

Adult Stem Cells and Diabetes

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INTRODUCTION

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 [1]. 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 [2]. Recently, reports have suggested that ASCs can be differentiated to alternative cell fates. For instance, insulin-producing cells have been derived from ectoderm precursors [3,4]. Neural stem cell showed an ability to expand *in vitro* and expressing pro-insulin mRNA. The result cells are sensitive to glucose concentration and respond to sulphonylurea. These cells secrete insulin C-peptide in response to glucose concentration, when they were transplanted in immune compromised rats without detectable tumor formation.

BONE –MARROW STEM CELLS

Hematopoietic stem cells may be capable of differentiation to insulin-expressing cells [5]. Bone-marrow cells express islets markers such as insulin and GLUT2 *in vivo*, when they transplanted in irradiated mice. This was confirmed by lineage tracing technique. However, Other investigators believe that data represent cell fusion without true endocrine cell neogenesis [6,7].

They suggested that injury pancreatic cells recruit bone marrow derived cells without expressing of insulin from these recruited cells. A 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 [8].

Studies have reported that mesenchymal stem cells isolated from rodent bone marrow or adipose tissue can differentiate into insulin-producing cells by using serum free medium that include nicotinamide and beta-mercap to ethanol. After conditional culture, pancreatic markers expressed while stem cell markers down-regulated. These cells can reduce glucose level in rat diabetic model [9,10].

Another group has reported that transfection of human bone marrow mesenchymal stem cells with a Pancreatic Andduodenal Homeobox Factor 1 (**PDX1**) construct yields insulin-secreting cells [11]. Similar findings demonstrated without genetic manipulation more recently. The cells expressed pancreatic markers in both mRNA and protein level [12]. Also, these resulting cells secreted insulin in a glucose-dependent manner and improved glucose levels on transplantation into nude mice with strep to zotocin-induced diabetes [12]. However, absence of a defined protocol for differentiation and inadequate insulin production continue to limit clinical potential of insulin-producing mesenchymal stem cells [13]. 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 [10].

UMBILICAL CORD BLOOD STEM CELLS (UCBSCS)

CD133+ and CD34+ cells were isolated from human umbilical cord blood and expanded in culture. Application of protocols used to differentiate mouse ESCs to insulin-secreting stem cells on these cells isolated from human umbilical cord blood has been employed to generate islet-like clusters which contain C-peptide and insulin [14]. Insulin-secreting cells generated from mesenchymal stem cells derived from human umbilical cord blood after treating tem with a combination of high-glucose, retinoic acid, nicotinamide, epidermal growth factor, and exendin-4 for 15-days which induce the cells to expressed pancreatic β -cell markers, including insulin, glucagon, Glut-2, PDX1, Pax4, and Ngn3. However, do not respond physiologically to a glucose challenge limiting therapeutic potential [15].

INDUCED PLURIPOTENT STEM CELLS (IPSCS)

Yamanaka's team was the first group to generate embryonic stem cell-like cells called Induced Pluripotent Stem Cells (**iPSCs**) from mouse fibroblast cells by introducing 4 transcription factors namely, OCT-3/4, SOX2, c-Myc, and Klf4 [16]. These cells showed embryonic stem cell morphology, growth pattern and expressed gene markers. In addition, they form teratomas after transplantation into nude mice. iPSCs could develop complete mouse when they injected into blastocysts.

One year later, the same group successfully reprogrammed human fibroblast cells to pluripotent stem cells using the same factors [17]. Reprogrammed cells express embryonic stem cell markers and differentiate into cell types of three germ layers *in vitro*. Manipulation of these Pluripotent Stem Cells (**iPSCs**) with growth factors has produced islet-like clusters which express islets markers including c-peptide, glucagon and release insulin in response to glucose stimulation [18,19]. Application of Good Manufacturing Practice (**GMP**) by using free-serum medium and culturing IPSCs without feeder layer cells are providing important steps for clinical application. Also, 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 on co genes in the reprogramming process has led to iPSCs forming teratomas in mice studies precluding their clinical applications [20]. Replacing proto-oncogenic factors and using viral-free vectors, such as plasmid containing the Complementary DNAs (**cdNAs**) of Oct3/4, Sox2, and Klf4 and the other containing the c-Myc, cDNA, may eliminate this concern. Moreover, studies have showed that manipulating of culture condition including oxygen concentration and supplement with growth factors, without genetic manipulation, induce endogenous transcription and translation of embryonic markers such as OCT4, SOX2 and NANOG. However, the efficiency of such protocol was limited [21-23].

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. 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'. Very known example of trans differentiation is conversion of *Drosophila* leg to wing by ectopic expression of Vestigial factor [24,25].

However, the role of cell division in transdifferentiation was controversial claiming that DNA replication, which is the key step in division, is not required for differentiation. Also, dilution of transcription factors, important factors in cellular specification, is accompanied with cellular division. Moreover, division of differentiated cells produces identical differentiated daughter cells in dynamic cells [26]. Somatic Nuclear Transfer (**SNT**) techniques demonstrate the potency of cells of re-programming. Similarly called Induced Pluripotent Stem Cells (**iPSCs**) approved reprogramming ability of cells reversing the developmental process from adult differentiated cells to pluripotent cells.

