New sources for insulin-producing cells

Hussain R. Al-Turaifi, *PhD, MSc.*

ABSTRACT

أن استبدال الخلايا يقدم العلاج الممكن لمرض السكري من النوع الأول. ولكن النقص الحاد في المتبرعين المناسبين يحد من تطبيق هذه النوع من العلاج. تركز الأبحاث الحديثة على مصادر جديدة لخلايا بيتا المفرزة للانسولين من بينها الخلايا الجذعية الجنينية أو البالغة وخلايا أعضاء الجسم الأخرى. أن أمكانية تكاثر خلايا بيتا المفرزة للانسولين لتكون خلايا منتجة للانسولين جديدة، بالإضافة إلى أمكانية تحويل الخلايا الأخرى في البنكرياس البالغ تعد آلية غير واضحة. كما أن وجود خلايا جذعية خاصة بالبنكرياس لايزال موضوعاً غامضاً. في هذه المراجعة سنوجز المصادر المحتملة لخلايا بيتا المفرزة للانسولين.

Cellular replacement offers the potential of a 'cure' for type 1 diabetes mellitus. Shortage of suitable donors limits widespread implementation of this approach. Recent research has been focused on potential new sources of beta-cells including embryonic and adult stem cells, and other organs cells. The contribution of beta-cell replication to new islet formation, in addition to the potential for transdifferentiation of pancreatic acini and ductal cells in adult human pancreas is not clear. The existence of true stem cells within pancreas remains contentious issue. In this review, we summarized the possible sources of new insulin-secreting cells.

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From the Department of Laboratory and Blood Bank, King Fahad Hofuf Hospital, Hofuf, Kingdom of Saudi Arabia.

Address correspondence and reprint request to: Dr. Hussain R. Al-Turaifi, Department of Laboratory and Blood Bank, King Fahad Hofuf Hospital, Hofuf, Kingdom of Saudi Arabia. Tel. +966 535787700. Fax. +966 (5) 754593. E-mail: hrturaifi@gmail.com

The first islet transplantation experiments were conducted in rats in 1973.[1] In 2000, researchers from Alberta University published their approach to islet transplantation known as the Edmonton protocol which led to a great advance in this field.[2] Several advantages such as less invasive procedure and potential to modulate the immunogenity of the islets after isolation with the hope of grafts without the need for permanent immunosuppression make islet transplantation preferable to pancreas transplantation.[3] On the other hand, each patient requires islets isolated from a median of 2 donors.[4] Thus, human islets donors will never meet the needs of all patients. New sources of ß-cells are urgently required. This review focuses on current strategies to obtain new insulin-producing cells and highlights the main possible sources including stem cells differentiation, ß-cell replication, manipulation of other pancreatic cells, and existence of pancreatic stem cell and transdifferentiation of cells from other organs.

Stem cells. The stem cell field of research is a relatively new area, which has attracted researchers in biomedical studies due to their tremendous expected ability to replace cells destroyed by degenerative diseases. Despite this, a precise definition of stemness is not completely clear. However, most definitions agree that stem cells must have 2 characteristics: firstly, self-renewal ability and secondly, potential to produce end-differentiated functional cells. There are many grades of potency describing the range of end-cells resulting from differentiation of particular stem cells: Totipotent = the ability to differentiate all kind of cells including trophectoderm. Pluripotent = the ability to produce all cells derived from the 3 germ layers, ectoderm, endoderm, and mesoderm. Multipotent = the ability to differentiate cells related to same germ layer. Stem cells are the undifferentiated cells that have the ability to renew and to differentiate multiple lineages. They are categorized chronologically depending on the time of isolation. For instance, embryonic stem cells (ESCs) from embryo, fetal stem cells (FSCs) from fetus, and adult stem cells (ASCs) from a post-natal organ. Moreover, these types are divided into other categories depending on origin such as mouse ESCs, human ESCs, umbilical cord stem cells, bone marrow stem cells, neural stem cells, hepatic stem cells, and so forth. Other terms such as progenitor or precursor are used to indicate that these cells have lower ability to renew themselves and more narrow range of differentiation. Stem cells are identified and characterized by expression

of markers which are not expressed in end-differentiated cells and which may play a role in their renewal ability.

