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Stem Cells in Diabetes Treatment

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Abstract

For years, researchers have painstakingly dissected this complicated disease 'Diabetes' caused by the destruction of insulin producing islet cells of the pancreas. Despite progress in understanding the underlying disease mechanisms for diabetes, there is still a paucity of effective therapies. Mesenchymal stem cells (MSCs) are pluripotent stromal cells that have the potential to give rise to cells of diverse lineages. Interestingly, MSCs can be found in virtually all postnatal tissues. The main criteria currently used to characterize and identify these cells are the capacity for self-renewal and differentiation into tissues of mesodermal origin, combined with a lack in expression of certain hematopoietic molecules. Because of their developmental plasticity, the notion of MSC-based therapeutic intervention has become an emerging strategy for the replacement of injured tissues. Investigators have been making slow, but steady, progress on experimental strategies for pancreatic transplantation and islet cell replacement. Now, researchers have turned their attention to adult stem cells that appear to be precursors to islet cells and embryonic stem cells that produce insulin. This review article describes the possible way to use stem cells in treatment of 'Diabetes'.

Keywords Mesenchymal stem cells, Diabetes, Pluripotency

INTRODUCTION

Diabetes mellitus, often simply referred to as diabetes, is a condition in which a person has a high blood sugar (glucose) level as a result of the body either not producing enough insulin, or because body cells do not properly respond to the insulin that is produced. Insulin is a hormone produced in the pancreas which enables body cells to absorb glucose, to turn into energy. If the body cells do not absorb the glucose, the glucose accumulates in the blood (hyperglycemia),

leading to various potential medical complications. [1-2] There are many types of diabetes the most common of which are [2] type 1 diabetes; results from the body's failure to produce insulin, and presently requires the person to inject insulin, type 2 diabetes; results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes combined with an absolute insulin deficiency, gestational diabetes; is when pregnant women, who have never had diabetes before, have a high blood glucose level during pregnancy. It may precede development of type 2 diabetes. Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes. All forms of diabetes have been treatable since insulin became medically available in 1921, and type 2 diabetes can be controlled with tablets, but it is chronic condition that usually cannot be cured. Pancreas transplants have been tried with limited success in type 1 diabetes; gastric bypass surgery has been successful in many with morbid obesity and type 2 diabetes and gestational diabetes usually resolves after delivery. Diabetes without proper treatments can cause many complications. Acute complications include hypoglycemia, diabetic ketoacidosis, or nonketotic hyperosmolar coma. Serious long-term complications include cardiovascular disease, chronic renal failure, retinal damage. Adequate treatment of diabetes is thus important, as well as blood pressure control and lifestyle factors such as smoking cesation and maintaining a healthy body weight. [2] As of 2000 at least 171 million people worldwide suffer from diabetes, or 2.8% of the population. [3] Type 2 diabetes is by far the most common, affecting 90 to 95% of the U.S. diabetes population. [4]

Several groups of researchers are investigating the use of fetal tissue as a potential source of islet progenitor cells. For example, using mice, researchers have compared the insulin content of implants from several sources of stem cells—fresh human fetal pancreatic tissue, purified human islets, and cultured islet tissue. They found that insulin content was initially higher in the fresh tissue and purified islets. However, with time, insulin concentration decreased in the whole tissue grafts, while it remained the same in the purified islet grafts. When cultured islets were implanted, however, their insulin content increased over the course of three months. The researchers concluded that precursor cells within the cultured islets were able to proliferate (continue to replicate) and differentiate (specialize) into functioning islet tissue, but that the purified islet cells (already differentiated) could not further proliferate when grafted. Importantly, the researchers found, however, that it was also difficult to expand cultures of fetal islet progenitor cells in culture. [5]

Patients with diabetes can show vast improvement after receiving transplants of insulin-producing islets from cadavers. Though they must take drugs to stall rejection of the transplanted cells, several hundred patients with the most severe type of diabetes have benefited from the procedure since it first became established in 2000. But the effects don't last. After two years, islet function begins to decline, and unless more cells are transplanted, patients eventually return to full insulin dependency. [6-8]

