

Generation of Insulin-Producing Cells from Stem Cells for Cell Replacement Therapy of Type 1 Diabetes

Shimon Efrat PhD

Department of Human Genetics and Molecular Medicine, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

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Abstract

Type 1 diabetes mellitus is caused by an autoimmune destruction of pancreatic islet beta cells, leading to insulin deficiency. Beta-cell replacement is considered the optimal treatment for type 1 diabetes, however it is severely limited by the shortage of human organ donors. An effective cell-replacement strategy depends on the development of an abundant supply of beta cells and their protection from recurring immune destruction. Stem/progenitor cells, which can be expanded in tissue culture and induced to differentiate into multiple cell types, represent an attractive source for generation of cells with beta-cell properties: insulin biosynthesis, storage, and regulated secretion in response to physiologic signals. Embryonic stem cells have been shown to spontaneously differentiate into insulin-producing cells at a low frequency, and this capacity could be further enhanced by tissue culture conditions, soluble agents, and expression of dominant transcription factor genes. Progenitor cells from fetal and adult tissues, such as liver and bone marrow, have also been shown capable of differentiation towards the beta-cell phenotype *in vivo*, or following expression of dominant transcription factors *in vitro*. These approaches offer novel ways for generation of cells for transplantation into patients with type 1 diabetes.

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Type 1 (insulin-dependent) diabetes is an autoimmune disease resulting from destruction of the pancreatic islet insulin-producing beta cells by the immune system. The current treatment of insulin does not represent a cure because insulin dosage is difficult to adjust, leading in the long run to complications in major organs. Restoration of a close-loop insulin delivery system through beta-cell replacement or a glucose-sensing insulin pump represents the most promising approaches for the cure of type 1 diabetes. Pancreas transplantation has been successful but is severely limited by the shortage of donors. With the recent development of new islet isolation and immunosuppression procedures, impressive success rates have been reported using islets from two to three donors per recipient [1]. This progress underscores the urgent need for development of alternative sources to human pancreas donors, namely abundant cultured human beta cells for transplantation. Since differentiated beta cells cannot be propagated in tissue culture, investigators have employed two main approaches to beta-cell expansion *in vitro*: reversible immortalization of mature beta cells, and differentiation of stem/progenitor cells [2]. While the first approach has been successful in rodent beta cells, so far it has met with difficulties in isolated human islets. The alternative to forced expansion of post-mitotic beta cells is the induction of differentiation of stem cells, which have a natural self-expansion

capacity, into insulin-producing cells. This induction can be achieved *in vitro* by a combination of tissue culture conditions, soluble factors, and dominant transcription factor genes, and complemented by further differentiation *in vivo* following transplantation [Figure 1]. The goal is not only induction of insulin biosynthesis, but also its correct processing, storage, and regulated secretion in response to physiologic signals, without which such cell therapy approaches would not be significantly advantageous over insulin administration.

Once sufficient cells are available, ways must be found to protect them from immune destruction following transplantation. In principal, the source of stem cells could be allogeneic, giving rise to a universal donor cell population that can be banked and thoroughly characterized *in vitro* before transplantation, or autologous – taken from one of the patient's own tissues and tailored for each patient separately. Although autologous cells can avoid allograft rejection, they are likely to face recurring autoimmunity, unless they lack the (largely unknown) antigenic targets of bona fide beta cells. It is also possible that beta-like cells derived from stem/progenitor cells may be more resistant, compared to beta cells, to agents secreted by the immune system. The likelihood is that beta-like cells manipulated in tissue culture, regardless of their source, will have to be protected to survive transplantation into a patient with an autoimmune predisposition against beta cells. The options being considered include cell encapsulation, improved immunosuppression, induction of donor-specific tolerance, and cell engineering with immunoprotective genes [2], or combinations

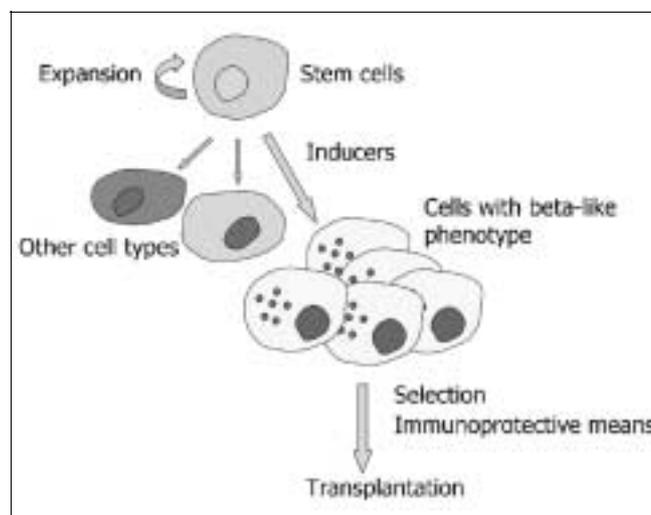


Figure 1. Scheme for development of insulin-producing cells from stem cells

thereof. Despite successes in animal models, at present none of these approaches can offer satisfactory protection in humans.