Embryonic stem cells. Embryonic stem cells are pluripotent cells, which have the capacity to generate the 3 germ layers. Embryonic stem cells were first successfully isolated from mouse blastocysts in 1981.[5] Human ESCs are derived from the inner cell mass at 5-7 days using 2 steps in vitro, or from 8 day blastocysts using 3 manipulation steps.[6] It has been noted that insulin expression occurs spontaneously in ESC spheroid structure aggregations.[7] However, understanding of signals and transcriptional networks regulating the development of pancreas has helped in manipulating ESCs to more efficiently generate ß-like cells. Insulin-producing cells have been generated from mouse embryonic cells using other differentiation approaches including transfection with pancreatic transcription factors; treatment with extracellular growth factors; and the use of the cell trapping system in which neomycin was expressed under the control of human insulin gene promoter.[8] These insulin-producing cells have the ability to normalize the blood glucose of streptozotocin-diabetic mice.[7] Likewise, human ESCs spontaneously differentiate to generate insulin-secreting cells which can be enriched by adding beta-fibroblast growth factor (bFGF) to supplement the medium.[9] More efficient endocrine cell neogenesis has been achieved from human ESCs through 5 pancreatic organogenesis defined differentiation stages using extracellular growth factors in vitro. These cells have the capability to synthesize all pancreatic hormones: insulin, glucagon, somatostatin, pancreatic polypeptide, and ghrelin.[10] However, maintained proliferative capacity of ESCs with the risk of development of teratocarcinoma, potential absence of pure defined single hormone positive phenotype and immune system rejection have limited their clinical potential.[11] In addition, ethical issue of generating ESCs from human limits there clinical applications generally.

Adult stem cells. Adult stem cells are found within tissues of adult organisms and are believed to have more restricted differentiation capacity than cells from the germ layer or the organ type that they are isolated. Adult stem cells have been isolated from different tissues including bone marrow, nose, kidney, liver, muscle, skin, brain, the retina and the limbus of the eye.[12] It has been proposed that ASCs may have a role in treating a wide range of diseases such as ischemic heart disease, spinal cord lesions, non-union of fractured bones, Parkinson's disease, Huntington disease in addition to type 1 diabetes mellitus.[13]

Recently, reports have suggested that ASCs can be differentiated to alternative cell fates. For instance, insulin-producing cells have been derived from ectoderm precursors.[14,15] In addition, hematopoietic stem cells may be capable of differentiation to insulin-expressing cells.[16] Other investigators believe that data represent cell fusion without true endocrine cell neogenesis.[17,18] An unifying hypothesis may be that bone marrow stem cells facilitate islet regeneration and/or replication by as yet unknown mechanisms without themselves providing a source of new insulin-secreting cells.[19] Studies have reported that mesenchymal stem cells isolated from rodent bone marrow or adipose tissue can differentiate into insulin-producing cells.[20,21] Another group has reported that transfection of human bone marrow mesenchymal stem cells with a pancreatic and duodenal homeobox factor 1 (PDX1) construct yields insulin-secreting cells,[22] Similar findings demonstrated without genetic manipulation more recently.[23] These resulting cells secreted insulin in a glucose-dependent manner and improved glucose levels on transplantation into nude mice with streptozotocin-induced diabetes.[23] However, absence of a defined protocol for differentiation and inadequate insulin production continue to limit clinical potential of insulin-producing mesenchymal stem cells.[24] In fact, mesodermal stem cells (hematopoietic and mesenchymal) have been reported to generate multiple lineages including liver, brain, lung, gastrointestinal tract and skin, as well as insulin, somatostatin, and glucagon-expressing cells.[21]