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.

Reprogramming technology was used to treat animal models. For example, ear hair cells formed from different cell type was used successfully to treat deaf animal [27].

Li et al. [28] 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).

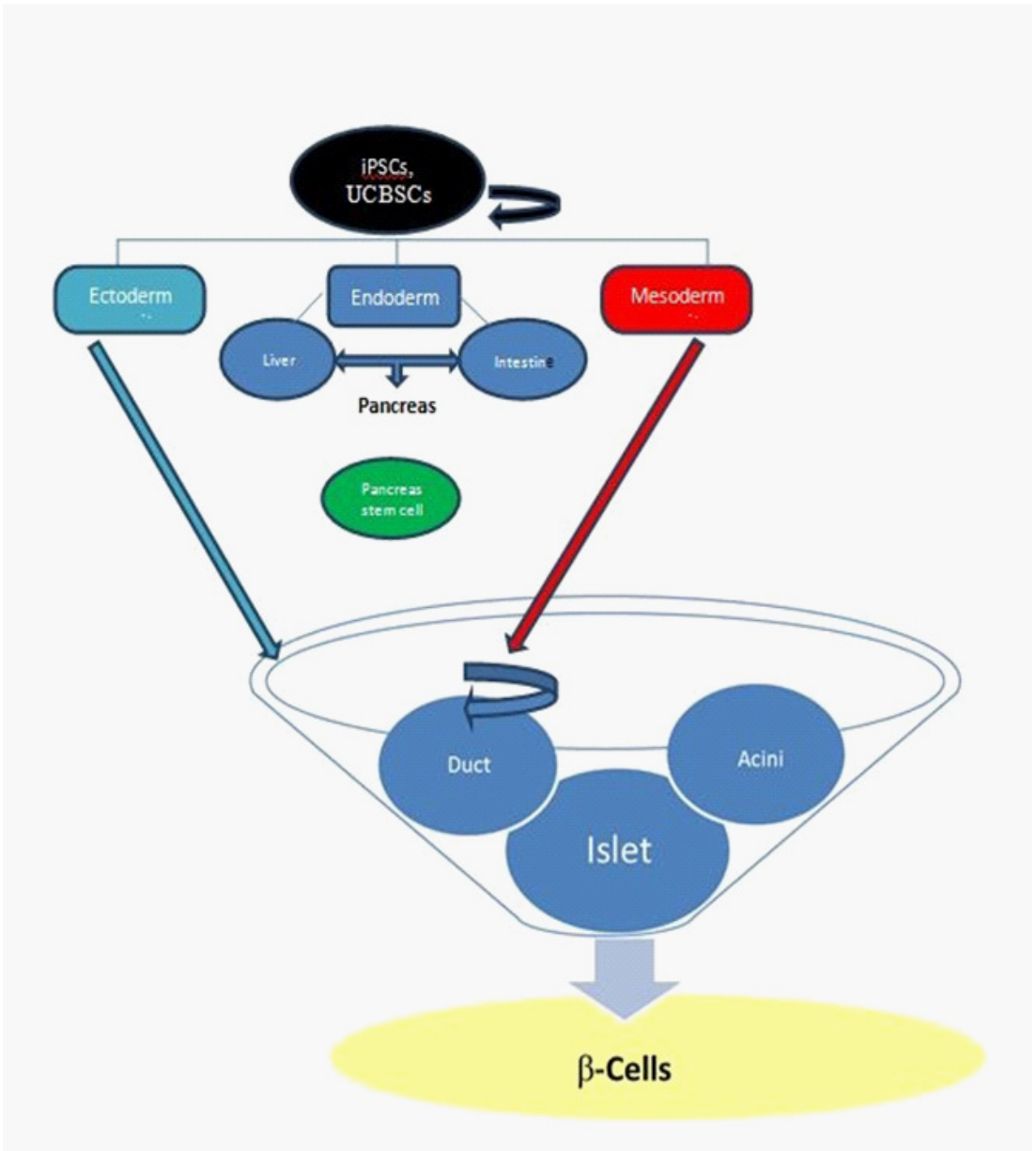


Figure 1: A simple diagram depicting the possible mechanisms for pancreatic β - cells generation. **UCBSCs:** Umbilical cord blood stem cells, **iPSCs:** Induced pluripotent stem cells.

LIVER TRANSDIFFERENTIATION

Expression of insulin in liver cells has been reported by several groups. Ferber et al [29] 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.

Ectopic expression of PDX-1 was reported in Non-Obese Diabetic (**CAD-NOD**) mice which promote production of insulin and decreasing glucose level of mice. Also, expression of PDX-1 associated with ameliorating of immunity which may modulate the autoimmune attack of trans-differentiated liver –pancreas cells [30].

Zalzman et al [31]. have also obtained this result employing human fetal liver cells. Transfection of human fetal live cells with PDX-1 gene induces production and storage of insulin which secreted in glucose response manner. Transplantation of transdifferentiated human fetal cells in diabetic mice normalizes glucose concentration. However, fulminant hepatitis was reported by transdifferentiation using PDX-1 factor.

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 transdifferentiation in previous studies with PDX1 complexes. Other islet markers such as glucagon, pancreatic polypeptide and somatostatin, and cell-specific glucokinase and sulfonylurea receptor were detected [32].

