

Stem Cell Sources to Treat Diabetes

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ABSTRACT

We review progress towards the goal of utilizing stem cells as a source of engineered pancreatic β -cells for therapy of diabetes. Protocols for the *in vitro* differentiation of embryonic stem (ES) cells based on normal developmental cues have generated β -like cells that produce high levels of insulin, albeit at low efficiency and without full responsiveness to extracellular levels of glucose. Induced pluripotent stem (iPS) cells also can yield insulin-producing cells following similar approaches. An important recent report shows that when transplanted into mice, human ES-derived cells with a phenotype corresponding to pancreatic endoderm matured to yield cells capable of maintaining near-normal regulation of blood sugar [Kroon et al., 2008]. Major hurdles that must be overcome to enable the broad clinical translation of these advances include teratoma formation by ES and iPS cells, and the need for immunosuppressive drugs. Classes of stem cells that can be expanded extensively in culture but do not form teratomas, such as amniotic fluid-derived stem cells and hepatic stem cells, offer possible alternatives for the production of β -like cells, but further evidence is required to document this potential. Generation of autologous iPS cells should prevent transplant rejection, but may prove prohibitively expensive. Banking strategies to identify small numbers of stem cell lines homozygous for major histocompatibility loci have been proposed to enable beneficial genetic matching that would decrease the need for immunosuppression. *J. Cell. Biochem.* 106: 507–511, 2009. © 2009 Wiley-Liss, Inc.

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Advances in the clinical transplantation of pancreatic islets of Langerhans to treat diabetes, coupled with the limited availability of islets from donated organs, have driven efforts to generate insulin-producing cells from renewable stem cells. A recent report in *Nature Biotechnology* strengthens the evidence that human embryonic stem (ES) cells can give rise to cells that secrete insulin in a glucose-responsive manner, the signature characteristic of pancreatic β -cells. This encourages expectations that cell therapy ultimately will cure many patients with diabetes. However, significant further advances will be necessary before ES cells can be considered a safe, robust source for such treatment. Efforts to develop optimal stem cell sources for diabetes therapy should remain active.

The discovery of insulin and its use to treat diabetes mellitus represents one of the great chapters in the history of medicine. However, from the earliest days of insulin therapy, it was apparent that injection of the hormone did not completely compensate for the loss of the insulin-producing β -cells of the pancreas. As Frederick Banting recognized in his Nobel Lecture, delivered in 1925, “Insulin

is not a cure for diabetes; it is a treatment” [Banting, 1965]. Although life-saving to individuals with Type 1 diabetes (T1DM), and beneficial to many with severe Type 2 diabetes (T2DM), even state-of-the-art insulin therapy does not prevent long-term complications, notably cardiovascular disease and damage to the microvasculature and nerves, associated with elevated blood glucose levels [Tripathi and Srivastava, 2006; Coccheri, 2007]. Moreover, patients with “brittle diabetes,” a subtype of T1DM, show unexplained metabolic instability despite carefully monitored insulin therapy, and suffer frequent episodes of hypoglycemia and/or ketoacidosis that can be life-threatening [Bertuzzi et al., 2007]. These individuals have benefitted from pancreas or pancreatic islet transplantation, in which the restoration of β -cells improves homeostasis. Indeed, it is in patients with brittle T1DM that islet transplantation has been assessed in an international trial of the Edmonton protocol, under which allogeneic islets are provided with chronic drug treatment to block immune rejection [Shapiro et al., 2006]. The results indicate that the procedure offers significant benefits and that patients who receive a sufficient number of islets

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reproducibly achieve independence from exogenous insulin, at least for a period of time. However, the scarce supply of organs from deceased donors and the risks associated with long-term immunosuppression place major constraints on this mode of therapy. Both limitations must be overcome in order to realize the dream of a widely available, cell-based cure for diabetes [Marzorati et al., 2007].

The development of pluripotent ES cell lines from the inner cell mass of early stage mouse embryos [Evans and Kaufman, 1981; Martin, 1981] and, 17 years later, human embryos [Thomson et al., 1998] offered the potential to generate any specialized cell type in large quantities. Yet, for many differentiated cells the practical achievement of this goal remains a daunting challenge. Early efforts established that ES cells could give rise to cells expressing lineage markers of the endocrine pancreas, including insulin, but yields were low and properties such as levels of hormone and regulation by glucose did not closely mirror those of mature pancreatic β -cells [Soria et al., 2000; Lumelsky et al., 2001; Kania et al., 2004; Ku et al., 2004]. A major step forward came as investigators identified known developmental cues that could induce ES cells to replicate key aspects of the segregation of specific germ layers that occurs at gastrulation in the normal embryo. Since the pancreas derives from the endoderm, an important breakthrough was to induce differentiation of ES cells to mesoendoderm (progenitor of both mesoderm and endoderm) and definitive endoderm (distinct from the extraembryonic visceral endoderm, which appears earlier and does not contribute to adult organ structures). This was achieved by exposing mouse or human ES cells to activin A, a member of the TGF- β family closely related to the “appropriate” developmental signal, nodal, or to nodal itself, and in the absence of overriding signals from serum or other growth factors [Kubo et al., 2004; D’Amour et al., 2005; Pfendler et al., 2005; McLean et al., 2007].

