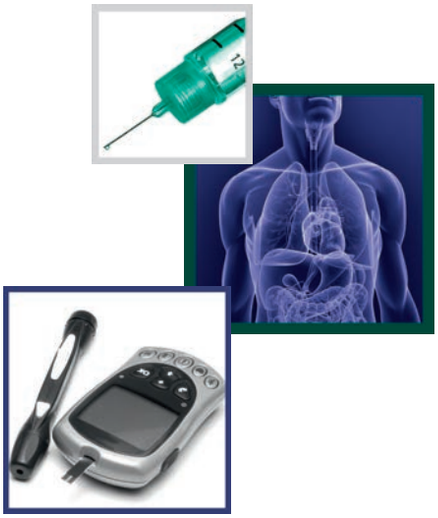
EDITORIAL



Insulin-secreting cell regeneration: a dream or reality?

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"Turning the dream of regenerating β-cells into a reality requires an understanding of normal β-cell ontogeny and how to mimic and evaluate it."

Autoimmune destruction of insulin-producing β-cells leads to Type 1 diabetes (T1D). The global challenge to find a cure for T1D centers on transplanting or regenerating insulin-producing cells, while at the same time preventing disease recurrence. This quest for cure dominates prevention in the public's mind, although in reality there can be no cure of an autoimmune disease without prevention. The impetus comes from the demands of daily insulin injections to treat T1D and the inability of this approach to attain physiologic control of blood glucose, in many cases leading to longer-term complications secondary to hyperglycemia. Although transplantation of the pancreas or purified islets allows near-normal maintenance of blood glucose, it comes at a high technical and financial cost and is severely limited by the shortage of organ donors. In addition, long-term insulin-independence appears to be the exception rather than the rule [1]. Alternative sources of β-cells, primarily embryonic or adult stem/progenitor cells, have, therefore, been pursued [2–5]. Turning the dream of regenerating β-cells into a reality requires an understanding of normal β-cell ontogeny and how to mimic and evaluate it.

The β-cell regeneration progress has to match the known molecular identity of a developing pancreas. Over the last 30 years, pancreatic endocrine cell lineages have been mapped through the role of specialized growth factors and sequential activation/inhibition of signaling pathways. Thus, early specification of dorsal and ventral pancreatic buds from foregut endoderm requires Hedgehog and FGF signaling [6]. Growth and branching morphogenesis involve FGF, Notch, Wnt, TGF-β and EGF pathways [7]. These pathways are activated by interactions with surrounding tissues, causing expression of specialized transcription factors. Sets of transcription factors form transcriptome signatures responsible for tissue transformation. Although incomplete, these groups are used to identify whether cultivated cells reached late-definitive endoderm, pancreatic progenitor or immature β-cell stages of development.

In addition, the β-cell regeneration progress has to match known β-cell function. Cellular response to incretins, such as glucose and exenatide, is a valuable feature. It displays insulin production, reveals presence of secretory networks and detects dose-dependent insulin

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release. Delivery of an alternative β-cell source into an animal model of T1D diabetes tests whether transplants can survive and reverse hyperglycemia, as well as maintain euglycemia. These checks are used to uncover the extent of alternative β-cell source functional 'maturity'. Equivalents of endogenous β-cell molecular identity and function will signify success of proposed T1D treatments.