More recently, insulin expression has been reported in liver cells transfected with non-viral vectors (nucleofection) expressing PDX1 and/or Ngn3 [33].

Another group have demonstrated that an immune reaction to the adenoviral back-bone itself may decrease blood glucose level in mice [34]. Others have shown that the common bile duct may be a source of insulin-producing cells, they demonstrated that bile duct epithelium naturally develop β cells that contain insulin in granules secreted in response to glucose stimulation. Other islet markers such as glucagon, pancreatic polypeptide and somatostatin are also detected. Exocrine cells did not observe within bile duct. Expanded intra hepatic biliary epithelial cells *in vitro* could be transdifferentiated to insulin- producing cells by transfection with PDX-1 and NeuroD1. Formed cell express other β cells genes including GLUT2 and prohormone convertase 1 and 2 [35,36].

INTESTINAL TRANSDIFFERENTIATION

Failures of Ngn3 expression lose the Expression of Isl1, Pax4, Pax6, and NeuroD which lead to fail to generate any pancreatic endocrine cells [37]. Expression of one of the important transcription factors required for pancreatic islet embryogenesis, Ngn3, has also been detected in the intestine and stomach [38]. Thus, intestinal cells are a candidate source of β -cells. Transfection of intestinal epithelium cells with PDX-1 or Isl-1 genes transdifferentiated them to

insulin-producing cells. Direction of stomach cells to produce insulin lead to impair development of gastrin- (G cells) and somatostatin (D cells) -secreting cells. serotonin- (enterochromaffin EC cells), histamine- (enterochromaffin-like ECL cells) and ghrelin (X/A cells) -expressing cells are still developed [39].

These cells expressed amylin, glucokinase, and Nkx6.1, and develop insulin storage gradually [40]. However, these cells secreted insulin in a non-glucose-regulated manner. In addition to PDX1, Maf A gene over expression is able to produce insulin from intestinal cells, which reversed diabetic animals [41].

NEURAL PROGENITOR CELL TRANSDIFFERENTIATION

Although neural cells and islet cells are derived from different germ layers, ectoderm and endoderm respectively, hypothalamic neurons in fact express the insulin gene [42].

The action of insulin in brain cells was not clear. Indeed, Insulin-related peptides are synthesized in brain and serve as neurotransmitters or neuro modulators. Insulin secretion in brain cells is regulated by glucose resembling pancreas manner. Also, serotonin regulate neural insulin secretion directly in the hypothalamus.

On other hand, mesenchymal cells derived from islets Express desmin, vimentin, glial fibrillary acidic protein and nestin which is a neuroectoderm marker. [43,44].

Scientists produce insulin from a human neurosphere cell line through a 4 stage growth factors manipulation protocol. 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 β -cells. These cells are glucose-responsive insulin-producing cells and express islet regulatory genes. Moreover, they did not form detectable tumors [44].

Derivation of New β -Cells from The Pancreas

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

Islet portion

Islet of Langerhans represents approximately 1-2% (around one million cells in healthy individual) of pancreatic tissue. Islets composed of different cells that secrete hormones including beta (β)-cells secrete insulin, alpha (α)-cells secrete glucagon, delta (δ)-cells secrete somatostatin, PP-cells secrete pancreatic polypeptide and ϵ - cells secrete ghrelin.

Beta-cell replication

Dor et al [45] 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 Tran's location to the nucleus by tamoxifen treatment and expressed only in pancreatic β -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 which indicate all new insulin-producing cells generated from existing β -cells and not from HPAP negative cells including other islet cells, epithelial ductal cells, exocrine cells or stem cells. However, this view has been challenged by other researchers who demonstrated that human β -cells have low replication ability, at least *in vitro* [46].

β -cells from human and rat islets were sorted by using their high contents of zinc via Newport Green stain which exclude ductal and dead cells. Culturing of human and rat islets in different conditions demonstrated that human β -cells do not have the ability for proliferation in contrast to rat β -cells. Human Growth Hormone (**hGH**) and the glucagon-like peptide-1 analogue liraglutide are the best growth factor that enhanced proliferation of rat beta cells. This finding was shown by using proliferation markers such as Ki67 and Brd U with insulin staining [47].

Alternative mechanisms for new islet-derived β -cells

Several groups have studied how the islet portion of pancreas might be a source of new β -cells. Gershengorn et al [48] suggested that human islet-derived cells, which have fibroblast growth pattern *in vitro* and do not express hormones markers, 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 [48]

Likewise, Ouziel-Yahalom et al [49] 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 β -cells markers, insulin, PDX-1, beta2, Nkx2.2, Glut2 and Pax6 decreased significantly after passage 3. Re-differentiation of the cells was achieved by beta cellul in, activin-A, and exendin-4 treatment *in vitro*. Beta cellul in re-differentiate 43% of PHID to insulin producing cells. Furthermore, Gao et al sorted human islets cells by using Mini MACS (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 β -cells [50].

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 Trans genic mice did not express β -cell specific lineage labels [51].

By contrast, similar lineage-tracing technology applied inhuman islets has most recently confirmed that β -cells take part in the *in vitro* EMT process. [52] These findings underline the potential for important species differences between rodents and humans.

