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In search of a surrogate: engineering human beta cell lines for therapy

Brittney N. Newby, **Naohiro Terada**, and **Clayton E. Mathews**
Department of Pathology, Immunology, and Laboratory Medicine, University of Florida, Gainesville, FL 32610-0275, USA

Abstract

Replacement of insulin-producing cells is a promising therapy for the restoration of the beta cell mass that is destroyed in patients with type 1 diabetes (T1D). However, the use of large amounts of islets per transplant, coupled with the scarcity of donor tissue, diminishes its feasibility. Here we briefly discuss current progress in developing ideal functional beta cells as well as the rationale for developing renewable sources of insulin-producing cells that can be transplanted.

Keywords

type 1 diabetes; islet cell transplantation; beta cells; induced pluripotent stem cell; embryonic stem cell

Introduction

T1D is a debilitating disease that arises from an immune-mediated assault on the insulin-producing pancreatic beta cells. Current thought is that beta cell injury leads to a decline in beta cell function as well as mass, leaving patients with the inability adequately to regulate blood glucose. Since the discovery of insulin in the 1920s, exogenous insulin replacement remains the mainstay of treatment. Progress in the field has led to new approaches to the development of therapies such as insulin analogs, better means for monitoring blood glucose, and the development of medical devices such as pumps, pens, and the artificial pancreas [1]. Although current therapy has significantly improved the life expectancy of patients, insulin is not a cure and does not protect patients from numerous downstream complications such as cardiovascular disease and neuropathy.

The trials and tribulations of beta cell replacement

The mainstream considers T1D an autoimmune disease and therefore continued efforts are made to identify therapeutic approaches that will modulate the immune system and induce tolerance to beta cells [2]. Trials targeting the immune system, even those that do so aggressively, have not been complete successes. An example is the cyclosporine trial [3], where remission was induced but in only a quarter of those on therapy. The overall limited

Corresponding author: Mathews, C.E. (clayton.mathews@pathology.ufl.edu).

success of these trials could result from the lack of sufficient beta cell mass at onset to rescue most patients. This would suggest that a combined approach of immunotherapy with beta cell replacement could induce insulin independence.

Replenishing beta cell mass through pancreatic islet transplantation provides a favorable approach to restoration of metabolic control in these patients. However, beta cell replacement using cells obtained from organ donors presents a difficulty. The number of cases of T1D in the USA may be as many as 3 million, with approximately 30 000 new cases diagnosed each year (see http://www.jdrf.org). By contrast, although 1376 pancreata were obtained from deceased organ donors, the number of individuals that received a pancreas transplant in 2013 was merely 256. These data highlight the difficulty that all patients in need of an organ transplant face: that is, although there are over 100 million registered organ donors and approximately 2.7 million people die in the USA each year, less than 14 000 were suitable as organ donors in 2013 (see http://www.unos.org). With the incidence of T1D increasing at a rate of 3% per year, the divide between transplantable organs or cells available for transplant and the number of patients with T1D results in this type of therapy being an impossibility for all of those affected with this disease. Therefore, renewable sources of beta cells provide the potential for cell replacement in a significantly wider patient group.

Lessons learned from current beta cell lines

Human beta cells act as the quintessential metabolic sensor by employing elegant mechanisms to orchestrate the lowering of blood glucose levels. Most progress in uncovering the mechanisms of beta cell metabolism, growth, and function has been derived from studies using rodent models or isolated human islets. Although we have been able to learn a great deal from these studies, there is a body of evidence that illustrates marked differences between human and rodent beta cells. Further, recent evidence points to significant differences between isolated islets and those found *in situ* [4] that could result from the stress of islet isolation. Therefore, a need exists for the development of functional human pancreatic beta cells that are established cell lines or those that can be generated from stem cells. However, progress on this front has been hindered by the lack of access to tissue and inconsistent islet isolation and culture methods, plus difficulty developing approaches for genetic alteration [5]. Ideally, a human pancreatic beta cell line should maintain insulin content, express relevant immunological markers, and remain metabolically active with utilization of appropriate secretory mechanisms.