Umbilical cord blood stem cells (UCBSCs). Application of protocols used to differentiate mouse ESCs to insulin-secreting stem cells on cells isolated from human umbilical cord blood has been employed to generate islet-like clusters which contain C-peptide and insulin.[25] However, insulin-secreting cells generated from mesenchymal stem cells derived from human umbilical cord blood do not respond physiologically to a glucose challenge limiting therapeutic potential.[26]

Induced pluripotent stem cells (iPSCs). Yamanaka's team was the first group to generate embryonic stem cell-like cells from mouse fibroblast cells by introducing 4 transcription factors namely, OCT-3/4, SOX_2, c-Myc, and Klf4.[27] One year later, the same group successfully reprogrammed human fibroblast cells to pluripotent stem cells using the same factors.[28] Manipulation of these pluripotent stem cells (iPSCs) with growth factors has produced islet-like clusters which release insulin in response to glucose stimulation.[29,30] Generation of iPSCs from a patient's own somatic cells may overcome immunity and ethical issues concerned with ESCs but will not avoid concerns regarding recurrence of the

autoimmune process initially leading to diabetes if new insulin-secreting cells can be successfully derived. Use of viral vectors potentially activating oncogenes in the reprogramming process has led to iPSCs forming teratomas in mice studies precluding their clinical applications.[31] Replacing proto-oncogenic factors and using viral-free vectors may eliminate this concern.[32,34]

Transdifferentiation. In development and maintenance of adult organs, cells may travel long pathways before acquiring their final phenotype. It had been thought that differentiated cells maintained a single distinct phenotype for life. On the contrary, researchers have now demonstrated that cells may dedifferentiate to earlier immature stage.[35] Furthermore, changes in master transcription factor gene expression can lead to the conversion of well differentiated cells to another phenotype in a process called 'transdifferentiation'.[36,37] Theoretically, transdifferentiation can occur between cell types related to each other, at least within the same germ layer of origin, much easier than between cell types from different tissues or germ layers.[38] Li et al[39] has summarized different models of transdifferentiation including conversion of myoblasts to adipocytes; pancreas to liver and vice versa. Indeed, insulin-producing cells have been derived by a transdifferentiation process from several tissues (**Figure 1**).

Liver transdifferentiation. Expression of insulin in liver cells has been reported by several groups. Ferber et al[40] have demonstrated the ability of liver cells to express insulin by introducing the PDX-1 gene via in vivo adenoviral vector delivery with normalization of hyperglycemia in mice with Type 1 diabetes.[41] Zalzman et al[42] have also obtained this result employing human fetal liver cells. Similarly, in vivo transduction of liver cells with adenoviral Beta1/NeuroD vectors in combination with betacellulin treatment induced insulin-producing cells, but without inflammation due to exocrine pancreatic transdifferentiaton in previous studies with PDX1 complexes.[43] More recently, insulin expression has been reported in liver cells transfected with non-viral vectors expressing PDX1 and/or Ngn3.[44] Another group have demonstrated that an immune reaction to the adenoviral back-bone itself may decrease blood glucose level in mice.[45] Others have shown that the common bile duct may be a source of insulin-producing cells.[46,47]

Intestinal transdifferentiation. Expression of one of the important transcription factors required for pancreatic islet embryogenesis, Ngn3,[48] has also been detected in the intestine and stomach.[49] Thus, intestinal cells are a candidate source of ß-cells. Transfection of intestinal epithelium cells with PDX-1 or Isl-1 genes transdifferentiated them to insulin-producing cells.[50,51] However, these cells secreted insulin in a non-glucose-regulated manner. In addition to PDX1, MafA gene overexpression is able to produce insulin from intestinal cells, which reversed diabetic animals.[52]

Neural progenitor cell trandifferentiation. Although neural cells and islet cells are derived from different germ layers, ectoderm and endoderm respectively, hypothalamic neurons in fact express the insulin gene.[53] In addition, mesenchymal cells derived from islets