Stem Cells

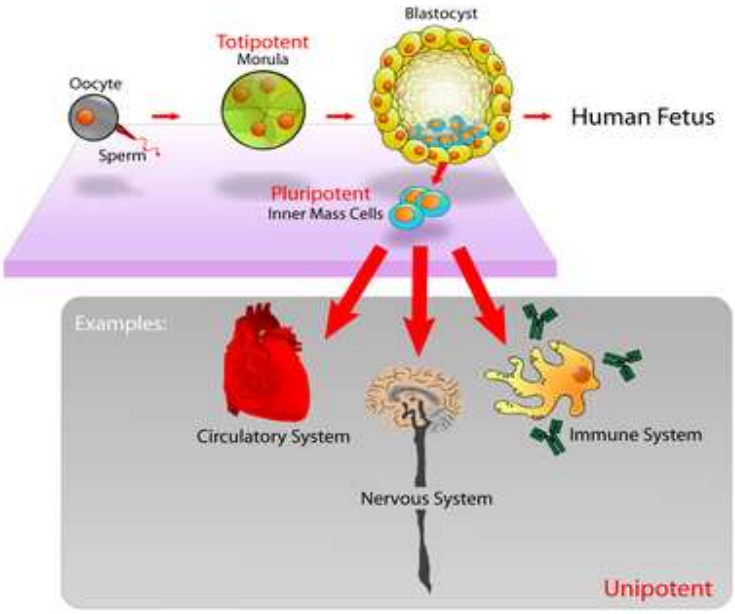
Stem cells are cells found in most, if not all, multi-cellular organisms. They are characterized by the ability to renew themselves through mitotic cell division and differentiating into a diverse range of specialized cell types. Research in the stem cell field grew out of findings by Canadian scientists Ernest A. McCulloch and James E. till in the 1960s. [9-10] The two broad types of

mammalian stem cells are, embryonic stem cells that are isolated from the inner cell mass of blastocysts, and adult stem cells that are found in adult tissues. In a developing embryo, stem cells can differentiate into all of the specialized embryonic tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing specialized cells, but also maintain the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues.

Stem cells can now be grown and transformed into specialized cells with characteristics consistent with cells of various tissues such as muscles or nerves through cell culture. Highly plastic adult stem cells from a variety of sources, including umbilical cord blood and bone marrow, are routinely used in medical therapies. Embryonic cell lines and autologous embryonic stem cells generated through therapeutic cloning have also been proposed as promising candidates for future therapies. [11]

The classical definition of a stem cell requires that it possess two properties, self-renewal- the ability to go through numerous cycles of cell division while maintaining the undifferentiated state and potency- the capacity to differentiate into specialized cell types. In the strictest sense, this requires stem cells to be either totipotent or pluripotent- to be able to give rise to any mature cell type, although multipotent or unipotent progenitor cells are sometimes referred to as stem cells. Potency specifies the differentiation potential (the potential to differentiate into different cell types) of the stem cells. Totipotent stem cells can differentiate into embryonic and extraembryonic cell types. Such cells can construct a complete, viable, organism. [12] These cells are produced from the fusion of an egg and sperm cell. Cells produced by the first few divisions of the fertilized egg are also totipotent. [13] Pluripotent stem cells are the descendants of totipotent cells and can differentiate into nearly all cells, that is cells derived from any of the three germ layers. [14]

Figure 1 Development of different types of stem cells



Multipotent stem cells can differentiate into a number of cells, but only those of a closely related family of cells. Oligopotent stem cells can differentiate into only a few cells, such as lymphoid or myeloid stem cells. Unipotent cells can produce only one cell type, their own, but have the property of self-renewal which distinguishes them from non-stem cells (e.g. muscle stem cells). [12]