A team led by Emmanuel Baetge at Novocell, Inc. continued in stepwise fashion to identify culture conditions that would drive the further differentiation of human ES cell-derived definitive endoderm through subsequent stages on the desired path—posterior foregut, pancreatic endoderm, progenitors of endocrine pancreas, and hormone-producing endocrine cells [D’Amour et al., 2006]. At the end about 7% of cells expressed high levels of proinsulin that was processed, albeit inefficiently, to insulin and C-peptide. Insulin secretion was not responsive to glucose levels, but could be increased by other compounds known to act on β -cells in fetal pancreas, which also respond poorly to glucose. Two other groups, using somewhat different factor “cocktails,” downstream from definitive endoderm, published confirmation that cells able to synthesize and secrete insulin and other pancreatic hormones can be derived from human ES cells [J. Jiang et al., 2007; W. Jiang et al., 2007]. These teams observed at least a modest response of insulin secretion to elevated glucose, perhaps in part due to maturation of cells for longer periods and under three-dimensional culture conditions that promoted the formation of “islet-like” cell clusters [J. Jiang et al., 2007; W. Jiang et al., 2007].

The new report from the Novocell group extends to a demonstration that human ES cells can serve as a source of functional insulin-producing cells capable of maintaining glucose stably at normal levels in mice lacking their own β -cells [Kroon et al., 2008].

They used a somewhat simplified procedure to differentiate human ES cells in culture to the pancreatic endoderm stage, and then implanted these cells in the epididymal fat pads of mice (a severely immune deficient strain, to avoid rejection). Several months later the mice were treated with the toxin streptozotocin, under conditions that would destroy mouse but not human β -cells. While control animals became diabetic, those that had received the cell implants maintained near-normal regulation of blood sugar, and expressed human insulin and C-peptide. If the implanted cells were excised, the animals became diabetic. The data indicated that the human ES-derived cells had differentiated to a functional β -cell-like state. Experts in pancreatic islet physiology have noted that the ES-derived endocrine cells may still have a somewhat immature phenotype [Ricordi and Edlund, 2008]. Nonetheless, the work stands as a remarkable proof of principle study for the potential clinical use of stem cells as a renewable source of pancreatic β -cells.

A successful strategy for therapeutic implementation of human ES cell-based therapy for diabetes will have to overcome several important hurdles. First, there is a safety concern because undifferentiated ES cells give rise to teratoma tumors. Not surprisingly, Kroon et al. found that more than 15% of animals that received transplants developed teratomas or similar cell growths. It will be essential to insure that there are no residual pluripotent ES cells present in populations implanted into patients. It is possible that some of the intermediate stage cells, downstream from pluripotent stem cells but still multipotent and highly proliferative, would also be tumorigenic. Implanted cells possibly could be modified by the insertion of a “suicide” gene that would allow selective killing of any ES cell-derived tumors [Schuldiner et al., 2003]. However, no such approach is likely to be 100% efficient, and genetic manipulation of cells carries its own risks of inducing unwanted mutations through vector insertion.

It is also unclear what fraction of human ES cell lines will efficiently give rise to pancreatic progenitors and, ultimately, functional β -cells. Although comparisons of nearly five dozen human ES lines, developed around the world, revealed strong similarities in the expression of a variety of cellular markers [Adewumi et al., 2007], at a functional level the cells may prove surprisingly heterogeneous. Indeed, Douglas Melton and colleagues found that 17 independent lines tested showed highly different patterns of differentiation to various cell types, and only three of these had a strong propensity to give rise to pancreatic lineage cells [Osafune et al., 2008].

While it would be possible to choose a single “best” human ES line to advance toward clinical use in diabetes, having a battery of independently derived ES cells may facilitate genetic matching of donor cells to patients. It is usually assumed that therapies using cells from unrelated donors will necessitate life-long immunosuppressive treatment to prevent graft rejection, unless novel technologies to induce tolerance to genetically mismatched grafts prove successful [Marzorati et al., 2007]. However, it is not unreasonable to expect that grafts of cells grown and differentiated in culture will be intrinsically less immunogenic than transplants of tissue or organs from deceased individuals, which include cells and factors that provoke inflammatory responses. It therefore may prove

possible at least to use less stringent immunosuppression to avoid rejection of stem cell-derived transplants.

