the immune system with specific reference to membrane encapsulation and islet transplantation, Ann. N. Y. Acad. Sci. 944 (2001) 226–239.
[2] T. Berney, L. Buhler, A. Caulfield, J. Oberholzer, C. Toso, R. Alejandro, D.K. Cooper, C. Ricordi, P. Morel, Transplantation of islets of Langerhans: new developments, Swiss Med. Wkly. 132 (2001) 671–680.
[3] A.M. Shapiro, J.R. Lakey, E.A. Ryan, G.S. Korobutt, E. Toth, G.L. Warnock, N.M. Kneteman, R.V. Rajotte, Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen, N. Engl. J. Med. 343 (2000) 230–238.
[4] V.K. Ramiya, M. Maraist, K.E. Arfors, D.A. Schatz, A.B. Peck, J.G. Cornelius, Reversal of insulin-dependent diabetes using islets generated in vitro from pancreatic stem cells, Nat. Med. 6 (2000) 278–282.
[5] N. Lumelsky, O. Blondel, P. Laeng, I. Velasco, R. Ravin, R. McKay, Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets, Science 292 (2001) 1389–1394.
[6] H. Zulewski, E.J. Abraham, M.J. Gerlach, P.B. Daniel, W. Moritz, B. Muller, M. Vallejo, M.K. Thomas, J.F. Habener, Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine exocrine and hepatic phenotypes, Diabetes 50 (2000) 521–533.
[7] B. Soria, E. Roche, G. Berna, T. Leon-Quinto, J.A. Reig, F. Martin, Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice, Diabetes 49 (2000) 157–162.
[8] S. Assady, G. Maor, M. Amit, J. Itskovitz-Eldor, K.L. Skorecki, M. Tzukerman, Insulin production by human embryonic stem cells, Diabetes 50 (2001) 1691–1697.
[9] T. Yamaoka, Gene therapy for diabetes mellitus, Curr. Mol. Med. 1 (2001) 325–337.
[10] Y. Jiang, B.N. Jahagirdar, R.L. Reinhardt, R.E. Schwartz, C.D. Keene, X.R. Ortiz-Gonzalez, M. Reyes, T. Lenvik, T. Lund, M. Blackstad, J. Du, S. Aldrich, A. Jisberg, W.C. Low, D.A. Largaespada, C.M. Verfaillie, Pluripotency of mesenchymal stem cells derived from adult marrow, Nature 418 (2002) 41–49.
[11] N. Terada, T. Hamazaki, M. Oka, M. Hoki, D.M. Mastalerz, Y. Nakano, E.M. Meyer, L. Morel, B.E. Petersen, E.W. Scott, Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion, Nature 416 (2002) 542–545.
[12] Q.L. Ying, J. Nichols, E.P. Evans, A.G. Smith, Changing potency by spontaneous fusion, Nature 416 (2002) 545–548.
[13] S. Ferber, A. Halkin, H. Cohen, I. Ber, Y. Einav, I. Goldberg, I. Barshack, R. Seijffers, J. Kopolovic, N. Kaiser, A. Karasik, Pancreatic and duodenal homeobox gene1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia, Nat. Med. 6 (2000) 568–572.