Embryonic stem cell lines (ES cell lines) are cultures of cells derived from the epiblast tissue of the inner cell mass of a blastocyst or earlier morula stage embryos. [15] A blastocyst is an early stage embryo, approximately four to five days old in humans and consisting of 50–150 cells. ES cells are pluripotent and give rise during development to all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm. In other words, they can develop into each of the more than 200 cell types of the adult body when given sufficient and necessary stimulation for a specific cell type. They do not contribute to the extra-embryonic membranes or the placenta. Nearly all research to date has taken place using mouse embryonic stem cells (mES) or human embryonic stem cells (hES). Both have the essential stem cell characteristics, yet they require very different environments in order to maintain an undifferentiated state. Mouse ES cells are grown on a layer of gelatin and require the presence of leukemia inhibitory factor (LIF). [16] Human ES cells are grown on a feeder layer of mouse embryonic fibroblasts (MEFs) and require the presence of basic fibroblast growth factor (bFGF or FGF-2). [17] Without optimal culture conditions or genetic manipulation, [18] embryonic stem cells will rapidly differentiate. A human embryonic stem cell is also defined by the presence of several transcription factors and cell surface proteins. The transcription factors Oct-4, Nanog, and Sox2 form the core regulatory network that ensures the suppression of genes that lead to differentiation and the maintenance of pluripotency. [19] The cell surface antigens most commonly used to identify hES cells are the glycolipids SSEA3 and SSEA4 and the keratan sulfate antigens Tra-1-60 and Tra-1-81. The molecular definition of a stem cell includes many more proteins and continues to be a topic of research. [20] After nearly ten years of research, [21] there are no approved treatments using embryonic stem cells. The first human trial was approved by the US Food & Drug Administration in January 2009. [22] ES cells, being pluripotent cells, require specific signals for correct differentiation, if injected directly into another body ES cells will differentiate into many different types of cells, causing a teratoma. Differentiating ES cells into usable cells while avoiding transplant rejection are just a few of the hurdles that embryonic stem cell researchers still face. [23] Many nations currently have moratoria on either ES cell research or the production of new ES cell lines. Because of their combined abilities of unlimited expansion and pluripotency, embryonic stem cells remain a theoretically potential source for regenerative medicine and tissue replacement after injury or disease.

Fetal stem cells are primitive cell types found in the organs of fetuses. [24] The classification of fetal stem cells remains unclear and this type of stem cell is currently often grouped into an adult stem cell. However, a more clear distinction between the two cell types appears necessary. Adult stem cell refers to any cell which is found in a developed organism that has two properties: the ability to divide and create another cell like itself and also divide and create a cell more differentiated than itself. Also known as somatic stem cells and germline stem cells, they can be found in children, as well as adults. [25] Pluripotent adult stem cells are rare and generally small in number but can be found in a number of tissues including umbilical cord blood. [26] A great deal of adult stem cell research has focused on clarifying their capacity to divide or self-renew

indefinitely and their differentiation potential. [27] In mice, pluripotent stem cells are directly generated from adult fibroblast cultures. Unfortunately, many mice don't live long with stem cell organs. [28] Most adult stem cells are lineage-restricted (multipotent) and are generally referred to by their tissue origin (mesenchymal stem cell, adipose-derived stem cell, endothelial stem cell, etc.). [29-30] Adult stem cell treatments have been successfully used for many years to treat leukemia and related bone/blood cancers through bone marrow transplants. [31] Adult stem cells are also used in veterinary medicine to treat tendon and ligament injuries in horses. [32] The use of adult stem cells in research and therapy is not as controversial as embryonic stem cells, because the production of adult stem cells does not require the destruction of an embryo. Additionally, because in some instances adult stem cells can be obtained from the intended recipient, (an autograft) the risk of rejection is essentially non-existent in these situations. Consequently, more US government funding is being provided for adult stem cell research. [33]

