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Stem cells as a therapeutic target for diabetes

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1. ABSTRACT

The rapidly increasing number of diabetes patients across the world poses a great challenge to the current therapeutic approach. The traditional method of exogenous supply of insulin has ephemeral effect and often causes lethal hypoglycemia that demands to develop a novel strategy. Recent investigations on regeneration of insulin producing cells (IPCs) revealed that in addition to primary source i.e., pancreatic beta cells, IPCs can be derived from several alternative sources including embryonic, adult, mesenchymal and hematopoietic stem cells via the process of proliferation, dedifferentiation, neogenesis, nuclear reprogramming and transdifferentiation. There is considerable success in insulin independency of diabetes patient after transplantation of whole pancreas and / or the islet cells. However, the major challenge for regenerative therapy is to obtain a large source of islet / beta cells donor. Recent advances in the directed differentiation of stem cells generated a promising hope for a better and permanent insulin independency for diabetes. In this review we discussed stem cells as a potential future therapeutic target for the treatment of diabetes and associated diseases.

2. INTRODUCTION

Diabetes mellitus (DM) is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. It affects more than 200 million of adult population worldwide and is projected to affect at least 5% of global adult population by the year 2025 (1; 2). Diabetes can be categorized into three major types- (a) Type 1 diabetes: it is also known as juvenile-onset diabetes and is characterized by beta-cell destruction, typically by an autoimmune T cell-mediated mechanism, which usually leads to an absolute deficiency of insulin in the body required for glucose metabolism. About 5-10% of Americans who were diagnosed with diabetes have Type1 diabetes. (b) Type 2 diabetes: it is also known as adult-onset diabetes and is characterized by inability of insulin to properly metabolize glucose. Combined with insulin deficiency, it scored about 90–95% of diabetes patients in USA. It is commonly linked to obesity, which can cause insulin resistance. Despite the different pathogenic mechanisms of Type 1 and Type 2 diabetes, they share common symptoms including glucose intolerance, hyperglycemia, hyperlipidaemia and similar

complications, and (c) Gestational diabetes: it appears during the second trimester of gestation causing high blood glucose level and disappears after the birth of the baby. It is uncontrolled and affects both the baby and the mother. However, proper diet, exercise and medication can reduce its effect. Gestational diabetes is reported in approximately 5-10% of pregnant women. The total number of diabetes patient in USA is approximately 23.6 millions (http://www.diabetes.org/about-diabetes.jsp). It stands sixth in the leading causes of mortality in USA even after current medication of insulin injection and oral hypoglycemic pills. Additionally, it is also implicated in the other pathologies such as adult blindness, kidney failure, amputation of leg and feet, pregnancy complications and heart attack (http://www.kellogg.umich.edu/patientcare/conditions/diabetes.html). The association of diabetes with micro-and macro-vascular complications and cardiomyopathy makes it a major cause of morbidity and mortality in the world (3-5). The alarming rate of increase in the incidence of Type 1 diabetes is not only limited to Europe and America (6) but also includes other countries of the world (7).

The major strategy of the current medication for decreasing the blood glucose level in diabetes is exogenous supply of insulin. Although it is successful in decreasing the blood glucose level in hyperglycemic patients, it is neither capable of completely mimicking endogenously secreted insulin released from pancreatic beta-cells, which is tightly regulated for maintaining the optimum level of blood glucose nor is safe as it often causes hypoglycemic coma. Thus, strategies to promote either the expansion of existing beta-cells within the body or the supply of stem cell derived insulin-producing cells would provide a future treatment option for the patients with complicated diabetes. Stem cells are self-renewing, clonogenic and multipotent cells having tremendous potential for the treatment of several human diseases, and potential source for regenerative medicine and tissue replacement after injury or disease. They are classified as embryonic and adult stem cells based on their respective origins; from blastocyst -stage embryos and from niches of mature adult tissues and bone marrow (8). Since these cells can be used to replenish the dead cells of different organs, they can be used in therapy of diseases such as myocardial infarction in heart where cardiomyocytes dies and diabetes where insulin producing pancreatic beta-cells either die or become defective. This review aims to provide an overview of the most current progress in this exciting area and will cover development of pancreatic beta-cells, their regeneration from different stem cell lineages, the regulatory role of microRNAs in diabetes, the therapeutic challenges and strategies to deal with it.