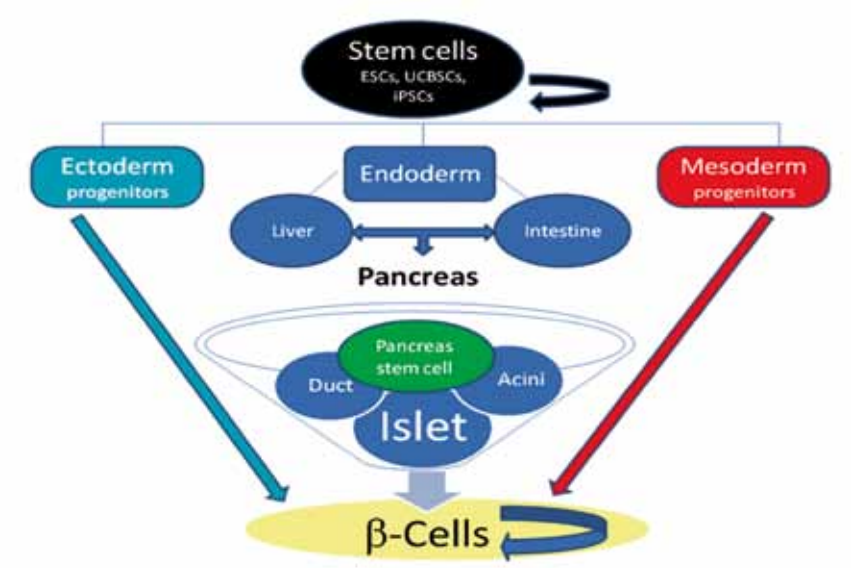

Figure 1 - A simple diagram depicting the possible mechanisms for pancreatic ß-cells generation. ESCs - embryonic stem cells, UCBSCs - umblical cord blood stem cells, iPSCs - induced pluripotent stem cells.

express nestin, a neuroectoderm marker.[54,55] Insulin-producing cells have been developed from a human neurosphere cell line through a 4 stage growth factors manipulation protocol.[15] Despite the fact that the newly formed insulin-secreting cells ameliorate hyperglycemia in diabetic mice, the insulin content in these cells represented only 0.3% of that in human ß-cells.

Derivation of new ß-cells from the pancreas. Both the islet tissue composed of endocrine cells and the exocrine portion of the pancreas composed from acini and ductal epithelial cells most likely are important candidates as a source of new insulin-producing cells.

1) Islet portion. ***Beta-cell replication.*** Dor et al[56] have shown that many new adult pancreatic Beta-cells are formed by self-duplication. They proved this theory in mice by using a transgenic strain in which cre-recombinase driven by the insulin promoter linked to the estrogen-receptor (ER) is activated by translocation to the nucleus by tamoxifen treatment and expressed only in pancreatic ß-cells. Cre recombination leads to fate marking by expression of human placental alkaline phosphatase (HPAP). This is expressed only by insulin-producing cells present at the time of tamoxifen injection and their progeny. At the end of the in vivo pulse-chase experiment, all islets still contained HPAP positive cells.[56] However, this view has been challenged by other researchers who demonstrated that human ß-cells have low replication ability, at least in vitro.[57] Culturing of human and rat islets in different conditions demonstrated that human ß-cells do not have the ability for proliferation in contrast to rat ß-cells. This finding was shown by using proliferation markers such as Ki67 and BrdU with insulin staining.[58]

