Mini Review

Stem cell therapy for diabetes

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ABSTRACT

Stem cell therapy holds immense promise for the treatment of patients with diabetes mellitus. Research on the ability of human embryonic stem cells to differentiate into islet cells has defined the developmental stages and transcription factors involved in this process. However, the clinical applications of human embryonic stem cells are limited by ethical concerns, as well as the potential for teratoma formation. As a consequence, alternative forms of stem cell therapies, such as induced pluripotent stem cells, umbilical cord stem cells and bone marrow-derived mesenchymal stem cells, have become an area of intense study. Recent advances in stem cell therapy may turn this into a realistic treatment for diabetes in the near future.

Key words: Embryonic stem cell, induced pluripotent stem cell, mesenchymal stem cell, diabetes

This lecture is based on a recent review.[1]

The increasing burden of diabetes worldwide is well-known, and the effects on health care costs and in human suffering, morbidity, and mortality will be primarily felt in the developing nations including India, China, and countries in Africa. New drugs are being developed at a rapid pace, and the last few years have seen several new classes of compounds for the treatment of diabetes e.g. glucagon-like peptide (GLP-1) mimetics, dipeptidyl-peptidase-4 (DPP-4) inhibitors, sodium glucose transporter-2 (SGLT2) inhibitors. New surgical treatments have also become increasingly available and advocated as effective therapies for diabetes. Gastric restriction surgery, gastric bypass surgery, simultaneous pancreas-kidney transplantation, pancreatic and islet transplantation have all been introduced in recent years. To avoid the trauma of a major operation, there have been many studies on the transplantation of isolated islets removed from a cadaveric pancreas. There was encouragement from the "Edmonton protocol" described by Shapiro and colleagues in the New England Journal in 2000. The islets were injected into the portal vein and patients, especially those suffering from dangerous, hypoglycemic unawareness, were treated before they had developed severe complications of diabetes, especially renal complications. While the early results were promising, with some 70% of the patients requiring no insulin injections after two years, at five years, most of these patients had deteriorated and required insulin supplements, despite some having received more than one transplant of islets. In the more recent series of patients, the Edmonton group has reported better long-term results with the use of the monoclonal anti-lymphocyte antibody, Campath 1H given as an induction agent, 45% of patients being insulin-independent at five years, and 75% had detectable C-peptide.

However, cadavaric pancreata and islets compete for the same source and are limited in number, and so, neither treatment could readily be offered to the vast majority of diabetic patients. Some have attempted to use an alternative source, for example, encapsulated islets from neonatal or adult pigs. This is still very experimental and will be a far away alternative with many technical and possibly ethical obstacles to overcome.

More recently, with the successes in the development of pluripotent adult stem cells (from Yamanaka, awarded the 2012 Nobel prize for medicine for developing induced pluripotent stem cells – iPSCs), new approaches to seek a methods that may be more accessible and available have been attempted. Much hope was derived initially from

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embryonic stem cell (ESC) research, since these cells can be persuaded to multiply and develop into any tissue, but the process was expensive, and the problem of teratoma formation from these stem cells proved extremely difficult to overcome. Many of the important factors related to fetal development are not understood and cannot be reproduced. However, some progress has been made, and (occasionally) cells been persuaded to secrete insulin, but so far, there have been very minimal therapeutic application.

Scientists are now aware that to persuade a cell to produce insulin is only one step in what may be a long and difficult journey. Islets cells are highly specialized to have not only a basal release of insulin but also to respond rapidly to changes in blood glucose concentration. With insulin, the process and regulation of switching off secretion is as important as the switching on secretion.

A variety of approaches has been made with different starting points. The stem cell reproduces itself and can then also divide asymmetrically and form another cell type: This is known as differentiation. Although initially they were thought to be available only from embryos, non-embryonic stem cells can now be obtained without too much difficulty from neonatal tissue, umbilical cord, and also from a variety of adult tissues including bone marrow, skin, and fat. These stem cells can be expanded and made to differentiate, but their repertoire is restricted compared with embryonic stem cells: *oligo-* or *pluri-* as opposed to *toti*-potent embryonic stem cells. Even more, recently, there has been much interest in the process of direct cell trans-differentiation, in which a committed and fully differentiated cell, for example a liver cell, is changed directly to another cell type, for example an islet beta-cell, without induction of de-differentiation back to a stem cell stage.

Yamanaka, in 2006, was able to produce pluripotent stem cells from mouse neonatal and adult fibroblast cultures by adding a cocktail of four defined factors.[2] This led to a series of other studies developing the process, which was shown to be repeatable with human tissue as well as laboratory mice. The use of iPS cells avoided the ethical constraints of using human embryos, but there have been other problems and obstacles still. There have been emerging reports of iPS cells becoming antigenic to an autologous or isologous host, and the cells can accumulate DNA abnormalities and even retain epigenetic memory of the cell type of origin and thus have a tendency to revert back. Like embryonic stem cells, iPS cells can form teratoma, especially if differentiation is not complete.

