REVIEW ON STEM CELLS AND THEIR THERAPY ON DIABETES

G.Bharathkumar*, 2K.Kapil, 3R.Prashanthreedy, 4D.Shyamala, 5P.Keerthi

B.Pharmacy scholar, Samskruti College of Pharmacy, Hyderabad.

Abstract
Stem cells are undifferentiated or ‘blank’ cells found in the human body that have the potential to develop into many different cell types that carry out different functions. The defining characteristic of human stem cells is their ability to self-renew (provide an exact copy of themselves) while maintaining the potential to develop into other types of cells, such as blood, brain or heart. Although stem cells from different sources all share these general characteristics, there are also significant differences among them. Stem cell therapy is an intervention strategy that introduces new adult stem cells into damaged tissue in order to treat disease or injury. Many medical researchers believe that stem cell treatments have the potential to change the face of human disease and alleviate suffering. The ability of stem cells to self-renew and give rise to subsequent generations with variable degrees of differentiation capacities, offers significant potential for generation of tissues that can potentially replace diseased and damaged areas in the body, with minimal risk of rejection and side effects.

Key words: Stem cells, langerhans, islets, insulin, diabetic, undifferentiated.

Corresponding Author:
G.Bharath Kumar
Samskruti College of Pharmacy
Email: bharathk147@gmail.com
Contact: +91-8341130049

Available online: www.ijipsr.com  September Issue
INTRODUCTION:

Stem cells are undifferentiated or "blank" cells found in the human bodies that have the potential to develop into many different cell types that carry out different functions. [1] Most cells in the human body are differentiated [2] that means they are built to function in a particular organ system and carry out a specific function. A red blood cell, for example, is designed to carry oxygen, while a white blood cell is designed to fight off disease. These differentiated cells result from the process of cell division, a process that begins with undifferentiated stem cells.

Pluripotent stem cells, found in embryos, can give rise to all the cells found in the human body – cells as diverse as those found in the brain, bone, heart and skin. [3]

Multipotent stem cells, found in adults or in babies' umbilical cords, have a more limited capacity. Their development is limited to the cells that make up the organ system that they originated from. For example, a multipotent stem cell in the bone marrow can develop into a red blood cell, a blood platelet or a white blood cell, but not into a skin cell or brain cell [4].

Researchers believe that stem cells, especially pluripotent stem cells, hold much potential for medical therapies and medical research such as:

- Growing replacement cells or whole replacement organs. Human stem cells can be used to generate specialized cells in a laboratory and then be transplanted to replace damaged cells in the body. These could be used to treat a range of conditions from Parkinson disease to heart failure to spinal injuries. For example, in the case of a spinal injury, neural stem cells could be generated to replace damaged tissue [5].
- "Patching" organs that don't work properly - like helping a diabetic person's pancreas produce insulin. The newest therapies in research on stem cells and diabetes involve generating islet cells that produce insulin to replace those that a diabetic person's immune system destroys[6]
- In the study of human development, stem cells could help researchers determine why, in the early stages of development, some cells become cancerous or how genetic diseases develop. This could lead to answers as to how they might be prevented [7]
For research purposes, stem cells may be useful as a testing ground for new drugs before they are used on humans. Stem cells may be more accurate for research results than using animal subjects, as well as solve the ethical dilemma of using animals for medical testing.

Stem cell research has the potential to bring new treatment options to patients with Alzheimer's, Parkinson's disease, heart disease, burns, diabetes, and spinal cord injuries.

**Types of Stem Cells**

The body is made up of about 200 different kinds of specialised cells such as muscle cells, nerve cells, fat cells and skin cells. All specialised cells originate from stem cells. A stem cell is a cell that is not yet specialised. The process of specialisation is called differentiation and once the differentiation pathway of a stem cell has been decided, it can no longer become another type of cell [8]. A stem cell that can become every type of cell in the body is called pluripotent whilst a stem cell that can become only some types of cells is called multipotent. Stem cells are found in the early embryo, the fetus, placenta, umbilical cord, and in many different tissues of the adult body.