3. PANCREAS AND BETA-CELL DEVELOPMENT

Before discussing stem cell based therapies for diabetes, it is important to understand how pancreas develops. Pancreas is a complex endoderm-derived organ, which consists of two major functional entities namely exocrine cells and duct cells that exert exocrine and endocrine activities. The exocrine cells constitute more than 90-95% of the total pancreatic cell mass including acinar cells that produce digestive enzymes such as lipases, carbohydrases and amlyases; and duct cells that provide conduits to the gut for the enzymes (9). In the pancreatic tissue 1-2% of the endocrine organ consists of hundreds of thousands endocrine clusters that ranges from less than 50 to more than 500μM in diameter and scattered into the tissue. They play a key role in establishing normoglycemia in the body.

Five different endocrine cell types are known in the pancreas, and each specialized in production and secretion of specific pancreatic hormone that are essential for the regulation of glucose homeostasis in the blood. They are alpha-cells secreting glucagon, beta-cells producing insulin, delta-cells producing somatostatin, PP-cells secreting pancreatic polypeptide, and ε-cells producing ghrelin (10, 11). In human pancreas islet cells contains approximately 50 to 63 % beta-cells, 15 to 30 % alpha-cells, 3 to 5 % delta-cells, ~1 % ghrelin cells and ~1 % PP cells (12). Pancreas is a combination of lobulated, branched acinar gland that forms the exocrine pancreas and embedded in the acinar gland, and the Islets of Langerhans that constitute the endocrine pancreas. Considerable progress has been made over the last century to understand the cellular organization of the adult pancreas and the morphological changes that occur during pancreas development. In recent years, tremendous work has been performed to gather information about the molecular mechanisms that regulate pancreas organogenesis, epithelial cell differentiation and beta-cell replacement therapy. The development of pancreas includes generation of endoderm / gut endothelium, pancreatic differentiation, endocrine specification, and ultimately beta-cell differentiation. Pancreas development is controlled by a complex interaction of signaling pathways and transcription factors that determine early pancreatic specification as well as the later differentiation of exocrine and endocrine lineages (Figure 1).

During development the three germ layers-ectoderm, mesoderm and endoderm are formed through intensive cell migration at the stage of gastrulation (13, 14). The definitive endoderm from which the pancreas arises begins as a flat sheet of cells that is specified during gastrulation. The anterior part of the definitive endoderm gives rise to the foregut, liver and lungs, while the posterior part becomes the midgut and hindgut (13). Genes required for definitive endoderm formation include *Wnt / beta-catenin*, *Nodal*, *GATA4/6*, *FoxA2* and *Mix* (15; 16) and several members of the Sox family including *Sox17* (17).

Specification of the pancreatic field occurs around embryonic day 8.5 (E8.5) in mouse and 3 weeks in human. After the domains are specified and initiate morphogenetic budding, the dorsal and ventral pancreatic buds merge to create the gland. The development of the pancreas is orchestrated by a series of inductive interactions between endoderm and mesoderm derived tissues, including the notochord, blood vessels and gut mesoderm (18). These interactions can lead to the differentiation of endoderm to a pancreatic fate. Pancreatic epithelial cells