Although disappointing, most cell lines have proven to be ineffective models for functional studies of beta cell insulin-secretory mechanisms and in summary are likely to be inappropriate for cell-replacement purposes. However, these cell lines have proven to be valuable tools for other *in vitro* applications, especially immunological studies [6]. Specifically, the adult beta cell-derived βLox5 cell line has been essential in uncovering the role of mitochondria in Fas and proinflammatory cytokine-mediated beta cell death [7]. Additionally, βLox5 cells express relevant T1D autoantigens and, similar to primary islets, display upregulation of major histocompatibility complex (MHC) class I on priming with interferons, making them excellent beta cell surrogates in cell-mediated lymphocytotoxicity

assays. Another cell line, HP62, derived from transfection of human islets with SV40, has been utilized to study cytokine-mediated regulation of adhesion molecule expression on the surface of beta cells [6].

Other human pancreatic beta cell lines show some potential for utilization in functional studies. The year 2011 marked the emergence of two new beta cell lines, 1.1B4 and EndoC-βH1. The 1.1B4 cell line was derived from electrofusion of the exocrine pancreatic cell line PANC-1 and freshly isolated human pancreatic beta cells. Characterization of these cells revealed stable insulin content for at least 40 passages and glucose-stimulated insulin secretion [5]. Recently, Vasu *et al.* demonstrated decreased viability and beta cell function of the 1.1B4 cell line in response to proinflammatory cytokines, highlighting the potential of these cells for use as a beta cell model [8]. Utilizing a step-wise process, the cell line EndoC-βH1 was derived from fetal pancreatic tissue, transduced with a lentiviral vector for SV40T under the regulation of the insulin promoter so that only insulin-producing cells would become immortalized. After transplantation in severe combined immunodeficiency (SCID) mice, insulinomas derived from the fetal tissue were explanted, cultured, and characterized. Interestingly, these cells have significantly higher insulin content compared with other human beta cell lines, although the level is not comparable with that of primary beta cells [9]. Although EndoC-βH1 cells appear to display beta cell-like characteristics, they still express oncogenes, making them vastly different from primary beta cells. Therefore, Scharfmann *et al.* recently published the development of the conditionally immortalized EndoC-βH2 cells. After excision of SV40LT and hTERT using cre-recombinase, proliferation markedly decreases, with concurrent increases in insulin mRNA expression and beta cell transcription factors and proteins as well as increased insulin secretion, suggesting that EndoC-βH2 cells may behave more similarly to beta cells [10]. These newly developed cell lines represent unique tools and provide a glimmer of hope for functional and immunological beta cell studies to come.

Regenerative medicine: a glimpse into the future

The use of human embryonic stem cells (hESCs) presents another potential avenue for the production of a transplantable source of beta cells. hESCs are a population of cells that represent endless replicative potential. Recent progress in our understanding of human beta cell development suggests that mimicking developmental cues, through induced expression of relevant transcription factors or through the supplementation of soluble growth factors, can guide the differentiation of hESCs into pancreatic progenitors. The development of these hESCs into fully functional insulin-producing beta cells *in vitro* has not yet been described; however, in 2012 Schulz *et al.* were able to guide differentiation of the hESC line CyT49 into islet-like cells that on transplantation into mice protected against streptozotocin-induced diabetes [11]. Although recent data suggest that further differentiation of hESC-derived cells may occur *in vivo*, the use of hESCs presents several issues, including ethical dilemmas as well as the risk of teratoma formation and potential growth of unwanted cell types after transplantation [12]. There is also considerable interest in utilizing induced pluripotent stem cells (iPSCs) or somatic cells that have dedifferentiated to a state of pluripotency after nuclear reprogramming [13] and thwart the ethical quandary presented by hESCs. iPSC biology is advancing rapidly and approaches that use cell-surface markers can purify