Stem cells are often divided into two groups: tissue specific stem cells (often referred to as adult stem cells) and pluripotent stem cells (including embryonic stem cells and induced pluripotent stem cells). Tissue specific stem cells are derived from, or resident in, fetal or adult tissue, and can usually only give rise to the cells of that tissue, thus they are considered multipotent. Embryonic stem cells, derived from a small group of cells within the very early embryo, and their new counterpart induced pluripotent stem (iPS) cells are considered pluripotent as they can become every type of cell in the body [9]

**Tissue Specific Stem Cells**

Tissue specific stem cells are undifferentiated cells found in the tissues and organs of the body. They are capable of self-renewal. Their differentiation is mainly restricted to forming the cell types of that tissue or organ. The chief role of tissue specific stem cells is to maintain and repair the tissue in which they are found. Skin stem cells, for example, give rise to new skin cells, ensuring that old or damaged skin cells are replenished.

It now appears that all tissues probably contain adult stem cells. Most tissues contain only tiny numbers of stem cells. The exception is bone marrow and umbilical cord blood which contains relatively high
numbers of stem cells. In each tissue, adult stem cells are used to produce new mature cells as old ones die in the natural processes of ageing. They may also be activated by disease or injury[10,11] Due to their small numbers isolation of adult stem cells is difficult but they have been successfully isolated from the brain, bone marrow, blood, muscle, skin, lung, pancreas and liver. To date the majority of research has been carried out on haematopoietic stem cells isolated from bone marrow and umbilical cord blood and on mesenchymal stem cells which can also be sourced from the bone marrow and some other tissues [12] Haematopoietic stem cells are the stem cells from which all blood cells and many of the cells of our adult immune system are derived. These are the stem cells with the longest history of clinical use in treating disorders such as leukaemia via bone marrow transplants. There has recently been much interest in whether haematopoietic stem cells can be caused to differentiate into non blood cells, such as heart muscle cells or even nerve cells[13] Mesenchymal stem cells can be found in the bone marrow but are also found in several other sites in the body such as the placenta. Mesenchymal stem cells are particularly interesting to researchers because in addition to their capacity to differentiate into the multiple cell types listed above, they also have anti-inflammatory and immune-suppressing properties.Blood can be collected from the umbilical cord of a newborn baby shortly after birth. This blood is rich in haematopoietic stem cells that can be used to generate blood cells and cells of the immune system.

**Pluripotent Stem Cells**

**Embryonic Stem Cells**

Discovered in 1998, human embryonic stem cells (hESCs) are the most primitive type of stem cell and can replicate and generate every cell type of the human body.

Human embryonic stem cells are derived from human blastocysts (early stage embryos) that are five to seven days old. In Australia these blastocysts are donated for research with consent from patients who have completed treatment for infertility, and have surplus embryos. At this stage of development the blastocyst is a hollow ball of about 150 cells and no bigger than a pinhead. Figure 1 demonstrates the different parts of the blastocyst, showing that next to a large internal cavity (C), is a small group of approximately 30 cells called the inner cell mass (ICM). The outer layer is the trophectoderm (T). The inner cell mass is what ultimately becomes the embryo, and the trophectoderm becomes the placenta [15].
Blastocyst

ICM

The inner cell mass cells are able to develop into any type of cell in our body and can contribute to all the cells and tissues of the adult organism. These types of cells are called pluripotent and it is this pluripotency that makes them of interest to research and therapy [16]. Embryonic stem cells are isolated from the blastocyst when the inner cell mass is removed and cultured in the laboratory. During this process the blastocyst is destroyed.

Once the cells have been isolated they can be grown continuously in a laboratory culture dish that contains a nutrient-rich culture medium. As the stem cells divide and spread over the surface of the dish some are removed to populate fresh subcultures to form a stem cell line. Because these cells have the ability to keep dividing (self-renewing), large numbers of embryonic stem cells can be grown in the laboratory and also frozen for future use[17].Therefore, established hESC lines can be maintained in laboratories for research, shared between researchers and maybe ultimately used in cell-based therapies.

Somatic Cell Nuclear Transfer (SCNT) or Therapeutic Cloning

SCNT refers to the removal of a nucleus, which contains the genetic material or DNA, from virtually any cell of the body and its transfer by injection into an unfertilised egg (oocyte) from which the nucleus has also been removed. The newly reconstituted entity is then stimulated to start dividing. After 5-7 days in culture, embryonic stem cells can then be removed and used to create many embryonic stem cells in culture. These embryonic stem cell lines are genetically identical to the cell from which the DNA was originally removed [18].