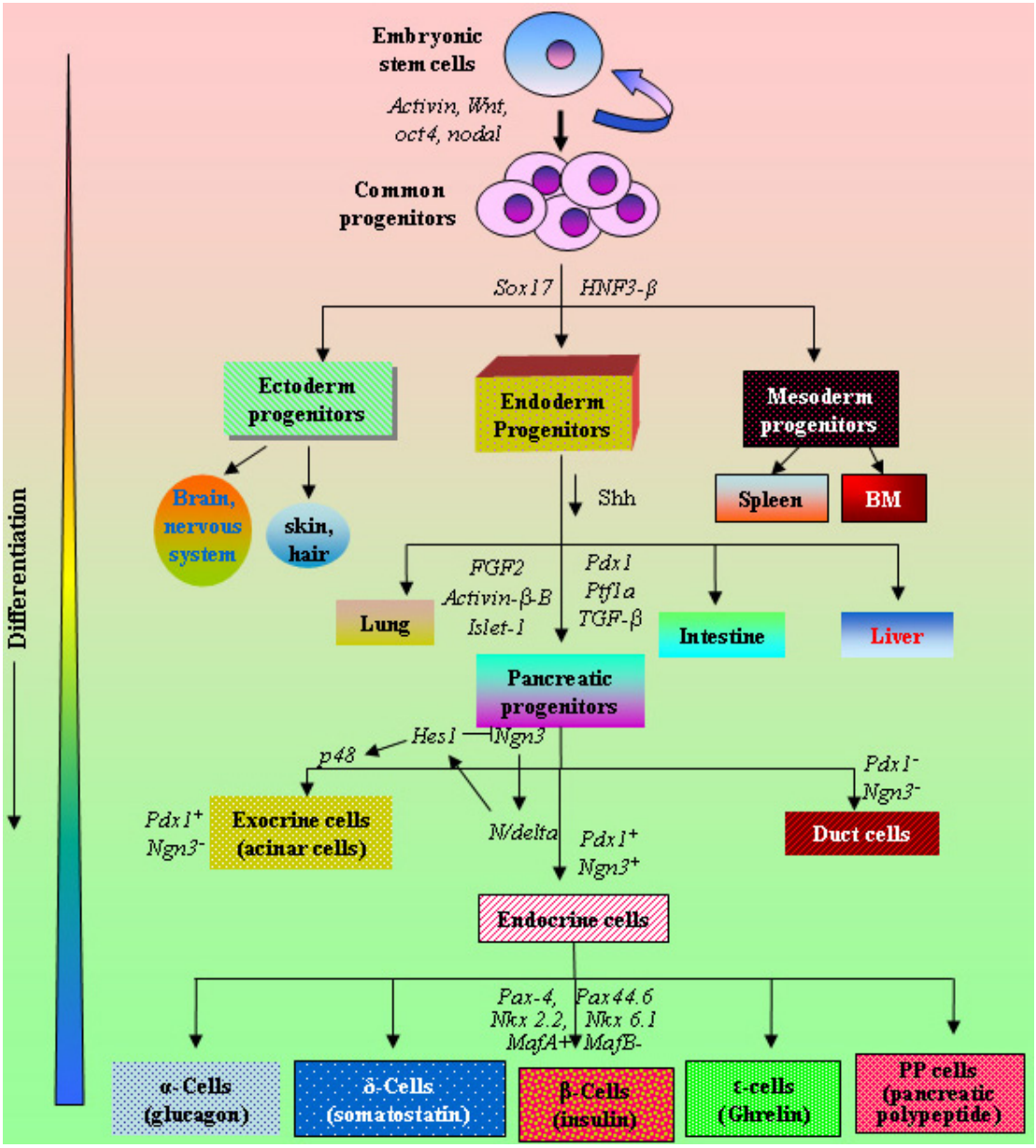

Figure 1. A schematic overview of the cell lineage determination during pancreas development. Pancreas consists of three types of epithelial cells- exocrine, endocrine and duct cells. The exocrine tissue is composed of acinar cells that secrete pancreatic enzymes that are delivered to the intestine to facilitate the digestion of food. Scattered throughout the exocrine tissue are many thousands of clusters of endocrine cells known as islets of Langerhans. Within the islets, alpha- cells produce glucagon while beta- cells, delta -cells, epsilon -cells and gamma-cells produce insulin, somatostatin, ghrelin and pancreatic polypeptides respectively. Transcription factors involved in the specification of the various lineages are shown in italics.

proliferate, branch and differentiate toward several types of cells in the pancreas. Insulin and glucagon can be detected as early as E9.5 and other hormone-secreting cells become first evident at E13. Pdx-1expressing cells give rise to endocrine, exocrine and ductal cells demonstrating that Pdx-1 represents a marker of all pancreatic lineages. Inactivation of Pdx-1 after bud formation prevents both islet and acinar cell differentiation. The expansion and differentiation of pancreatic progenitor cells is regulated by Notch signaling. Further, the Notch signaling pathway determines endocrine fate by the expression of the 'pro-endocrine' gene, neurogenin3 (Ngn3). At the end stage of islet formation and maturation, mutual interaction between vascular endothelial cells and endocrine cells promotes islet angiogenesis that is vital for the functional islets. Many transcription factors such as Pdx1, ISL LIM homeobox 1 (Isl-1), Ngn3, NK2 homeobox 2 (Nkx2.2), NK6 homeobox 1 (Nkx6.1), neurogenic differentiation factor (NeuroD), Hlxb9, paired box gene (Pax)-4, MafA and Pax-6 have been reported as islet differentiation factors. Ngn3 is a key