Recently, Russ et al proof that pancreatic progenitor differentiation protocols promote precocious endocrine commitment, ultimately resulting in the generation of non-functional poly hormonal cells by using retinoic acid followed by combined EGF/KGF efficiently which generate glucose-responsive beta-like cells *in vitro* that exhibit key features of bona fide human beta cells, remain functional after short-term transplantation, and reduce blood glucose levels in diabetic mice [53].

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. Several factors regulate the function of acinar cells including food intake, hormones and neuro-transmitters. Both endocrine and exocrine cells derive from a common pool of progenitors present in the foregut endoderm. Genetic and growth factors directed the pancreatic progenitors to form exocrine or endocrine cells. Acinar cell could be differentiated into an embryonic progenitor-like phenotype in suspension culture. [54] Mashima et al. [55] 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. Treating AR42J cells with activin-A alone converted them to neuron like cells which express pancreatic poly peptide at the mRNA level only and not for insulin or glucagon. whereas, 10% of these cells were transdifferentiated to insulin-secreting cells by beta cellulin, a member of the epidermal growth factor, in addition to activin-A. Insulin was secreted from these cells in response to concentration of potassium, tolbutamide, cabachol and glucagon-like peptide-1 [56]. Several transcription factors are changed during the transdifferentiation process; however, activin-A regulates mainly the expression of neurogenin3 and PAX4 [57]. However, introduction or down regulation of PAX4 did not induce morphological or expression change of acinar cell line which indicate the key role of neurogenin3. Smad proteins, PAX4, and others are also involved [58-61]. 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 [62]. Palgi work confirm that *Nkx2.2* independently with growth factors can regulate the conversion of AR42J to polypeptide expressing cells. Others demonstrated that AR42J lack the ability to store, the cells could not or convert pro insulin to insulin after glucagon-like peptide- and culturing on matrigel coated medium. *Glu2*, *PDX1* and insulin mRNA were expressed, and pro insulin synthesis and secretion were confirmed by immunochemistry and enzyme-linked techniques. However, the cells lack convertase enzymes, which cleave pro insulin to C-peptide and insulin [63]. *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 β -cells structurally, which normalized blood glucose levels in diabetic mice [64].

Ductal cells

Ductal cells are simple columnar epithelial cells that secrete bicarbonate and water. Several lines of evidence support the suggestion that new β -cells are derived from the ductal compartment. For example, during embryogenesis, islets develop from epithelial precursor cells. This is mediated by a cascade of extra-cellular mesodermic signals followed by signals from mesenchyme, pancreatic epithelium and many of the transcription factors that are expressed in ductal epithelial cells are required for endocrine development. It appears that the epithelial stage maybe an intermediate level in the normal development. Possible sources of β -cells process of insulin-producing cells from islet precursor cells [48,65].

The Bonner-Weir groups are confident that the pancreatic ductal epithelium serves as a 'potential pool' of pancreatic stem cells [66]. 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 with three-dimensional structure of ductal cysts. Cells were increased 10–15 fold within 3–4 weeks culture and expressed insulin in addition to epithelial markers indicating incomplete differentiation. Moreover, these cells secrete insulin in response to glucose stimulation. The insulin secretion increased 23 fold when the medium glucose elevated from 5mM to 20 mM [67].

Similar results were obtained by treating these cells with GLP1 and GLP1 agonist (exendin-4). Both pancreatic β -cells and duct cells contain GLP1 receptors [68]. Zhao et al [69] separated human exocrine cells and treated them with streptozotocin and G418 to remove β -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, beta cellulose, 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 [69].

This study was claimed that firstly treatment with streptozotocin did not clear all β -cells, 90% of treated cells only express epithelial and mesenchymal markers, cytokeratin-19 and vimentin, respectively. Secondly, transplanted cells may contain residual β -cells or de-differentiated islet cells which could be replicated or re-differentiated *in vivo*.

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. Previous argument applied to this study as well [70].

Cell-lineage technique was used to directly prove that ductal cells could be a putative source of insulin-producing cells after birth and candidate cells for replacement therapy of diabetes. 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 β -cells *in vivo* after birth.

To examine which embryonic transcription factors induce effective trans-differentiation of adult mouse and human ductal to. Even though, NeuroD1 is the most effective factor to produce insulin-expressing cells from ductal cells, transfection of these cells with the transcription factors PDX1, Ngn3, NeuroD1 and Pax4 generated insulin-producing cells with higher efficiency than with NeuroD1 [71,72].

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. The result cells did not show phenotypic change. However, they synthesis insulin and secrete it depending on glucose concentration in media [73].

On the contrary, Hardikar et al [74] reported that serum free medium alone could induce PANC1 to aggregate by secreting Fibroblast Growth Factor (**FGF**) 2 that work as a paracrine chemo attractant, then transdifferentiate to insulin-producing cells. Our work could not repeat these experiments by wide range of GLP1 concentration with or without GLP1 transfection. Other growth factors, transcription factors and culturing techniques were used. Unfortunately they failed to induce human pancreatic ductal epithelial cell line to insulin-producing cells (unpublished work).

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.