SCNT may have applications in the creation of embryonic stem cells which can then be used for the development of patient- and disease-specific cell-based therapies as well as the production of stem cells with specific disease characteristics for research purposes. The use of a patient's own cells for tissue replacement through SCNT may overcome the problem of immune rejection that is a major complication of tissue or organ transplantation today.

SCNT is commonly referred to as therapeutic cloning. The word ‗cloning‘ often conjures up images of cloning an individual (reproductive cloning) such as the process used to create Dolly. Using SCNT to create a human embryo to implant into a uterus is illegal in Australia and many parts of the world [19]. The scientific community
overwhelmingly rejects reproductive cloning, but SCNT may provide an invaluable tool for basic research. However, whilst the technology has been proven in many species it has yet to produce a stem cell line in humans. A major breakthrough occurred in November 2007 when a group of scientists reported that they had successfully extracted stem cells from monkey embryos generated by SCNT.

**Induced Pluripotent Stem Cells**

In November 2007, a significant development occurred when scientists announced they had developed a new technology to cause mature human cells to resemble pluripotent stem cells similar in many ways to hESCs. These reprogrammed cells are referred to as induced pluripotent stem (iPS) cells [20].

Initially iPS cells were generated using viruses to genetically engineer mature cells to achieve a pluripotent status. The purpose of the virus was to insert reprogramming genes into a cell such as a skin cell and then culture the cells in the laboratory for 4-5 weeks after which a small number of iPS cells begin to appear. However technologies for reprogramming cells are moving very quickly and researchers are now investigating the use of new methods that do not remain in the cells causing permanent and potentially harmful changes [21]. These new technologies currently utilize different types of non-integrating viruses and chemicals and small molecules.

Similar to SCNT, this technology allows scientists a new method of creating diseased cells for research by using mature cells from a patient with a genetic disease, such as Huntington's disease, and turning those cells into iPS cells. Such disease-specific stem cells may enable disease investigation and drug development offering a unique opportunity to recreate both normal and diseased human tissue formation in the laboratory [22]. iPS technology also has the potential to produce genetically identical -patient specific‖ embryonic stem cell-like lines that would be recognized as -self‖ and not rejected by the patient they were made from, however there is much to be understood before this could be achieved.

Whilst the discovery of iPS cells is a significant breakthrough in the field of reprogramming, the use of iPS cells in the clinic is many years away - if it occurs at all - as several significant hurdles need to be overcome [23]. It is still unclear how genetically stable or safe iPS cells will be for potential clinical use. More research needs to be done into induced pluripotent stem cells to discover if they will offer the same equivalent research value as embryonic stem cells. Having only recently discovered these cells, scientist are yet to confirm if iPS cells have the ability to divide and remain chromosomally stable like embryonic stem cells over a long period of time [24].

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**September Issue**
Figure 1: Schematic Representation of Production of Stem Cells

Totipotent Stem Cells
Totipotent (toti = whole, potent = able to) stem cells can give rise to all cell types of an organism, including extra embryonic cells (i.e., cells that comprise supportive tissues of the embryo, such as the placenta). An example of a totipotent stem cell is the fertilized egg of the mammalian embryo (also called the zygote) [25]. This single stem cell undergoes a number of cell divisions to generate distinct embryonic tissues (embryo proper) and extraembryonic support tissues (the placenta and nutrient-providing yolk sac). During the first few cell divisions of embryonic development, the cells of the embryo remain totipotent. If at this time the embryo splits, the totipotent cells can continue to develop normally to yield identical (or monozygotic) twins [26].
Diabetes Therapy with Stem Cells