transcription factor required for islet cell development. Nkx2.2 is required for the final differentiation of beta-cells and production of insulin. Nkx6.1 and Pax-4 act as beta-cell determining factors. Pax- 6 is required for islet cell proliferation, morphology and beta-cell function. Transcriptional regulator Islet-1 (Isl-1) is essential for the maturation, proliferation and survival of the endocrine pancreas (19). MafA is a basic-leucine zipper transcription factor (20-22) that controls beta-cell-specific expression of the insulin gene through RIPE3b1 and thus acts as a potent transactivator for the insulin gene (22, 23). Additionally it is involved in the development and function of beta-cells as well as in the pathogenesis of diabetes (20, 21). MafB, an activator of the glucagon gene is expressed in the developing alpha- and beta-cells and regulates transcription of those key factors during development that are required for the production of mature alpha- and beta-cells (21). Heparan sulfate binds with several signaling molecules and regulates ligand-receptor interactions. It thus plays an essential role in embryonic development. It is also involved in the regulation of postnatal islet maturation, which is required to ensure normal insulin secretion (24). A recent study suggests that Dicer1 is important for maintaining the adult pancreas and regulates the differentiation of endocrine precursor cells (25). A number of signaling pathways including the Hedgehog, Fgf, Notch, Wnt, and TGF-beta control various aspects of pancreas and endocrine cell development as well as their proliferation and differentiation. Activin and growth differentiation factors (GDF) are involved in the endocrine and exocrine lineage specification (26-28). Vascular endothelial growth factor (VEGF) regulates insulin gene expression and beta -cell proliferation through laminin and maintains adult islet function. Tremendous progress has been made on pancreatic development, transcriptional regulation of pancreatic endocrine specification, growth and lineage allocation that contributes to our knowledge of how endogenous beta-cells develop and differentiate. Understanding pancreas organogenesis will provide a better clue for translational research for beta-cell regeneration.

4. DEVELOPMENT OF STEM CELL THERAPY FOR DIABETES

Stem cells are self-renewing, unspecialized cells that give rise to multiple specialized cell types through a process of differentiation. The adult endocrine pancreas has for a long time considered a quiescent cell population. Recent studies revealed that like other tissues adult endocrine pancreas is also a dynamic population of cells, where the amount of beta-cell mass is determined by the interplay of cell expansion and reduction mechanisms. Cell expansion can occur through beta-cell hypertrophy, self-replication, transdifferentiation and neogenesis. In contrast, cell reduction can results from beta-cell atrophy, death or loss of phenotypic stability. Thus, the development of strategies to avoid beta-cell mass reduction or to enhance beta-cell mass expansion, both *in vivo* and *in vitro* could provide a promising option for cell-based therapy of Type 1 and Type 2 diabetes.

The major approach to ameliorate the hyperglycemic condition is either by exogenous supply of insulin or induction of insulin producing cells (pancreatic beta-cells) either by differentiation of stem cells *in vivo* or transplantation of *ex vivo* differentiated cells in pancreas. The fact that exogenous insulin cannot maintain the optimum physiological level of glucose and is often accompanied by hypoglycemia, pancreas / pancreatic islet replacement therapy is considered as a better alternative. The transplantation of intact pancreas or the beta - cell mass can fulfill the need for achieving life long normoglycemia. Although there are several promising advancements in this direction (6), the major limiting factor is shortage of functional beta-cells from available donors (29). Therefore, the current strategy is focused mainly on regeneration of pancreatic beta- cells where the basic need is identification of biomarkers for these cells. The micro-environmental cues required for differentiation of stem cells into pancreatic beta- cells either *in vitro, ex vivo* or *in vivo* will promote the regeneration of large number of the cells required for therapy of diabetes. Recently, the success of mesenchymal stem cells to achieve this goal and mitigate the effect of hyperglycemia is quite enthusiastic (29-32). Several sources of stem / precursor cells have been suggested that can repopulate the damaged beta- cells such as differentiation of embryonic stem cell (ESC), hematopoietic stem cell (HSC), mesenchymal stem cell (MSC), resident stem cells and induced pluripotent cells (iPS) as well as transdifferentiation and neogenesis (Figure 2)