Alternative mechanisms for new islet-derived ß-cells. Several groups have studied how the islet portion of pancreas might be a source of new ß-cells. Gershengorn et al[59] suggested that human islet-derived cells are generated by epithelial-to-mesenchymal transition (EMT) and, after expansion, they re-differentiated to insulin-expressing epithelial cells on incubation in serum free medium.[59] Likewise, Ouziel-Yahalom et al[60] isolated islets and cultured them in CRML medium. These cells dedifferentiated on passaging to form cells termed proliferating human islet-derived cells (PHID) where the ß-cells markers, insulin, PDX-1, beta2, Nkx2.2, Glut2 and Pax6 decreased significantly after passage 3. Redifferentiation of the cells was achieved by betacellulin, activin-A, and exendin-4 treatment in vitro.[60] Furthermore, Gao et al[61] sorted human islets cells by using MiniMACS (magnetic cell separation system) with monoclonal anti-NCAM to eliminate endocrine cells. Thus, they demonstrated that human islet cells could de-differentiate into a duct-like phenotype and then re-differentiate into islet cells, as opposed to direct replication of ß-cells.[61] However, the EMT hypothesis has been opposed by other groups by using Cre-recombinase labelling of insulin and PDX1 promoters in transgenic mice. The fibroblast-like cells generated from the islet culture of these transgenic mice did not express ß-cell specific lineage labels.[62] By contrast, similar lineage-tracing technology applied in human islets has most recently confirmed that ß-cells take part in the in vitro EMT process.[63] These findings underline the potential for important species differences between rodents and humans.

2) Exocrine portion. ***Acinar cells.*** Acinar cells comprise 95% of the exocrine pancreas. They secrete a variety of digestive enzymes such as proteases, lipases and amylases. Mashima et al.[64] showed the ability of the rat acinar cell line (AR42J) to convert to insulin-producing cells by treatment with hepatocyte growth factor (HGF). This was enhanced by activin-A, a transforming growth factor.[65] Treating AR42J cells with activin-A alone converted them to neuron like cells which express insulin at the mRNA level only. Whereas, 10% of these cells were transdifferentiated to insulin-secreting cells by betacellulin, a member of the epidermal growth factor, in addition to activin-A.[65] Several transcription factors are changed during the transdifferentiation process; however, activin-A regulates mainly the expression of neurogenin3.[66] Smad proteins, PAX4, and others are also involved.[67-70] Palgi et al[71] were unable to confirm the capacity of AR42J to transdifferentiate to insulin-producing cells, even though, they transfected the AR42J-B13 sub-clone cells with the full length cDNAs of isl-1, Nkx6.1, Nkx2.2 and pdx-1 under the control of the CMV promoter.[71] others demonstrated that AR42J lack the ability to store or convert proinsulin to insulin after growth factors treatment.[72] In vivo transduction of the pancreas in mice with a vector containing Ngn3, PDX1 and MafA cDNAs converted exocrine cells (acini) to insulin-producing cells resembling islet ß-cells structurally, which normalized blood glucose levels in diabetic mice.[73]

Ductal cells. Ductal cells are simple columnar epithelial cells that secrete bicarbonate and water. Several lines of evidences support the suggestion that new ß-cells are derived from the ductal compartment. For example, during embryogenesis, islets develop from epithelial precursor cells.[74] This is mediated by extracellular signals and many of the transcription factors that are expressed in ductal epithelial cells are required for endocrine development. It appears that the epithelial stage may be an intermediate level in the normal development

process of insulin-producing cells from islet precursor cells.[59] The Bonner-Weir group are confident that the pancreatic ductal epithelium serves as a 'potential pool' of pancreatic stem cells.[75] They have cultured cells in vitro from a duct cell-rich fraction of human pancreas tissue separated by the Ficoll gradient method. These cells express cytokeratin-19 (a specific pancreatic duct cell marker) and PDX-1, but not insulin. After that, cells were cultivated by overlaying the cells with Matrigel, an extracellular matrix, forming duct-derived clusters that expressed insulin in addition to epithelial makers indicating incomplete differentiation. Moreover, these cells secrete insulin in response to glucose stimulation.[76] Similar results were obtained by treating theses cells with GLP1.[77] Zhao et al[78] separated human exocrine cells and treated them with streptozotocin and G418 to remove ß-cells and fibroblasts, respectively. Remaining cells were transdifferentiated to insulin-expressing cells by culturing them in serum free medium with GLP1 for 3 hours and treating them later with ABNG cocktail (Activin-A, betacellulin, nicotinamide and glucose). Insulin expression was significantly enhanced by transfection of the cells with a PDX1 gene. Insulin protein remained undetectable in vitro. When cells were transplanted into mice with streptozotocin-induced diabetes, however, they reversed hyperglycemia.[78] Another group has reported expression of PDX1 and nestin in dissected human pancreatic ducts with a similar phenotype to bone marrow-derived mesenchymal stem cells. These cells appear to secrete insulin when treated with Matrigel.[79]