A Source of Beta-Cells, the Need

Glucose homeostasis requires finely regulated insulin secretion by pancreatic beta-cells present in islets of Langerhans [27-29]. In health, under fasting basal conditions meal ingestion this rate increases by as much as 5–10-fold [30]. To accomplish this requires not only normally functioning beta-cells, but also a sufficient number of beta-cells, collectively often referred to as beta-cell mass. In health, the human pancreas contains approximately one million islets, each containing approximately two thousand beta-cells [31-34]. Thus, the beta-cells constitute 1.5% of the total pancreatic mass (1–2 g in total) [34]. While in humans, an up to 40% loss of beta-cells can be tolerated without a significant deterioration of glucose tolerance [35], a further reduction in beta-cell mass leads to hyperglycemia. Type 1 diabetes is caused by autoimmune-mediated destruction of beta-cells [36-42] once hyperglycemia develops as much as 90% of beta-cells have been lost. Some residual insulin secretion may persist in people with longstanding type 1 diabetes [43–46], consistent with autopsy studies reporting scattered beta-cells and occasional islets with beta-cells present in patients with long standing type 1 diabetes[12–14,25]. Therefore, beta-cell replacement is a potential therapy that might reverse rather than simply palliate both type 1 and type 2 diabetes. [41, 42]

Why embryonic stem cells?

Human ES cells are derived from the inner cell layer of the blastocyst (Fig. 1) [43, 45]. These cells subsequently give rise to all differentiated cells in the adult through a series of cell fate choices that involve self-renewal and differentiation [47–48]. Therefore they can theoretically be differentiated into any definitive cell type, including pancreatic beta-cells, when exposed to the appropriate signals in the correct sequence and over the appropriate time periods. Gives rise to the blastocyst, a structure comprised of an outer cell layer, the trophoectoderm, and the inner cell mass.

Obstacles associated with the use of embryonic stem cells

Despite the obvious appeal of ES cell-based beta-cell replacement therapy for diabetes, there are several major obstacles that must be overcome to before this approach can be realistically considered as a therapeutic option.
Ethical and religious sensitivities
There are ethical and religious sensitivities concerning use of human embryos that still hamper the broader use of ES cells for research purposes, and many governments ban or at least highly restrict this kind of research [52, 53].

Establish and expand cell lines
The first step before ES cells can be used as therapy is to obtain ES cell lines from human embryos and to expand these without loss of their pluripotential properties, or being contaminated in ways that would preclude use as a therapy. ES cell lines have been established from rodents, rabbits, pigs, primates and humans [48, 50, 54–60]. Human ES cells have typically been expanded as undifferentiated colonies on feeding layers of mouse embryonic fibroblasts. ES cells differentiate into ectodermal, mesodermal and endodermal structures after removal from this layer [50]. The use of mouse fibroblasts as feeding layers has contaminated some of the limited supply of human ES cells available with mouse genes, precluding their therapeutic potential. However, it is possible to expand human ES cells without mouse feeder layer cells so that in future human ES cells can be expanded without this problem, although obtaining new ES cell lines is limited by the unresolved religious and ethical concerns raised earlier. Thus successful culture of human ES cells over 30 passages has recently been described in the absence of mouse embryonic fibroblasts by using only serum replacement media and high concentrations of basic fibroblast growth factor [61].

Development:
The second step before ES cells can be considered as a therapeutic option for diabetes is to establish the means to drive development of the ES cells lines to differentiated and functioning beta-cells. In normal human development ES cells are exposed to numerous complexes and as yet minimally understood signals to generate fully differentiated beta-cells within the organelle, the islet of Langerhans. These include signals arising from both within the future developing beta-cells to activate transcriptional programs, but perhaps more importantly; neighboring cells that are not destined to form beta-cells can signal to influence progenitor cell differentiation into beta-cells [62–65]. Beta-cells develop from progenitor cells arising along with exocrine and ductal tissue in the evolving three-dimensional architecture of the primordial pancreatic bud from the foregut. During embryonic development the pancreas forms from a ventral and a dorsal bud,
protrusions of the primitive gut endoderm that fuse to form the pancreas. Subsequently, a cascade of transcription factors are activated which initiate further development and differentiation toward the specific cell types (Fig. 2). The cell fates choices of pancreatic progenitors are regulated by Notch signaling through HES1 activation [66]. The transcription factor neurogenin3 (NGN3) plays an essential role in the differentiation of endocrine cells from the pool of pancreatic progenitor cells [67, 69]. Since it takes more than 18 months to establish functional beta-cell mass in developing humans [70, 88], it is not yet clear if it will be possible to drive ES cells to a useful mass of beta-cells or beta and other cell type aggregates, ex vivo. A concern is the fact that human ES cells tend to undergo senescence and differentiation within days in culture thereby losing their pluripotency [71].