specific populations of insulin-producing cells that cells exhibit glucose-regulated insulin secretion after transplantation [14]. Further, the chance to isolate and then use autologous iPSCs derived from patients with diabetes could eliminate the need for further immunosuppression, although continuous autoimmunity remains a concern. Gene editing within patients' iPSCs, however, may potentially resolve the autoimmune concern. With recent advances in gene-editing technology using transcription activator-like effector nucleases (TALENs) or Cas9 nuclease, for example, it would be feasible to improve iPSC-derived beta cell function or survival after transplantation into patients with T1D. The derivation of patient iPSCs and the ability to generate fully functional beta cells would provide a unique resource to study the mechanisms underlying how genetic risk factors alter beta cell function or responses to immune stimuli.

With diabetes on the rise and the lack of efficacious mono- or combination immunotherapies, there is a glaring need for a source of functional human beta cells. Restoration of beta cell mass through transplantation represents a promising therapy; however, the dearth of transplantable donor tissue is a currently insurmountable hurdle for widespread therapeutic implementation. As the search for the optimal beta cell surrogate continues, research efforts should focus on improving and harnessing our current knowledge of beta cell development and differentiation, understanding the behavior of transplanted beta-like cells *in vivo*, and investigating the potential for use of current therapies to enhance engraftment of β-like cells. Regenerative medicine is a flourishing field that provides a promising outlook for the future; however, only time will tell whether this approach will provide the surrogate that patients and physicians have been waiting to arrive on the horizon (Box 1).

Box 1

Barricades to success

Engineering transplantable sources of insulin-secreting cells through cellular reprogramming and other recent technologies presents promising new therapeutic options for patients with diabetes. Recently, Yamada *et al.* described the development of pluripotent stem cells and downstream differentiation into cells that can synthesize insulin using somatic nuclear cell transfer from a patient with T1D. Although this paper, along with other publications, highlights exciting new techniques to generate pancreatic progenitors from patients, the authors fail to show convincing evidence regarding the ability of these neo-insulin-producing cells to exhibit glucose responsiveness [15]. The lack of glucose-stimulated insulin secretion by these or other recently produced cells emphasizes a glaring need on the road to translating these powerful regenerative technologies to the clinic – the emergence of a beta cell replacement that is truly functional.

Stem cells, attractive for their capacity to replicate extensively into various lineages, represent a plausible source of transplantable autologous insulin-secreting cells. The metamorphosis from a pluripotent stem cell into a fully functional mature beta cell has been an arduous task. The journey begins with the development of definitive endoderm and, through the expression of various developmental factors such as PDX-1, NKX-6.1,

and NGN3, yields a pancreatic progenitor. Unfortunately, functional analysis of these beta-like cells reveals an immature phenotype characterized by insulin secretion at low glucose levels without responsiveness to increases in the glucose concentration. Interestingly, the *in vivo* maturation of stem cell-derived surrogates has been shown to give rise to insulin-secreting cells that are able to regulate blood glucose levels in rodent recipients, suggesting that an *in vivo* environment, even a xenogeneic one, can provide cues for further maturation. The discovery of markers of functional beta cell maturation, such as Urocortin3, has been reported recently [16]. Although transplantation of pancreatic progenitors into human patients does not present a viable therapeutic option, characterization of the key mature beta cell markers and factors needed for induction of beta cell function is clearly vital.

As the search for a suitable surrogate continues, there are several other questions that need to be addressed. These issues include the development of non-genome-altering cellular-reprogramming methods, derivation of a standardized differentiation protocol aimed at generating the maximal number of endodermal pancreatic progenitor cells that is amenable to good manufacturing practices, and methods for protecting engrafted tissues from recurring autoimmunity and from the harmful effects of immunosuppressive drugs.

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