Nest in 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). Nest in-positive cells are located within large, small, and centrolobular ducts of the rat pancreas. Nest in-positive cells are able to expanded, generate liver and pancreas lineages *in vitro* transplanted into the diabetic mouse [75]. During embryogenesis of rat pancreas, nestin has been identified in immature duct, exocrine and endocrine cells which express c-Kit. This population of cells, c-Kit and nestin expressing cells showed increased immune positive for PDX1 and Glut2, which are mature beta-cell markers, in postnatal life. This findind suggest that these population cells represent endocrine precursor cells in postnatal pancreatic development in the rat [76].

Fetal human pancreas nestin positive cells express OCT4 and Ngn3. [34] Separating of these cells using collagenase digestion and culturing them *in vitro* with serum-free media supplemented with the cocktail of growth factors converted them to a mesenchymal stem cell phenotype which express mRNA of insulin, glucagon and pancreatic-duodenal homeo box gene-1, whereas the expression of nestin and neurogenin 3 disappeared. Moreover, insulin proteins were detected intracellularly [77].

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 [78]. 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 [79]. A similar phenotype was identified earlier in a cell population isolated from non-endocrine pancreatic cells by magnetic activated cell sorting using CXCR4 markers. This population, CXCR4-positive pancreatic cells, express markers of pancreatic endocrine progenitors (neurogenin-3, nestin) and markers of pluripotent stem cells (Oct-4, Nanog, ABCG2, CD133, CD117) [80].

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 [81]. Newcastle diabetes group isolated Islet Survivor Cells (**ISCs**) from islet-enriched fraction which was separated from the retrieved organ by digestion and density gradient centrifugation. These cells were characterized by RT-PCR, immune fluorescence staining, FACS, western blot and transfection studies with an OCT4 promoter-driven reporter. Nuclear expression of the pluripotency-associated stem cell marker complex OCT4/SOX2/NANOG was confirmed in ISCs. In pancreatic tissue, cell expressing OCT4, SOX2 and NANOG markers were localized within islet and exocrine portion. They are distinct from insulin expressing cells or ductal cells [82]. Moreover, small embryonic-like stem cells were detected within pancreatic human tissue (unpublished work).

Intravenous infusions of human Placenta-Derived MSC (**PD-MSC**) patients with Type 1 Diabetes (**T1D**) increase levels of insulin and C-peptide without fever, chills, liver damage and other side effects, where's renal function and cardiac function were improved after infusion [83].

Also, Stem Cell Educator therapy by human Cord Blood-Derived Multipotent Stem Cells (**CB-SCs**) markedly improve C-peptide levels, reduce the median glycated Hemoglobin A1C (**HbA1C**) values, and decrease the median daily dose of insulin in patients with Type 1 Diabetes (**T1D**) resulting clinical improvement in patient status [84]. Similarly, Autologous Hematopoietic Stem Cell Transplantation (**AHSCT**) preserved β -cell function in Chinese patients with new onset of Type 1 Diabetes (**T1D**) and diabetic ketoacidosis [85].

CONCLUSION

Despite, the important advances of generation of insulin-producing cells from different cells, the tumorigenicity, immune reactivity, 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 include in gmesenchymal, hematopoietic, umbilical cord blood derived stem cells to treat diabetes and its complication.

References

1. Tarnowski M, Sieron AL. Adult stem cells and their ability to differentiate. *Med Sci Monit.* 2006; 12: 154-163.
2. Tuch BE. Stem cells--a clinical update. *Aust Fam Physician.* 2006; 35: 719-721.
3. Burns CJ, Minger SL, Hall S, Milne H, Ramracheya RD. The in vitro differentiation of rat neural stem cells into an insulin-expressing phenotype. *Biochem Biophys Res Commun.* 2005; 326: 570-577.
4. Hori Y, Gu X, Xie X, Kim SK. Differentiation of insulin-producing cells from human neural progenitor cells. *PLoS Med.* 2005; 2: 103.
5. Ianus A, Holz GG, Theise ND, Hussain MA. In vivo derivation of glucose-competent pancreatic endocrine cells from bonemarrow without evidence of cell fusion. *J Clin Invest* 2003;111: 843-850.
6. Choi JB, Uchino H, Azuma K, Iwashita N, Tanaka Y. Little evidence of transdifferentiation of bone marrow-derived cells into pancreatic beta cells. *Diabetologia.* 2003; 46: 1366-1374.
7. Lechner A, Yang YG, Blacken RA, Wang L, Nolan AL. No evidence for significant transdifferentiation of bone marrow into pancreatic beta-cells in vivo. *Diabetes.* 2004; 53: 616-623.
8. Mathews V, Hanson PT, Ford E, Fujita J, Polonsky KS. Recruitment of bone marrow-derived endothelial cells to sites of pancreatic beta-cell injury. *Diabetes.* 2004; 53: 91-98.
9. Chen LB, Jiang XB, Yang L. Differentiation of rat marrow mesenchymal stem cells into pancreatic islet beta-cells. *World J Gastroenterol.* 2004; 10: 3016-3020.
10. Timper K, Seboek D, Eberhardt M, Linscheid P, Christ-Crain M. Human adipose tissue-derived mesenchymal stem cells differentiate into insulin, somatostatin and glucagon expressing cells. *Biochem Biophys Res Commun.* 2006; 341: 1135-1140.
11. Sun Y, Chen L, Hou XG, Hou WK, Dong JJ. Differentiation of bone marrow-derived mesenchymal stem cells from diabetic patients into insulin-producing cells in vitro. *Chin Med J (Engl).* 2007; 120: 771-776.
12. Xie QP, Huang H, Xu B, Dong X, Gao SL. Human bone marrow mesenchymal stem cells differentiate into insulin-producing cells upon microenvironmental manipulation in vitro. *Differentiation.* 2009; 77: 483-491.
13. Zulewski H. Stem cells with potential to generate insulin-producing cells in man. *Swiss Med Wkly.* 2007; 155: 60-67.
14. Denner L, Bodenbun Y, Zhao JG, Howe M, Cappo J. Directed engineering of umbilical cord blood stem cells to produce C-peptide and insulin. *Cell Prolif.* 2007; 40: 367-380.
15. Gao F, Wu DQ, Hu YH, Jin GX, Li GD. In vitro cultivation of islet-like cell clusters from human umbilical cord blood-derived mesenchymal stem cells. *Transl Res.* 2008; 151: 293-302.
16. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell.* 2006; 126: 663-676.
17. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; 131: 861-872.
18. Tateishi K, He J, Taranova O, Liang G, D'Alessio AC. Generation of insulin-secreting islet-like clusters from human skin fibroblasts. *J Biol Chem.* 2008; 283: 31601-31607.
19. Zhang D, Jiang W, Liu M, Sui X, Yin X. Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells. *Cell Res.* 2009; 19: 429-438.
20. Park IH, Zhao R, West JA, Yabuuchi A, Huo H. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature.* 2008; 451: 141-146.