Rejection:
Another obstacle that will have to be overcome before ES cells can be used as therapy is to protect the resulting beta-cells from rejection, to avoid the need for immuno suppression. Theoretically, ES cells can be manipulated ex vivo to induce immune-compatibility with the intended recipient [72, 50] avoiding the need for immuno suppression and its attendant side effects [(44, 45].

Progress to date
Several in vitro studies have demonstrated that it is possible to obtain cells that express insulin from human ES cells [63, 73]. Given the discussion of the challenges that must be overcome above, it is not surprising that functionally useful beta-cells have not yet been obtained from human ES cells [63, 74]. Cells that have been generated from human ES cells that express insulin have been minimally glucose responsive [63]. Expression of typical beta-cell markers, such as insulin, GLUT-2 and glucokinase, has been detected in some of these cells However, insulin staining in ES cell-derived tissue preparations should be interpreted with caution, because insulin staining can overestimate the proportion of beta-cells, when insulin is present in the culture medium [96]. Perhaps not surprisingly, preparations of human ES cells usually consist of a mixture of different cell types and purification of insulin-secreting cells from such cell clusters has proven to be technically difficult (64).

Islets or beta-cells
An unresolved question: Exactly what is the target form that beta-cells should take if successfully developed from human ES cells? Adult pancreatic islets have a complex
architecture, with the beta-cells being more preferentially located in the islet core and the other cell types, such as alpha-, delta- and PP-cells, being more abundant in the islet periphery [4, 8]. The main vascular supply of the islet comes from an arteriole that enters the islet from its beta-cell enriched core, from where the blood is being passed to the islet periphery. Also, beta-cells are tightly connected to each other via gap junctions, thereby allowing direct electrical coupling between neighboring cells [75]. This specific structure of the islets appears to be important for the coordinated discharge of the different islet hormones. Thus single beta-cells obtained from islets are much less responsive than beta-cells electrically coupled. Insulin in health is secreted in discrete secretory bursts, the primary mechanism through which insulin secreted is regulated being the modulation of burst mass. Moreover, insulin secreted per islet at a given stimulus of glucose is substantially increased once the islets are interconnected by a neural network. While it is already a considerable challenge to derive functional beta-cells from human ES cells, it would be altogether a more daunting challenge to recapitulate functional islets.

Figure 2. Beta-cell turnover in adult humans. Beta-cell mass is regulated by the new formation of cells via replication of existing beta-cells or new islet formation. From adult stem cells as well as by the rate of beta-cell apoptosis. Deficient beta-cell mass in patients with diabetes may therefore be restored by either enhancing new beta-cell formation or by inhibiting beta-cell apoptosis. From human ES cells. Should beta-cells derived from human ES cells be established in micro cell clusters, or as a single organ? Would such an aggregate of beta-cells be functional, and most importantly not lead to life threatening hypoglycemia?
Where shall we put them?

Assuming the limitations outlined above could be overcome, the next question arising would be where to best transplant such human ES-cell derived insulin-secreting cells, cell clusters or islets. Several considerations arise. Human ES cells have the potential to develop teratomas and possibly other cancers [76]. Should these cells or cell clusters therefore be encapsulated so that they cannot escape? If so the issues of how large should the capsules be where should they be implanted, how many would be required, and how long would the cells or cell clusters last in the capsules would have to be addressed. If the newly formed beta-cells, cell clusters or islets could be transplanted without concern of cancer, then the optimal tissue bed to transplant them would presumably have a relatively high oxygen tension since beta-cells are sensitive to anoxia. Also it would need to be established if the cells, cells clusters or islets should direct their secretion into the hepatic portal circulation or the systemic circulation. Given the pivotal role of the liver as the major insulin-responsive organ, it seems most desirable to mimic the physiologic route of intra-portal insulin delivery. The fact that intra-portal transplanted islets deliver their insulin directly to the liver rather than into the systemic circulation [77] may therefore provide an argument in favor of intra-portal islet transplantation. Alternatively, the intestinal mucosa may be an attractive transplantation site because of its rich vascularization and the high local concentrations of potential growth factors such as glucagon-like peptide 1 (GLP-1), gastric inhibitory polypeptide (GIP), GLP-2, gastrin, etc. [78-80], but the surgical implantation of beta-cell clusters into the gut may be technically more demanding.