Transfection of duct cells with the transcription factors PDX1, Ngn3, NeuroD1 and Pax4 generated insulin-producing cells with higher efficiency than with NeuroD1 alone.[80] Transgenic mice expressing Cre-recombinase under the control of carbonic anhydrase II (CAII) that is a marker of pancreatic ductal epithelial cells showed that CAII-expressing cells differentiated to acinar and endocrine cells after injury. This finding proved that ductal cells can (at least in mice) participate in neogenesis of ß-cells in vivo after birth.[81]

The rat pancreatic ductal epithelial cell line (ARIP) transdifferentiated to insulin-producing cells on treatment with GLP1, whereas the human pancreatic ductal epithelial cell line (PANC1) did not transdifferentiate on GLP1 treatment alone but only when transfected with PDX1.[82] On the contrary, Hardikar et al[83] reported that serum free medium alone could transdifferentiate PANC1 to insulin-producing cells.

Pancreatic stem/progenitor cells. Embryonic development of pancreas has shown that end-differentiated pancreatic cells are derived from stem/progenitor cells through sequential expression of specific transcription factors. Presence of these cells after birth in pancreas is not well documented. Several studies have set out to identify pancreatic stem/progenitor cells by tracking putative stem cell markers in pancreas.

Nestin filament, a neural stem cell marker, was detected within adult pancreas islet cells which neither express endocrine markers (insulin, glucagon, somatostatin and PP) nor ductal marker (CK19). Nestin-positive cells are able to generate liver and pancreas lineages in vitro.[84] During embryogensis of rat pancreas, nestin has been identified in immature duct, exocrine and endocrine cells which express c-Kit.[85] Fetal human pancreas nestin positive cells express OCT4 and Ngn3.[45] Culturing these cells in vitro converted them to a mesenchymal stem cell phenotype.[86]

In another study, CD133, a hematopoietic stem cell marker, was utilized to isolate CD133-expressing cells from adult pancreas using flow cytometer sorting. These cells exhibited an undifferentiated ductal phenotype which expressed c-Met. In vivo, these cells could generate all pancreatic lineages including insulin secreting cells.[87] Another group found that CD133 positive cell population isolated from human pancreas expressed other stem cell markers ABCG2, OCT4, Nanog and Rex1 as well as Ngn3.[88] A similar phenotype was identified earlier in a cell population isolated from non-endocrine pancreatic cells by magnetic activated cell sorting using CXCR4 markers.[89] By contrast, Gao group detected OCT4 positive cells in human adult pancreas within the duct compartment and coexpressing SOX2. However, these cells were distinct from CD133, CD34, insulin, and CK19 positive cells.[90]

Despite, the important advances of generation of insulin-producing cells from different cells, the tumorgenity, immunoreactivity, low efficacy of newly insulin-producing cells, and absence of robust and standard protocol that met general medical product (GMP) guidelines limit their clinical application. However, several clinical studies indicate the safety and effectiveness of using adult stem cell, including mesenchymal, hematopoietic, umbilical cord blood derived stem cells to treat diabetes and its complication.[91-94]

Recently, the International Society for Stem Cell Research (ISSCR) published a handbook that contains a list of guidelines to protect doctors, patients, and their families from unreliable commercial private stem cell clinics.[95]

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