21. Okita K, Nakagawa M, Hyunjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. *Science*. 2008; 322: 949-953.
22. Kaji K, Norrby K, Paca A, Mileikovsky M, Mohseni P. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. *Nature*. 2009; 458: 771-775.
23. Page RL, Ambady S, Holmes WF, Vilner L, Kole D. Induction of stem cell gene expression in adult human fibroblasts without transgenes. *Cloning Stem Cells*. 2009; 11: 417-426.
24. Beresford WA. Direct transdifferentiation: can cells change their phenotype without dividing? *Cell Differ Dev*. 1990; 29: 81-93.
25. Slack JM, Tosh D. Transdifferentiation and metaplasia--switching cell types. *Curr Opin Genet Dev*. 2001; 11: 581-586.
26. Tosh D, Slack JM. How cells change their phenotype. *Nat Rev Mol Cell Biol*. 2002; 3: 187-194.
27. Zhou Q, Melton DA. Extreme makeover: converting one cell into another. *Cell Stem Cell*. 2008; 3: 382-388.
28. Li WC, Yu WY, Quinlan JM, Burke ZD, Tosh D. The molecular basis of transdifferentiation. *J Cell Mol Med*. 2005; 9: 569-582.
29. Ferber S, Halkin A, Cohen H, Ber I, Einav Y. Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. *Nat Med*. 2000; 6: 568-572.
30. Shternhall-Ron K, Quintana FJ, Perl S, Meivar-Levy I, Barshack I. Ectopic PDX-1 expression in liver ameliorates type 1 diabetes. *J Autoimmun*. 2007; 28: 134-142.
31. Zalzman M, Gupta S, Giri RK, Berkovich I, Sappal BS. Reversal of hyperglycemia in mice by using human expandable insulin-producing cells differentiated from fetal liver progenitor cells. *Proc Natl Acad Sci U S A*. 2003; 100: 7253-7258.
32. Kojima H, Fujimiyama M, Matsumura K, Younan P, Imaeda H. NeuroD-beta-cellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice. *Nat Med*. 2003; 9: 596-603.
33. Motoyama H, Ogawa S, Kubo A, Miwa S, Nakayama J. In vitro reprogramming of adult hepatocytes into insulin-producing cells without viral vectors. *Biochem Biophys Res Commun*. 2009; 385: 123-128.
34. Wang H, Wang S, Hu J, Kong Y, Chen S. Oct4 is expressed in Nestin-positive cells as a marker for pancreatic endocrine progenitor. *Histochem Cell Biol*. 2009; 131: 553-563.
35. Dutton JR, Chillingworth NL, Eberhard D, Brannon CR, Hornsey MA. Beta cells occur naturally in extrahepatic bile ducts of mice. *J Cell Sci*. 2007; 120: 239-245.
36. Nagaya M, Katsuta H, Kaneto H, Bonner-Weir S, Weir GC. Adult mouse intrahepatic biliary epithelial cells induced in vitro become insulin-producing cells. *J Endocrinol*. 2009; 201:37-47.
37. Gradwohl G, Dierich A, LeMeur M, Guillemot F. neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. *Proc Natl Acad Sci U S A*. 2000; 97: 1607-1611.
38. Jenny M, Uhl C, Roche C, Duluc I, Guillermin V. Neurogenin3 is differentially required for endocrine cell fate specification in the intestinal and gastric epithelium. *EMBO J*. 2002; 21: 6338-6347.
39. Kojima H, Nakamura T, Fujita Y, Kishi A, Fujimiyama M. Combined expression of pancreatic duodenal homeobox 1 and islet factor 1 induces immature enterocytes to produce insulin. *Diabetes*. 2002; 51: 1398-1408.
40. Yoshida S, Kajimoto Y, Yasuda T, Watada H, Fujitani Y. PDX-1 induces differentiation of intestinal epithelioid IEC-6 into insulin-producing cells. *Diabetes*. 2002; 51: 2505-2513.
41. Nomura S, Nakamura T, Hashimoto T, Nishio Y, Maegawa H. MafA differentiates rat intestinal cells into insulin-producing cells. *Biochem Biophys Res Commun*. 2006; 349: 136-143.
42. Gerozissis K1. Brain insulin: regulation, mechanisms of action and functions. *Cell Mol Neurobiol*. 2003; 23: 1-25.
43. Lardon J, Rooman I, Bouwens L. Nestin expression in pancreatic stellate cells and angiogenic endothelial cells. *Histochem Cell Biol*. 2002; 117: 535-540.
44. Selander L, Edlund H. Nestin is expressed in mesenchymal and not epithelial cells of the developing mouse pancreas. *Mech Dev*. 2002; 113: 189-192.
45. Dor Y, Brown J, Martinez OI, Melton DA. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature*. 2004; 429: 41-46.
46. Scharfmann R. Expanding human beta cells. *Diabetologia*. 2008; 51: 692-693.
47. Parnaud G, Bosco D, Berney T, Pattou F, Kerr-Conte J. Proliferation of sorted human and rat beta cells. *Diabetologia*. 2008; 51: 91-100.