How long will they last?

Based on the recent experiences with islet transplantation in humans, the life span of an islet appears to be limited and most patients who had received islet transplantations have to revert to insulin treatment within less than five years [46]. Thus, to provide an ultimate cure for Diabetes, human ES cell-derived islets or beta-cells would either need to maintain their ability to proliferate, or the transplantation procedure would have to be repeated at regular intervals. Assuming the former possibility, one major challenge would be how to control the proliferative activity of these cells to maintain a physiologic balance between apoptosis and replication. Adult human beta-cells apparently exhibit some plasticity which allows them to respond to increasing secretory demands, e.g. caused by insulin resistance or obesity, by increasing both in number and
size [9]. It is unlikely that this complex regulation of beta-cell mass could resemble insulin-secreting cells derived from human ES-cells. Moreover, potential risks imposed by continued replication of ES-cell derived transplants include loss of cell cycle control and the induction of neoplastic cell growth. Consistent with this consideration, the formation of teratomas has been observed in insulin-producing cell lines derived from ES-cells. Therefore, maintaining a physiologic balance between replication and cell death appears to be a major challenge for beta-cell replacement therapies based on ES-cells.

NEW BETA-CELL FORMATION FROM ADULT STEM CELLS
There is an ongoing controversy regarding the existence of stem cells for new beta-cells in adult individuals [81-83]. Such adult stem cells have been suggested to reside in exocrine pancreatic parenchyma, pancreatic ducts, pancreatic islets, liver, spleen, and in bone marrow.

**Stem cells in exocrine ducts.**
Some studies support the idea of new islet formation from exocrine duct cells [84]. This concept first evolved from the budding of the endocrine pancreas observed during embryonic development [73], and has gained support from different lines of evidence: Thus, endocrine cells can frequently be detected in exocrine ducts in both rodents and humans, and islets adjacent to exocrine ducts are commonly found throughout the pancreas (Fig. 3)[9,10,14,]. Moreover, in rodents the number of these ducto-insular complexes is increased during conditions of high secretory demands, e.g. during chronic glucose infusion or after partial pancreatectomy implying compensatory new islet formation. In the adult human pancreas, exocrine ducts are often surrounded by areas of fibrous tissue, and the extent of this fibrosis is markedly increased in patients with type 1 diabetes [12, 13, and 25].

**Tran’s differentiation of exocrine cells**
Tran’s differentiation of exocrine acinar cells into beta-cells has been proposed as an alternative mechanism for new beta-cell formation in the adult human pancreas. This concept has been supported by the observation of single beta-cells scattered throughout the exocrine parenchyma in a patient with type 1 diabetes after immunosuppressive treatment. Moreover, a number of recent studies have shown that under *in vitro* conditions acinar tissue can be directed toward a beta-cell like phenotype [84, 23]. However, it is important to note that the appearance of scattered single beta-cells in exocrine parenchyma is a common finding in non-diabetic pancreas as well and that
the mere presence of these cells cannot directly prove that they were indeed originated from acinar cells [9, 14]. Tran’s differentiation.

**Bone marrow-derived stem cells**

A highly controversial question is, whether new beta-cells could originate from bone marrow derived cells. Janus et al performed experiments, in which bone marrow from male donor mice that expressed GFP as well as CRE under the INS2 promotor, was transplanted into female mice that were depleted of their own bone marrow by irradiation. They reported that after 4–6 wk, 1.7–3.0% of the islet beta-cells were derived from bone marrow cells. However, other investigators using similar experimental approaches failed to confirm these results.

**Replication as the primary mechanism of new beta-cell formation**

Recently, the existence of stem-cells for beta-cells in adult individuals has been challenged by the demonstration that in adult mice beta-cell replication almost exclusively accounts for the formation of new beta-cells [85]. Using lineage tracing experiments, Dore et al showed that the vast majority of adult beta-cells were derived from pre-existing beta-cells, thereby ruling out new-beta-cell formation from adult stem cells [50]. Moreover, recent studies in cyclin D2 knockout mice demonstrated that replication is the primary mechanism for maintaining postnatal beta-cell mass [71]. However, the frequency of -cell replication (Ki67 and insulin labeling) in adult human beta-cells is much lower than compared with that in rodents [9, 25]. It is therefore possible that -cell turnover in humans is accomplished from a different source than in rodents.