4.1. Tradition approach of treatment of diabetes

The regenerative therapy targets Type 1 diabetes, where beta-cells die and inadequate production of insulin causes diabetes. The best criteria to characterize Type 1 diabetes are to assess the presence of anti-islet cell-antibody (6). The other symptoms are severe insulitis and autoimmune destruction of pancreatic beta-cells leading to hyperglycemia (6). The traditional approach to treat this disease is injection of exogenous insulin and subsequent follow up of blood glucose level. However, the major drawback of this method is frequent incidence of hypoglycaemia in the patients that occurred due to inability of the exogenous insulin to mimic the physiology of secretion of endogenous insulin (6). The other promising approach is transplantation of pancreas (33, 34) and islet cells (35-37) for beta-cell replacement therapy. There was considerable success to treat diabetic patient from this approach. The follow up studies after transplantation of beta-cells from 2 to 5 years in different studies show great achievement for insulin independency (38-40). Nevertheless, this method has several limiting factors like need of immunosuppressant that always adds side effects, difficulty of obtaining transplant material and getting access to suitable organ donar (41).

4.2. Therapeutic potential of stem cells in diabetes

The latest approach using stem cells for treatment of diabetes was started in a clinical trials using autologous nonmyeloablative HSC transplantation by exploiting the immunomodulatory properties of stem cells (42, 43). The main strategy of their treatment was to inhibit