48. Gershengorn MC, Hardikar AA, Wei C, Geras-Raaka E, Marcus-Samuels B. Epithelial-to-mesenchymal transition generates proliferative human islet precursor cells. *Science*. 2004; 306: 2261-2264.
49. Ouziel-Yahalom L, Zalzman M, Anker-Kitai L, Knoller S, Bar Y. Expansion and redifferentiation of adult human pancreatic islet cells. *Biochem Biophys Res Commun*. 2006; 341: 291-298.
50. Gao R, Ustinov J, Korsgren O, Otonkoski T. In vitro neogenesis of human islets reflects the plasticity of differentiated human pancreatic cells. *Diabetologia*. 2005; 48: 2296-2304.
51. Billestrup N, Otonkoski T. Dedifferentiation for replication of human beta-cells: a division between mice and men? *Diabetes*. 2008; 57: 1457-1458.
52. Russ HA, Bar Y, Ravassard P, Efrat S. In vitro proliferation of cells derived from adult human beta-cells revealed by cell-lineage tracing. *Diabetes*. 2008; 57: 1575-1583.
53. Audrey V, Jennifer J, Thomas G, Gopika G, Mayya S, Tingxia G, Sapna P, Leena H, Vincenzo C, Robert B, Greg L, Peter A and Matthias H. Controlled induction of human pancreatic progenitors produces functional beta-like cells in vitro. *The embo journal*. 2015; 34.
54. Pinho AV, Rooman I, Reichert M, De Medts N, Bouwens L, Rustgi AK and Real FX. Adult pancreatic acinar cells dedifferentiate to an embryonic progenitor phenotype with concomitant activation of a senescence programme that is present in chronic pancreatitis. 2011; 60: 958-966.
55. Mashima H, Shibata H, Mine T, Kojima I. Formation of insulin-producing cells from pancreatic acinar AR42J cells by hepatocyte growth factor. *Endocrinology*. 1996; 137: 3969-3976.
56. Mashima H, Ohnishi H, Wakabayashi K, Mine T, Miyagawa J. Betacellulin and activin A coordinately convert amylase-secreting pancreatic AR42J cells into insulin-secreting cells. *J Clin Invest*. 1996; 97: 1647-1654.
57. Zhang YQ, Mashima H, Kojima I. Changes in the expression of transcription factors in pancreatic AR42J cells during differentiation into insulin-producing cells. *Diabetes*. 2001; 50: 10-14.
58. Zhang YQ, Kanzaki M, Furukawa M, Shibata H, Ozeki M. Involvement of Smad proteins in the differentiation of pancreatic AR42J cells induced by activin A. *Diabetologia*. 1999; 42: 719-727.
59. Ueda Y. Activin A increases Pax4 gene expression in pancreatic beta cell lines. *FEBS Lett*. 2000; 480: 101-105.
60. Zhu M, Breslin MB, Lan MS. Expression of a novel zinc-finger cDNA, IA-, is associated with rat AR42J cells differentiation into insulin-positive cells. *Pancreas*. 2002; 24: 139-145.
61. Smith SB, Gasa R, Watada H, Wang J, Griffen SC. Neurogenin3 and hepatic nuclear factor 1 cooperate in activating pancreatic expression of Pax4. *J Biol Chem*. 2003; 278: 38254-38259.
62. Palgi J, Stumpf E, Otonkoski T. Transcription factor expression and hormone production in pancreatic AR42J cells. *Mol Cell Endocrinol*. 2000; 165: 41-49.
63. Aldibbiat A, Marriott CE, Scougall KT, Campbell SC, Huang GC. Inability to process and store proinsulin in transdifferentiated pancreatic acinar cells lacking the regulated secretory pathway. *J Endocrinol*. 2008; 196: 33-43.
64. Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature*. 2008; 455: 627-632.
65. Scharfmann R. Control of early development of the pancreas in rodents and humans: implications of signals from the mesenchyme. *Diabetologia*. 2000; 43: 1083-1092.
66. Bonner-Weir S, Toschi E, Inada A, Reitz P, Fonseca SY. The pancreatic ductal epithelium serves as a potential pool of progenitor cells. *Pediatr Diabetes*. 2004; 5 Suppl 2: 16-22.
67. Bonner-Weir S, Taneja M, Weir GC, Tatarkiewicz K, Song KH. In vitro cultivation of human islets from expanded ductal tissue. *Proc Natl Acad Sci U S A*. 2000; 97: 7999-8004.
68. Xu G, Kaneto H, Lopez-Avalos MD, Weir GC, Bonner-Weir S. GLP-1/exendin-4 facilitates beta-cell neogenesis in rat and human pancreatic ducts. *Diabetes Res Clin Pract*. 2006; 73: 107-110.
69. Zhao M, Amiel SA, Christie MR, Rela M, Heaton N. Insulin-producing cells derived from human pancreatic non-endocrine cell cultures reverse streptozotocin-induced hyperglycaemia in mice. *Diabetologia*. 2005; 48: 2051-2061.
70. Lin HT, Chiou SH, Kao CL, Shyr YM, Hsu CJ. Characterization of pancreatic stem cells derived from adult human pancreas ducts by fluorescence activated cell sorting. *World J Gastroenterol*. 2006; 12: 4529-4535.
71. Noguchi H, Xu G, Matsumoto S, Kaneto H, Kobayashi N. Induction of pancreatic stem/progenitor cells into insulin-producing cells by adenoviral-mediated gene transfer technology. *Cell Transplant*. 2006; 15: 929-938.