**Strategies to replenish beta-cell mass from endogenous sources**

While the sources of new beta-cell formation remain to be elucidated, there is indirect evidence that ongoing beta-cell turnover may be present in adult humans. Beta-cell mass adaptively increases under conditions of high insulin resistance, e.g. in response to obesity or during pregnancy [9]. Assuming that adult beta-cells do turnover throughout life, beta-cell mass in patients with diabetes could theoretically be replenished by two different approaches: First, by enhancing new beta-cell formation, and second by inhibition of beta-cell apoptosis (Fig. 4). A number of approaches have been suggested to enhance beta-cell mass by increased beta-cell formation. Those include gut hormones, such as gastrin, GLP-1, or GIP, growth factors, such as hepatocyte growth factors, epidermal growth factor IGF-1 or -2 (IGF-1, IGF-2) [63-80], TGF-, and growth hormone, or other factors such as betacellulin. The alternative approach is to target
inhibition of excess beta-cell apoptosis in diabetes. Thus, given the putative ongoing beta-cell formation in adult life and the well documented increased beta-cell apoptosis in both type 1 and type 2 diabetes [9,25], reducing the rate of cell apoptosis may allow for beta-cell regeneration in these patients. In rodent models of type 1 diabetes, inhibition of beta-cell apoptosis leading to partial or total recovery of beta-cell mass has been achieved using many different approaches. In a recent review article, Roep and Atkinson were able to list 192 different approaches to cure diabetes in NOD mice. In humans, however, no intervention has accomplished comparable reversal of diabetes. To date the best outcome has been prevention or delay of the loss of cell function in patients with new-onset type 1 diabetes treated with CD-3 antibodies. In type 2 diabetes, beta-cell apoptosis has been suggested to be caused by the formation of toxic oligomers of hIAPP (9,29), free radicals, II and lipotoxicity[30 -34].

**Rates of beta-cell turnover in humans**

Successful use of the approach of suppression of beta-cell apoptosis to increase beta-cell mass from endogenous cell regeneration requires there to be sufficient new cell formation. Finegood *et al* attempted to quantitatively assess beta-cell turnover by using the frequencies of BrdU or thymidine incorporation in cells in rats. Based on these data, a turnover rate of 2% beta-cells per day was calculated in adult rats [86, 87-90]. However, using continuous long-term BrdU labeling in adult mice, only 1/1,400 beta-cells underwent replication per day. Assuming no additional input from new islet formation, Transdifferentiation or other potential sources, this would correspond to a proliferation rate of 0.0701% per day. Thus, even assuming a 0% rate of beta-cell death, recovery of a 50% deficit in beta-cell mass would be expected to occur after 1,429 d, a time period that far exceeds the typical life span of a mouse. In humans, similar calculations are difficult to perform, since BrdU labeling cannot be used for obvious reasons, but based on the reported frequencies of Ki67 labeling, the turnover rate of beta-cells seems to be even slower [9,25]. On the other hand, the increase in beta-cell mass observed in humans during pregnancy implies that this turnover rate can be increased by several-fold under certain conditions even in adult humans. [90]
CONCLUSIONS

Major achievements in the isolation, culture and targeted differentiation of ES cells prompt hopes that it will one day be possible to replenish beta-cell mass in patients with diabetes using ES cell-derived engineered insulin-producing cells. However, in light of ethical concerns and technical obstacles still to be overcome, this approach is unlikely to available as a therapy in the near future. Therefore, alternative methods to increase the number of insulin-secreting beta-cells in patients with diabetes should continue to be explored. The role of adult stem cells in the formation of new beta-cells is controversial, and the origin of such cells is unclear. In rodents the plasticity of the endocrine pancreas in adults and its ability to compensate for an experimental reduction of beta-cell mass suggests that there is ongoing regulated -cell turnover in adults which can be targeted to reverse diabetes. The potential for this approach in humans is much less clear but is the basis of active investigation at present.

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