Despite this, there has been very little success in directing differentiation of iPSCs to form islet beta-cells in sufficient quantity that will secrete and stop secretion in response to changes in blood glucose levels.

Another approach that has been tried is to combine gene therapy with stem cells. Some progress has been made in trying to express the desired insulin gene in more primitive undifferentiated cells by coaxing stem cells with differentiation factors *in vitro* and then by direct gene transfection using plasmids or a viral vector. We, and others, have used a human insulin gene construct and introduced *ex vivo* or *in vivo* into cells by direct electroporation (in *ex vivo* cells obviously) or by viral vectors. The adenovirus, adeno-associated virus, and various retro viruses have been most studied, especially the Lentivirus. However, any type of genetic engineering raises fears not only of infection from the virus but also of the unmasking of onco-genes, leading to malignancy, and there are strict regulations how to proceed to avoid these risks.

We have been interested in umbilical cord stem cells and in mesenchymal stem cells as targets for combined stem cell and gene therapy. These cells can be obtained in a reasonably easy and reproducible manner from otherwise discarded umbilical cord, or readily accessible bone marrow, selecting out the cells using various standard techniques. Fat, amnion, and umbilical cord blood are also sources, from which mesnechymal stem cells can be derived. After a proliferative phase, the cells take up an appearance similar to a carpet of fibroblasts, which can differentiate into bone, cartilage, or fat cells. Although mesenchymal stem cells from the various sources mentioned may look similar, their differentiation potentials are idiosyncratic and differ, which makes it inappropriate and difficult to think of them as a uniform source of target cells. Neonatal amnion cells and umbilical cord cells have low immunogenicity and do not express HLA class II antigens. They also secrete factors that inhibit immune reactions, for example, soluble HLA-G. Although immunogenicity is reduced significantly, they are still not autologous and, therefore, there remains a risk for allograft rejection. They have the advantage that they could be multiplied, frozen, and banked in large numbers and could be used in patients already needing immunosuppressive agents, for examples those having renal transplants.

In Singapore, our studies of umbilical cord-derived amnion cells have shown some success in having expression of insulin and glucagon genes, but little or no secretion of insulin *in vitro*. Together with insulin gene transfection *in vitro*, after peritoneal transplantation into sterptozotocin-induced diabetic mice, there was some improvement in glucose levels.[3] Our colleagues in Singapore[4,5] have used another model of autologous hepatocytes from streptozotocin-induced diabetic pigs. These separated

hepatocytes were successfully transfected *ex-vivo* with a human insulin gene construct by electrophoration, and then the cells were injected directly back into the liver parenchyma using multiple separate injections. The pigs were cured of their diabetes for up to nine months - which is a remarkable achievement. As these were autotransplantations, no immunosuppressive drugs were necessary, but the liver cells were obtained from large open surgical biopsies. This necessity of surgical removal of liver tissue would limit its applicability, but nevertheless has been a good proof of concept study. In the context of autoimmune diabetes, the risk of recurrent disease may well persist unless the target of autoimmune attack could be defined and eliminated. In these porcine experiments, the human insulin gene with a glucose sensing promoter EGR-1 was used. There was no virus involved, and the plasmid does not integrate. Division of the transfected cell would dilute gene activity, but large numbers of plasmid can be produced cheaply. The same group of workers successfully transfected bone marrow mesenchymal stem cells with the human insulin gene plasmid using the same EGR-1 promoter and electrophoration. This cured diabetic mice after direct intra-hepatic and intra-peritoneal injection.

Finally, there should be caution in interpreting the results of these and other reports of cell and gene therapy for diabetes. In gene transfection and/or transplantation of insulin-producing cells or clusters in the diabetic rodent, there have been many reports in the literature, but only a few of these claims have been reproduced in independent laboratories. We have suggested the need to satisfy "The Seven Pillars of Credibility" as essential criteria in the evaluation of claims of success in the use of stem cell and/or gene therapy for diabetes.[1]

1. Cure of hyperglycemia
2. Response to glucose tolerance test
3. Evidence of appropriate C-peptide secretion
4. Weight gain
5. Prompt return of diabetes when the transfecting gene and/or insulin producing cells are removed
6. No islet regeneration of stereptozotocin-treated animals and no re-generation of pancreas in pancreatectomized animals
7. Presence of insulin storage granules in the treated cells

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References

1. Calne RY, Gan SU, Lee KO. Stem cell and gene therapies for diabetes mellitus. Nat Rev Endocrinol 2010;6:173-7.
2. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663-76.
3. Zhou Y, Gan SU, Lin G. Characterization of human umbilical cord lining derived epithelial cells and transplant potential. Cell Transplant 2012;20:1827-41.
4. Chen NK, Sivalingam J, Tan SY, Kon OL. Plasmid-electroporated primary hepatocytes acquire quasi-physiological secretion of human insulin and restore euglycemia. Gene Ther 2005;12:655-7.
5. Chen NK, Wong JS, Kee IH, Lai SH, Thng CH, Ng WH, *et al*. Nonvirally modified autologous primary hepatocytes correct diabetes and prevent target organ injury in a large preclinical model. PLoS One 2008;3:e1734.

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