New technologies for reprogramming of adult cells to an induced pluripotent state (“iPS cells”), similar to ES cells, may change the landscape of future β -cell therapy [Okita et al., 2007; Park et al., 2007; Takahashi et al., 2007; Yu et al., 2007; Wernig et al., 2007]. Theoretically, it should become straightforward to make autologous iPS cells starting with readily accessible skin or blood cells from any individual, and to use these as a source of transplantable cells, obviating the need for immunosuppression. At a practical level it is not clear that this degree of individualized medicine can be made cost effective for patient populations that could eventually number in the hundreds of thousands or millions. However, assessments of cell banking strategies have indicated that as few as 10 pluripotent cell lines carefully chosen for homozygosity of the most common alleles at the major histocompatibility (MHC) genetic loci would provide considerable practical benefit for transplantation matching [Taylor et al., 2005]. Obtaining such a bank of MHC-homozygous pluripotent cells would be logistically and ethically difficult if making ES cell lines from embryos. On the other hand, it would appear quite feasible to identify the homozygous individuals from, for example, volunteers for bone marrow donation who are tested for their MHC genotype. A study of the British population indicated that approximately 10,000 individuals would suffice to find donors for the desired set of 10 cell lines [Taylor et al., 2005]. This would allow beneficial matching of “off the shelf” donor cells to a majority of potential transplant recipients, and thereby significantly decrease the need for immunosuppression.

It remains to be shown that iPS cells have the same capacity as human ES cells for differentiation toward β -cells. However, a very recent report documents that human iPS cells indeed can give rise to insulin-producing cells [Tateishi et al., 2008]. The iPS cells were induced to yield pancreatic islet-like clusters using one of the culture protocols previously described for human ES cells [J. Jiang et al., 2007]. As with ES cells, only a subset of the iPS cell clones appeared able to differentiate in this manner. Like ES cells, iPS cells form teratomas, so this problem remains to be solved. It is entirely conceivable that means will be found to reprogram directly cells to a post-ES stage that can still be propagated extensively in culture, but would not be tumorigenic. Definitive endoderm would be an attractive candidate for such an intermediate stage.

Other categories of stem cells also may be suitable for diabetes therapy. A number of reports have indicated that some markers of the β -cell lineage, including insulin, can be induced in mesenchymal stem cells (MSCs) from bone marrow, umbilical cord blood cells, or other adult stem cell sources, particularly after introduction of a gene for a pancreatic lineage transcription factor such as Pdx-1 [Korbling et al., 2005; Moriscot et al., 2005; Denner et al., 2007; Karnieli et al., 2007; Li et al., 2007; Sun et al., 2007; Chao et al., 2008; Gao et al., 2008; Liu and Han, 2008]. None of these studies has achieved the degree of validation of lineage markers or the long-term restoration of normal blood glucose seen in the current study from human ES cells.

Again, it also remains uncertain whether individualized autologous cell sources or small numbers of broadly expandable cell lines will be better suited to reach large numbers of patients.

Populations such as MSCs can be expanded to a much lesser extent than ES or iPS cells. However, our laboratory has described stem cells from human amniotic fluid (AFS cells) that are capable of extensive expansion in culture, do not form teratomas or other tumors, and can give rise to differentiated cells expressing markers of each of the three germ layers [De Coppi et al., 2007]. Recent studies of the perivascular compartment, indicating that is the source of MSCs and that it may contain longer-lived multipotent stem cells, could also point to a potential new source for β -cells [Crisan et al., 2008; de Silva Meirelles et al., 2008]. If it proves possible to generate highly functional β -cells from AFS cells or another comparable source, this would have many of the advantages of ES cell-derived therapy but with a decreased risk of post-transplant cancers.

The derivation of β -cells from ES cells initiating through definitive endoderm also raises the question of whether endogenous endodermal stem cells might be identified that would also be readily induced to the pancreatic lineage, preferably without need for genetic manipulation by introduction of pancreatic transcription factors. A strong candidate for such an endodermal stem cell is the hepatic stem cell (HpSC) obtained from fetal or adult human liver by immunoselection for CD326 (EpCAM) [Schmelzer et al., 2007]. These HpSCs can be expanded extensively in culture and do not form teratomas [Wauthier et al., 2008]. Whether they can be directed efficiently to follow a differentiation pathway to endocrine pancreas remains to be determined.

Whatever cell source proves optimal, there is growing reason for optimism that the replacement of islet transplantation from deceased organ donors by stem cell-derived highly functional β -cells is an achievable goal. This will bring us a large step closer to the goal of curative therapy for diabetes.

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