Mesenchymal stem cells (MSC) in diabetes

Although regenerative capabilities of MSCs have been a driving force in launching initial studies testing their therapeutic effectiveness, the immunomodulatory properties of MSCs have recently become equally exciting for investigators in terms of examining their potential implications in a variety of disease models. MSCs have been tested in rodent animal models to treat diseases where immunomodulation is thought to be the main operative mechanism. However, it is also important to note that even in the studies focusing on the plasticity of MSCs, the benefit effects observed could also have been due to the immunomodulatory capacities of MSCs. [34-35]

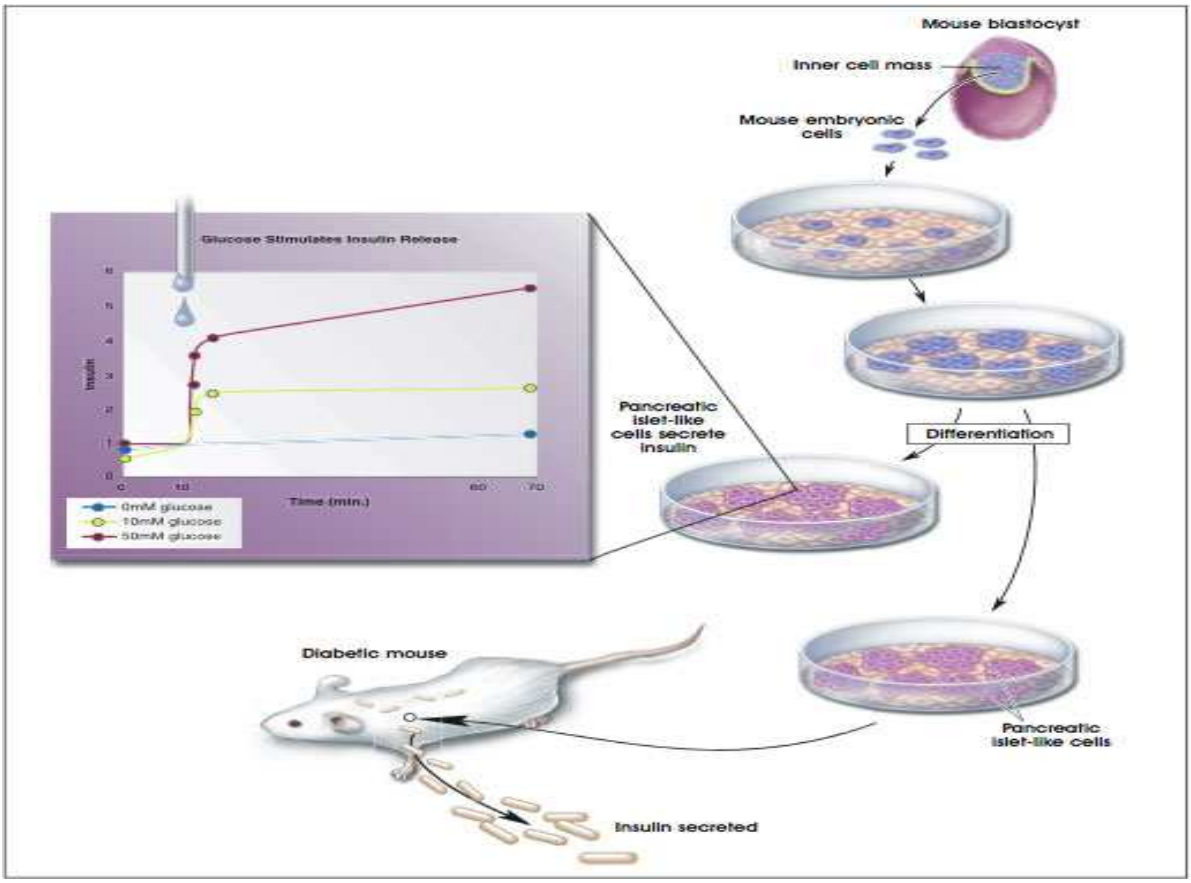
Notably, the MSC literature is lacking in reports on the use of MSCs in animal models of diabetes. Lee *et al.* used immunodeficient recipient mice (NOD.SCID), chemically rendered diabetic by streptozotocin injections, to study the effect of human MSCs in the development of diabetes. Infusion of hMSCs reduced glycemic levels and increased peripheral insulin levels. Human DNA infused as hMSCs was detected in the pancreas as well as in the kidney. [36] Among early reports responsible for stirring this interest was a study by Bartholomew *et al.* [37] in which the investigators demonstrated that donor MSC administered intravenously to MHC-mismatched recipient baboons before placement of second and third party skin grafts led to prolonged allograft survival.