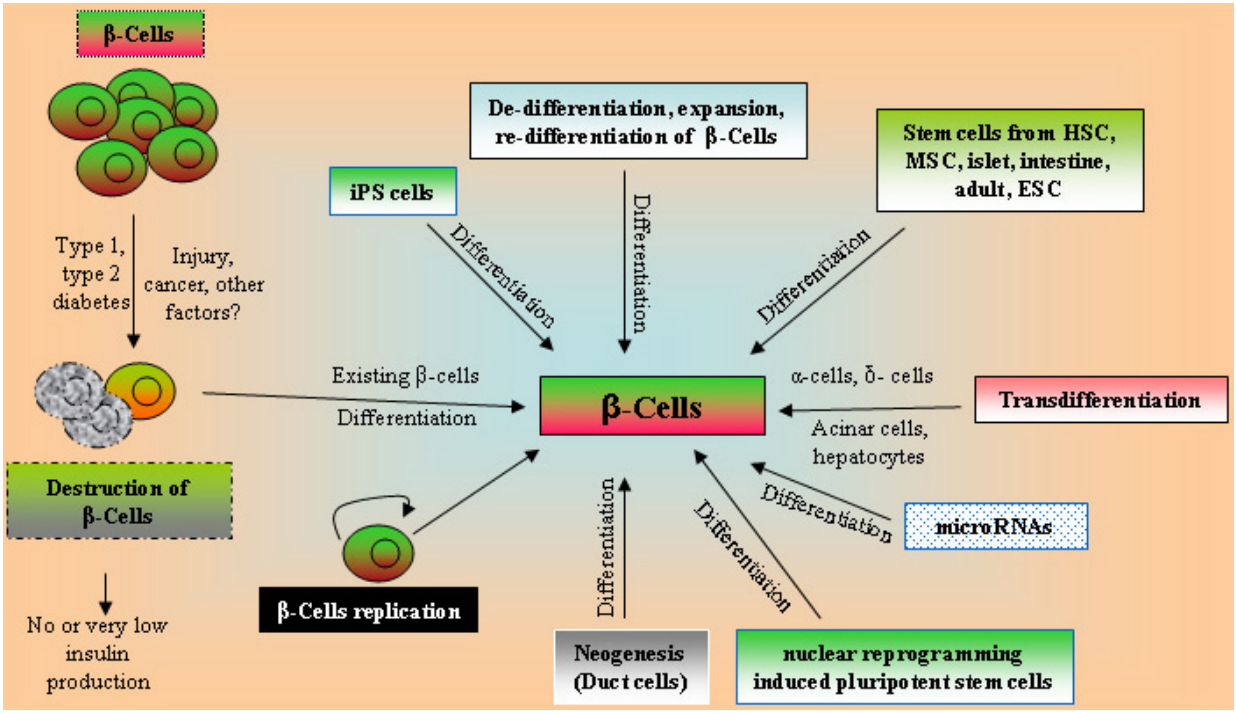

Figure 2. A schematic diagram depicting different possible resources and mechanisms for beta-cells regeneration. During Type 1 and Type 2 diabetes, most of the beta -cells are destroyed and no or very low insulin is produced. It may be treated by beta-cell regeneration by employing several alternative resources. Details are given in the text.

the autoimmune destruction of beta-cells with immunosuppressive drugs and to replenish the destroyed immune cells by using autologous HSC, which in turn reconstitute the normal immune system (42). More than a year and half follow up studies of patients with nonmyeloablative HSC transplantation revealed that the patients are insulin free. Further, the constant monitoring of their c-peptide level corroborated that insulin free condition of patients was due to the preservation of beta-cell mass (43). The important caveat from this therapeutic approach was to promote beta-cell regeneration to overcome autoimmunity and to ameliorate endogenous insulin secretion to maintain normoglycemia. MSC having immunomodulatory properties and power to differentiate into insulin-secreting cells is a promising therapeutic target for diabetes (6).

A number of studies have suggested the existence of stem cells within the pancreas that can give rise to insulin producing cells (44-64). There are other evidences suggesting that trans-differentiation of liver cells can generate beta-cells (65-71). Several other studies reported that bone marrow derived stem cells can be differentiated into insulin-expressing cells (72-78). Neural progenitor cells from the brain also have the capacity to differentiate into insulin expressing cells (45). In addition to these cells, there are other highly proliferative and pluripotent cells that are derived from inner cell mass of the blastocyst and are recognized as ESCs. They have the capacity to differentiate into all three embryonic germ layers. Accumulating evidences suggest that ESC can differentiate into cells with an insulin-expressing phenotype (79-88). Other sources for beta-cell regeneration are pancreas-derived multipotent progenitor (60, 89, 90), pancreatic duct cells (91), splenocytes (92, 93) and umbilical cord blood cells (94-96).

Stem cell-derived insulin-producing cells could be a renewable source of insulin-producing cells for cell transplantation. To enhance the maturation process of human embryonic stem cells (hESCs)-derived insulin-producing cells, recent investigations used genetic manipulation methodologies to deliver specific pancreatic transcription factors or developmental control genes to hESCs (49, 97). hESCs are derived from the inner cell mass of pre-implantation blastocyst and have potential for self-renewal, differentiation into all embryonic cell types, and unlimited expansion without compromising its differentiation capacity. Previous studies on beta-cells generation from hESCs were focused on the selection of cells positive for nestin (98, 99). It served as a biomarker for stem / progenitor cell populations in other tissues (100). However, recently it turns out to be a biomarker for neural and pancreatic exocrine progenitors, and does not mark endocrine progenitor cells (100, 101). The first report that insulin-secreting cells can be generated from spontaneous differentiation using hESCs come from Assady *et al.* (102). Later on Lavon *et al.* (97) demonstrated that the constitutive expression of Pdx-1 enhances the differentiation of hESCs toward pancreatic endocrine and exocrine cell types. The expression of Pdx-1 also increased the expression of several transcription factors that are

downstream to it such as Ngn3, PAX4, NKX2.2 and ISL1. Further, reprogramming of rat hepatic stem cell into functional insulin-producing cells by over expression of Pdx-1 and their delivery into diabetic mice with a lentivirus demonstrated that Pdx-1 is effective in converting hepatic stem cells into pancreatic endocrine precursor cells; and it is able to generate insulin-producing cells and restore euglycemia (103).