Embryonic stem (ES) cells are a frequent research choice for β-cell therapy. They are pluripotent, with the capacity to become self-renewing and any type of differentiated cell. ES cell differentiation is a multipath road with plenty of 'false' trails. Nonetheless, insulin production was demonstrated when hormone-negative mouse ES cells were cultivated *in vitro* [2,8–10]. Insulin secretion in response to glucose further strengthened these results [2,7]. Protocol modifications achieved increases in insulin synthesis [2,10], reversal of hyperglycemia [10] and focused on removal of tumorigenic cells from a transplant population [11]. However, for a long time the main obstacles included scaling up and difficulty replicating mouse data in experiments on human ES cells. Recently, coculture with organ-matched mesenchyme revealed that human ES (hES) cell-derived precursors maintain self-renewal capacity together with more than a million-fold expansion [9]. Still, the insulin-secreting capacity was only acquired by transplantation into immunodeficient mice, and testing these hES cells in diabetic mice was not reported. Another recent report employed 3D *in vitro* cultivation of hES cells to generate islet-like clusters with a high content of insulin-producing cells, which reversed hyperglycemia in streptozotocin-induced diabetic immunodeificient mice and maintained normoglycemia for 3 months [10]. Appearance of these new methods points to progress, a combination of which should generate large quantities of insulin-producing cells that can be tested in larger animal models of T1D. However, limited access to hES cells and ethical issues, such as the destruction of an embryo, objections to use of embryonic tissue for research and therapeutic purposes, tumor formation after transplantation and possible need for antirejection treatment for alloreactivity, militate against the application of ES cells for β-cell regeneration. These ES cell-associated drawbacks curb research enthusiasm, turning scientific gaze to other solutions.

The field of induced pluripotency is very recent and its novel technology quickly evolved to nonviral cell reprogramming to increase efficiency and improve safety [12]. Use of Oct3/4, Sox2, Klf4 and c-Myc transcription factors to convert somatic cells into induced pluripotent stem cells voids the need for embryonic tissues. However, the association with faster aging, mutations and oncogene activation limit induced pluripotent stem cell application to the cure of T1D [13]. Additionally, pluripotency positions cells where naturally selected ES cells begin. This alternative, therefore, requires more effort and increases the chances of faltering in the passage to β-cell regeneration.

Other options come in the form of adult stem cells identified and selected from various tissues. They are restricted in their capabilities, but their plasticity points to transdifferentiation capacity. Mesoderm-derived hemopoietic stem cell (HSC) and mesenchymal stem cells (MSC) have been considered for T1D cell therapy. The ease of HSC and MSC isolation and propagation secured their popularity in β-cell research. Enticing these stem cells to endoderm lineage produced mixed results. Growth factor treatments revealed inefficient differentiation along β-cell lineage that only improved by transplantation into mouse models of T1D. Thus, lowered blood glucose was reported in diabetic mice upon transplantation of mouse adipose-derived insulin-producing cells [14] and normoglycemia was maintained with transplantation of human bone marrow-derived mesenchymal cells [15]. These results, just like ES cell work, have not progressed beyond tests in mouse T1D models. However, credit is given to HSC and MSC immunomodulatory properties, with prospects of mixed cell therapy, partnered to another potential β-cell alternative [13].

Even less attention has been given to adult stem cells within the pancreas. These cells are already located in the pancreatic niche, ready to respond to regenerative cues. Evidence for injury-induced stem/precursor cell regeneration into β-cells within pancreatic ducts has been reported in rodents [16,17]. Recent findings also reveal precursor expansion in mouse models of T1D and stem/precursor cell capacity to form glucose-sensitive insulin-secreting cells *in vitro* [18]. Despite the multitude of work emphasizing mouse pancreatic stem/precursor cell potential, very little research was done in a human pancreas. These studies have so far revealed autoimmunity associated β-cell regeneration [19], duct cell replication [20] and provided limited

isolation/characterization of human pancreatic stem/progenitor cells [3].

Transdifferentiation of exocrine tissue and plasticity of α-cells represent alternative β-cell therapy. However, the necessity for viral agents [5] to transform acinar cells to an endocrine phenotype and variable degree of α-cell conversion to an insulin-producing phenotype [21], highlight safety concerns and knowledge limitations on pancreas adaptability to injury.

The diversity of published results should narrow the search for a successful form of β-cell therapy. It is also clear that plasticity of stem cells from various tissues is favored over the already preprogrammed capacity of pancreatic precursors. Reversing cell development is an attractive way forward, but suffers from incomplete knowledge of β-cell ontogeny. Limited financial availability and adherence to 'fashionable' stem cell alternatives neglect pancreas potential for β-cell regeneration. β-cell regeneration is real but, even with β-cell alternatives of human origin, successes of T1D therapy have been restricted to small animal models. Progress made in recent years positions ES cells as the most likely contender for β-cell therapy. For now, the safety and potency of published results has no definite substitute to insulin injections or donor tissue transplantation.

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