Researchers in Spain reported using mouse embryonic stem cells that were engineered to allow researchers to select for cells that were differentiating into insulin-producing cells. [38] Bernat Soria and his colleagues at the Universidad Miguel Hernandez in San Juan, Alicante, Spain, added DNA containing part of the insulin gene to embryonic cells from mice. The insulin gene was linked to another gene that rendered the mice resistant to an antibiotic drug. By growing the cells in the presence of an antibiotic, only those cells that were activating the insulin promoter were able to survive. The cells were cloned and then cultured under varying conditions. Cells cultured in the presence of low concentrations of glucose differentiated and were able to respond to changes in glucose concentration by increasing insulin secretion nearly sevenfold. The researchers then implanted the cells into the spleens of diabetic mice and found that symptoms of diabetes were reversed.

Ron McKay and his colleagues described a series of experiments in which they induced mouse embryonic cells to differentiate into insulin-secreting structures that resembled pancreatic islets.

[39] McKay and his colleagues started with embryonic stem cells and let them form embryoid bodies—an aggregate of cells containing all three embryonic germ layers. They then selected a population of cells from the embryoid bodies that expressed the neural marker nestin (Mouse embryonic stem cells). Using a sophisticated five-stage culturing technique, the researchers were able to induce the cells to form islet-like clusters that resembled those found in native pancreatic islets. The cells responded to normal glucose concentrations by secreting insulin, although insulin amounts were lower than those secreted by normal islet cells (Figure 3). When the cells were injected into diabetic mice, they survived, although they did not reverse the symptoms of diabetes.

Figure 2 Development of insulin secreting pancreatic-like cells from mouse embryonic stem cells



Mouse embryonic stem cells were derived from the inner cell mass of the early embryo (blastocyst) and cultured under specific conditions. The embryonic stem cells (in blue) were then expanded and differentiated. Cells with markers consistent with islet cells were selected for further differentiation and characterization. When these cells (in purple) were grown in culture, they spontaneously formed three-dimentional clusters similar in structure to normal pancreatic islets. The cells produced and secreted insulin. As depicted in figure 2, the pancreatic islet-like cells showed an increase in release of insulin as the glucose concentration of the culture media was increased. When the pancreatic islet-like cells were implanted in the shoulder of diabetic

mice, the cells became vascularized, synthesized insulin, and maintained physical characteristics similar to pancreatic islets.

Melton, Nissim Benvinisty of the Hebrew University in Jerusalem, and Josef Itskovitz-Eldor of the Technion in Haifa, Israel, reported that human embryonic stem cells could be manipulated in culture to express the PDX-1 gene, a gene that controls insulin transcription. [40] In these experiments, researchers cultured human embryonic stem cells and allowed them to spontaneously form embryoid bodies (clumps of embryonic stem cells composed of many types of cells from all three germ layers). The embryoid bodies were then treated with various growth factors, including nerve growth factor. The researchers found that both untreated embryoid bodies and those treated with nerve growth factor expressed PDX-1.