4.3. Transcription factors involved in converting MSC to insulin producing cells

Transplantation of adult human bone marrow-derived mesenchymal stem cells (hMSCs) could be a promising source to replenish insulin-producing cells because hMSCs have the suppressive effects on T cell responses to alloantigen and thus offer a novel cell-based approach for the prevention of autoimmune diabetes and for islet cell transplantation (6;104-107). Dedifferentiation is the process whereby mature cells become less differentiated and acquire the ability to differentiate into different cell types. As opposed to dedifferentiation, transdifferentiation is the process through which differentiated cells are stimulated to become a different mature cell type. hMSCs can be induced to differentiate into functional insulin-producing cells when Pdx-1 is introduced via recombinant adenoviral vector (108). Furthermore, Pdx-1 modified hMSCs seemed to contribute to the regeneration of pancreatic islets after cell transplantation in STZ-induced diabetic mice. Mouse bone marrow derived stem cells when treated with fetal calf serum and high concentrations of glucose for 4 months, were differentiated (or transdifferentiated) into functional beta-cells (109;110). Contrary to this, negative results are also documented (109). Thus genetically modified hMSCs are a potential cell source for cell replacement therapy for diabetes. It is also reported that progenitor cells in close proximity to ductal epithelium can differentiate into beta-cells because of cues from the large number of beta- cells in the pancreas (55,104,106,111). By using adenovirus to mediate Pdx-1, Neurogenin3 (Ngn3), NeuroD or Pax-4 expression in duct cells, Noguchi *et al.* (55) demonstrated that NeuroD was the most effective inducer of insulin expression in primary duct cells and suggested that the over expression NeuroD facilitates pancreatic stem / progenitor cell differentiation into insulin-producing cells in pancreas. Kodama *et al.* (93) have shown that live donor male or labeled splenocytes administered to diabetic NOD females contain cells that rapidly differentiate into islet and ductal epithelial cells within the pancreas. They found that treatment with irradiated splenocytes was also followed by islet regeneration, but at a slower rate. Further, they were persistent, functional, and apparent in all NOD hosts with permanent disease reversal. Nagaya, *et al.* (112) demonstrated that a sub-population of intra-hepatic biliary epithelial cells (IHBECs) can be induced to a beta-like phenotype. Recently, another interesting stem cell called human umbilical cord blood (UCB-MSCs) has been used as a source for beta-cells. They offer several advantages over other cells in terms of availability at higher frequencies and their unusually broad differentiation potential (113;114). Recently, Gao *et al.* (95) reported that MSC derived from UCB-MSCs can be used as new and potential stem cells in the treatment of diabetes.

The beta-cell populations of the endocrine pancreas may expand by either of two processes-replication or neogenesis. While replication requires the existence of an already differentiated beta-cell, neogenesis depends on the presence of active stem cells. Dor *et al.* (115) observed cell lineage using a transgenic mouse strain, in which the insulin promoter regulates the expression of a tamoxifen-dependent Cre recombinase to mark adult progenitor cells. Using this system, they were able to distinguish between existing beta-cells and new beta-cells that differentiated from stem cells. They found that beta-cells were derived only from the duplication of existing beta-cells. Based on this finding they suggested that only beta-cells can produce new beta-cells rather than being derived from pluripotent adult precursor cells (115). This was subsequently confirmed by Teta *et al.* (116), who used a DNA analogue- based lineage-tracing technique as well as other investigators (117-119). The autopsy studies in human provide strong supportive evidence that beta-cell replication is the primary mechanism underlying beta-cell expansion (120). Recently, it has been also documented that all beta-cells contribute equally to islet growth and maintenance. It is speculated that for tissues lacking an adult stem cell can be replenished equally by replication of all differentiated cells (121). Although, beta-cell replication alone may be sufficient to account for maintaining the mass of the pancreas, there are strong evidences supporting that new beta-cells can be generated by a process of neogenesis from a stem-cell population residing in the pancreatic duct (91). Al-Abdullah *et al.* (122) reported that copper deprivation contributes to the neogenesis of pancreatic alpha- and beta- cells in the ductules and acinar tissue of adult pancreas in rat model; and that transplanted stem cells maintain their functional capacity in the recipient after transplantation. Several other studies demonstrated that transcriptional regulation involving pdx1 is essential for endocrine neogenesis *in vivo* and *in vitro*, and that ectopic expression of pdx1 in the pancreas could induce endocrine neogenesis (84, 97,108). Taniguch *et al.* (123) demonstrated that adenovirus-mediated expression of pdx-1 can activate the endogenous pdx-1 gene, leading to beta-cell neogenesis and ductal proliferation. It has been shown that new beta-cell can be formed from non- beta-cells located in the lining of the duct during regeneration of the pancreas in response to duct ligation. Further, it was found that duct ligation induces an increased number of cells expressing Ngn3 (124). Recently, PaSCs (pancreatic stellate cells) have been identified in the pancreas that express the ABCG2 transporter and are able to secrete insulin after cell differentiation (125).