72. Inada A, Nienaber C, Katsuta H, Fujitani Y, Levine J. Carbonic anhydrase II-positive pancreatic cells are progenitors for both endocrine and exocrine pancreas after birth. *Proc Natl Acad Sci U S A*. 2008; 105: 19915-19919.
73. Hui H, Wright C, Perfetti R. Glucagon-like peptide 1 induces differentiation of islet duodenal homeobox-1-positive pancreatic ductal cells into insulin-secreting cells. *Diabetes*. 2001; 50: 785-796.
74. Hardikar AA, Marcus-Samuels B, Geras-Raaka E, Raaka BM, Gershengorn MC. Human pancreatic precursor cells secrete FGF2 to stimulate clustering into hormone-expressing islet-like cell aggregates. *Proc Natl Acad Sci U S A*. 2003; 100: 7117-7122.
75. Zulewski H, Abraham EJ, Gerlach MJ, Daniel PB, Moritz W. Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. *Diabetes*. 2001; 50: 521-533.
76. Yashpal NK, Li J, Wang R. Characterization of c-Kit and nestin expression during islet cell development in the prenatal and postnatal rat pancreas. *Dev Dyn*. 2004; 229: 813-825.
77. Zhang L, Hong TP, Hu J, Liu YN, Wu YH. Nestin-positive progenitor cells isolated from human fetal pancreas have phenotypic markers identical to mesenchymal stem cells. *World J Gastroenterol*. 2005; 11: 2906-2911.
78. Oshima Y, Suzuki A, Kawashimo K, Ishikawa M, Ohkohchi N. Isolation of mouse pancreatic ductal progenitor cells expressing CD133 and c-Met by flow cytometric cell sorting. *Gastroenterology*. 2007; 132: 720-732.
79. Koblas T, Pektorova L, Zacharovova K, Berkova Z, Girman P. Differentiation of CD133-positive pancreatic cells into insulin-producing islet-like cell clusters. *Transplant Proc*. 2008; 40: 415-418.
80. Koblas T, Zacharovová K, Berková Z, Mindlová M, Girman P. Isolation and characterization of human CXCR4-positive pancreatic cells. *Folia Biol (Praha)*. 2007; 53: 13-22.
81. Zhao M, Amiel SA, Christie MR, Muiesan P, Srinivasan P. Evidence for the presence of stem cell-like progenitor cells in human adult pancreas. *J Endocrinol*. 2007; 195: 407-414.
82. White MG, Al-Turaifi HR, Holliman GN, Aldibbiat A, Mahmoud A. Pluripotency-associated stem cell marker expression in proliferative cell cultures derived from adult human pancreas. *J Endocrinol*. 2011; 211: 169-176.
83. Jiang R, Han Z, Zhuo G, Qu X, Li X. Transplantation of placenta-derived mesenchymal stem cells in type 2 diabetes: a pilot study. *Front Med*. 2011; 5: 94-100.
84. Zhao Y, Jiang Z, Zhao T, Ye M, Hu C, Yin Z, et al. Reversal of type 1 diabetes via islet β cell regeneration following immunomodulation by cord blood-derived multipotent stem cells. *BMC Med* 2012; 10:13.
85. Li L, Shen S, Ouyang J, Hu Y, Hu L. Autologous hematopoietic stem cell transplantation modulates immunocompetent cells and improves β -cell function in Chinese patients with new onset of type 1 diabetes. *J Clin Endocrinol Metab*. 2012; 97: 1729-1736.