Insulin-producing cells from embryonic stem cells

There are two major types of stem cells: embryonic stem cells and tissue-derived stem cells. Embryonic stem cells are pluripotent cells derived from the inner cell mass of embryos at the blastocyst stage. These cells are immortal and can be propagated in tissue culture indefinitely under conditions that maintain their pluripotency. They are capable of spontaneous differentiation into virtually all cell types. The development of human embryonic stem cell lines opened new prospects for the generation of various cell types for cell replacement therapy. Both human and mouse embryonic stem cells undergoing spontaneous differentiation have been shown to generate, among many other cell types, a small percentage of insulin-producing cells [3,4]. Enrichment and selection protocols [5,6], as well as introduction of the beta-cell transcription factor Pax4 [7], improved the efficiency of generating insulin-producing cells from mouse embryonic stem cells. However, these cells produce low amounts of insulin, compared to beta cells, and their potential use in transplantation has met with ethical objections as well as concerns regarding the risk of teratoma formation. Nevertheless, there is a strong potential for developing differentiated beta-like cells from human embryonic stem cells, although much more work is needed to realize it.

Insulin-producing cells from pancreatic stem cells

Several fetal and adult tissues have been shown to contain stem cells that are responsible for tissue maintenance and renewal. The obvious place to look for adult tissue stem cells capable of differentiation into beta cells is the pancreas. Studies of experimental models of pancreas injury revealed that the epithelium of pancreatic ducts serves as a source of cells capable of islet neogenesis in the adult, and may constitute the pancreatic stem cells from which normal renewal of islets occurs throughout life. Recent reports have demonstrated the capacity of duct cells from both human [8] and mouse [9] pancreas to differentiate into insulin-producing cells in tissue culture. Stem cells have also been described within pancreatic islets [10]. While these findings are encouraging, the efficiency of expansion of these cell types in tissue culture and their rate of differentiation into insulin-producing cells need to be greatly improved to allow generation of significant cell numbers for transplantation.

Insulin-producing cells from non-pancreatic tissue stem cells

Tissue stem cells were thought to be restricted, compared with embryonic stem cells, with respect to their replication capacity and the cell types they can generate. In recent years the cell commitment concept has been challenged by reports demonstrating that cells from adult organs can give rise to unrelated cell types, both *in vivo* and in culture, given appropriate stimuli. It is unclear at present whether these phenomena represent bona fide trans-

differentiation or the persistence of embryonic pluripotent cells in adult tissues. While the natural plasticity of adult tissue stem cells is still being debated, it has been clearly demonstrated that committed cells can be at least partly reprogrammed with dominant genes that activate a cascade of developmental events. For example, mouse [11] and *Xenopus* [12] liver cells, as well as rat enterocytes [13], were shown to activate beta-cell gene expression following expression of Pdx1, a homeobox factor that plays key roles in pancreas development and gene expression in mature beta cells. Similarly, expression of neuroD, another beta-cell transcription factor, in mouse liver cells *in vivo* resulted in reversal of hyperglycemia [14]. Both liver and intestine share with pancreas a common embryonic origin in the primordial gut.

The encouraging results obtained with mouse liver cells prompted us to evaluate the potential of human liver stem/progenitor cells to be reprogrammed into insulin-producing cells by dominant transcription factors that direct the development of endocrine pancreas. Using the *Pdx1* gene we recently showed that cultured human fetal liver cells can be induced to produce and store mature insulin in significant amounts, about a third of those produced by normal beta cells, release it in response to physiologic glucose levels, and replace beta-cell function in streptozotocin-treated non-obese diabetic severe combined immunodeficient mice [15]. The modified cells expressed multiple beta-cell genes, however they also activated genes expressed in other islet cells and exocrine pancreas and continued to express some hepatic genes.

Another promising source of tissue stem cells is the bone marrow. In recent years bone marrow cells were shown to contain, in addition to hematopoietic stem cells, other stem cells, termed stromal or mesenchymal stem cells, with pluripotent capacities [16]. Bone marrow transplantation resulted in differentiation of the transplanted cells into a variety of ectodermal, mesodermal and endodermal tissues in both mice and humans. Although such results were shown in some cases to be caused by cell fusion, multiple rigorous studies support the wide differentiation potential of bone marrow stem cells. Two recent works have demonstrated that grafted mouse bone marrow cells can differentiate into endocrine pancreas cells [17] as well as induce regeneration of endogenous islets in streptozotocin-diabetic mice [18]. Cell fusion was specifically excluded in the first study [17]. It is possible that in patients with type 1 diabetes the endogenous bone marrow serves as a natural source of stem cells for continuous islet renewal in the pancreas, however newly formed beta cells are rapidly destroyed by autoimmunity. By using beta-like cells developed from autologous bone marrow cells by forced differentiation in tissue culture, and placed in a non-pancreatic site, this destruction may be avoided since these cells may not express the beta-cell autoantigens that are the target for autoimmunity. In addition, such cells may be more resistant to apoptosis induced by cytokines and free radicals, compared to normal beta cells that express particularly low levels of free radical scavenging enzymes.

Most normal human cells grown in tissue culture, including tissue stem cells, demonstrate a limited replication capacity before entering senescence. This has been attributed to telomere short-

ening in the absence of telomerase activity [19]. Activation or overexpression of the catalytic subunit of human telomerase (hTERT) has been shown to be effective in extending the replication capacity of a number of human cell types [20], without compromising their ability to undergo contact inhibition in culture, changing their karyotypes, or increasing their neoplastic potential *in vivo* [21–23]. Thus introduction of the *hTERT* gene into tissue stem cells to extend their replication capacity in tissue culture may allow the expansion of tissue stem cells to the same extent as that of embryonic stem cells.

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Correspondence: Dr. S. Efrat, Dept. of Human Genetics and Molecular Medicine, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv 69978, Israel.
 Phone: (972-3) 640-7701
 Fax: (972-3) 640-9950
 email sefrat@post.tau.ac.il