Table 1 Mesenchymal stem cells (MSC) in various animal models of diseases

S. No.	Therapy	Outcome	Reference
1	Kidney ischemia reperfusion injury	Syngeneic murine-MSCs are helpful in the restoration of tubular epithelial cells with an anti-inflammatory effect	[35]
2	STZ diabetes	Human-MSC grafted kidney and pancreas in STZ NOD.SCID mice ameliorating diabetes and kidney disease	[36]
3	Multiple sclerosis model (EAE)	Syngeneic murine-MSCs are home to inflamed lymphoid tissues reducing disease progression	[38]
4	Arthritis	Allogenic murine-MSCs reduce joint inflammation and increase Treg generation	[39]
5	Heart transplantation	Allogenic rat-MSCs injected intravenously migrated to the heart during chronic rejection	[41]
6	Myocardial infarction	Syngeneic rat-MSCs showed an anti-inflammation role in ischemic heart disease	[43]
7	Acute lung injury	Syngeneic intrapulmonary murine-MSCs decrease the severity of endotoxin-induced acute lung injury and improve survival in mice	[44]
8	Chronic lung injury	Syngeneic murine-MSCs protect lung tissue from bleomycin-induced injury with anti-inflammatory effect	[45]
9	Acute hepatic failure	Human-MSCs protect against hepatocyte death and increase survival in mice after the injections of the hepatotoxin D-galactosamine	[46]
10	Heart transplantation	Allogenic rat-MSCs co-injected with cyclosporine accelerate rejection	[46]
11	GHVD	Allogenic rat-MSCs prevent lethal GVHD	[48]
12	GVHD	Allogenic murine-MSCs did not improve GVHD	[48]
13	BM transplantation	Donor-MSCs increase rejection of allogeneic donor bone marrow cells	[49]

Future trends

Type 1 diabetes may prove to be especially difficult to cure, because the cells are destroyed when the body's own immune system attacks and destroys them. This autoimmunity must be

overcome if researchers hope to use transplanted cells to replace the damaged ones. Many researchers believe that at least initially, immunosuppressive therapy similar to that used in the Edmonton protocol will be beneficial. A potential advantage of embryonic cells is that, in theory, they could be engineered to express the appropriate genes that would allow them to escape or reduce detection by the immune system. Others have suggested that a technology should be developed to encapsulate or embed islet cells derived from islet stem or progenitor cells in a material that would allow small molecules such as insulin to pass through freely, but would not allow interactions between the islet cells and cells of the immune system. Such encapsulated cells could secrete insulin into the blood stream, but remain inaccessible to the immune system. Before any cell-based therapy to treat diabetes makes it to the clinic, many safety issues must be addressed. A major consideration is whether any precursor or stem-like cells transplanted into the body might revert to a more pluripotent state and induce the formation of tumors. These risks would seemingly be lessened if fully differentiated cells are used in transplantation. But before any kind of human islet-precursor cells can be used therapeutically, a renewable source of human stem cells must be developed. Although many progenitor cells have been identified in adult tissue, few of these cells can be cultured for multiple generations. Embryonic stem cells show the greatest promise for generating cell lines that will be free of contaminants and that can self renew. However, most researchers agree that until a therapeutically useful source of human islet cells is developed, all avenues of research should be exhaustively investigated, including both adult and embryonic sources of tissue.

CONCLUSION

For human stem cell-based therapy to become a reality for patients with diabetes, several important steps must be accomplished. Legislation in the United States must be changed to allow generation of new human stem cell lines that have not been compromised by co-culture with mouse cells and that offer distinct cell phenotypes to facilitate graft acceptance. The molecular mechanisms of cellular self-renewal must be understood more deeply so that we can efficiently maintain human stem cell lines in their pluripotent state. In addition, present culture methods must be improved to generate sufficient cells for clinical use: After stem cells enter the differentiation pathway, their time clock starts and they begin to lose telomerase activity and the capacity to replicate indefinitely. We must therefore learn how to maintain the stem cells in their pluripotent state for clinical use and to induce the differentiation process when needed for transplantation. Efficient, safe protocols must be designed for inducing b cell differentiation so that these clinically differentiated cells can normalize blood glucose levels the same way spontaneously differentiated b cells normalize blood glucose levels.

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