4.4. Generation of insulin-secreting cells through nuclear reprogramming and induced pluripotent stem (iPS) cells

Accumulative evidences suggest that islet cell transplantation for patients with diabetes holds great promise for achieving insulin independency. However, the extreme shortage of matched organ donors and the immuno-rejection has made it difficult for this treatment to

be used for the general diabetic population. Recent success in generating insulin-secreting islet-like cells from hESCs coupled with the success in deriving hESC-like induced pluripotent stem (iPS) cells from human fibroblasts have opened an emerging possibility of patient-specific treatment, where insulin-secreting islet-like cells could be derived from the patient's somatic cells by reprogramming the cell fate through defined factors. iPS cells are pluripotent that are capable of differentiating into a variety of different somatic cell types and are artificially derived by reprogramming a somatic cell (126, 127). Takahashi and Yamanaka (127) was the first to discover that viral transfection of four genes (*Oct 3/4, Sox2, c-Myc*, and *KLF4*) into an adult mouse fibroblast population can lead to the appearance of some cells with the characteristics of ESCs. Tateishi *et al.* (128) demonstrated that skin fibroblast-derived iPS cells have the potential to be differentiated into islet-like clusters through definitive and pancreatic endoderm. Zhou *et al.* (129) identify a specific combination of three transcription factors (Ngn-3, Pdx-1 and MafA) that reprograms differentiated pancreatic exocrine cells in adult mice into cells that closely resemble beta-cells. The induced beta-cells are indistinguishable from endogenous islet beta-cells in size, shape and ultrastructure. Stadtfeld *et al.* (130) used inducible lentiviruses to express *Oct4, Sox2, c-myc,* and *Klf4* in pancreatic beta-cells to assess whether a defined terminally differentiated cell type remains amenable to reprogramming. Their results provide evidence that terminally differentiated cells can be reprogrammed into pluripotent cells suggesting that *in vitro* reprogramming is not restricted to certain cell types or differentiation stages. Recently, Zhang *et al.* (131) reported a highly efficient approach to induce hESCs and iPS cells to differentiate into mature insulin-producing cells in a chemical-defined culture system. The differentiated hESCs obtained by this approach comprised nearly 25% insulin-positive cells as assayed by flow cytometry analysis and released insulin / C-peptide in response to glucose stimuli in a manner comparable to that of adult human islets. Most of these insulin-producing cells co-expressed mature beta-cell -specific markers, such as NKX6-1 and PDX1 indicating a similar gene expression pattern to adult islet beta-cell *in vivo*. Further they demonstrated that EGF facilitates the expansion of PDX1-positive pancreatic progenitors. The above studies confirmed that insulin-secreting cells can be generated from skin fibroblasts, raising the possibility that patient-specific iPS cells could potentially provide a treatment for diabetes in future.

5. MICRO-RNAS IN DIABETES

MicroRNAs (miRNAs) are a novel group of highly conserved, endogenous, 22-23 nucleotide non-coding RNAs that are involved in precise regulation of biological functions by negatively modulating the gene expression either through promotion of mRNA degradation or through translational repression of proteins (132,133). The tremendous potential of these tiny regulators has been recently documented in many cellular pathways including development, cell differentiation, proliferation and apoptosis, and are also manifested in diverse diseases including cardiovascular, different types of cancer as well as diabetes (133-137). It has been reported that miRNAs are critical in regulation of these complex diseases and they may be exploited as targets for therapeutic intervention. Understanding the regulatory mechanisms of miRNAs in insulin secretion and glucose homeostasis may unravel a better understanding of pancreatic cell biology and diabetes Pathophysiology. And it opens a new window for novel therapeutic targets that includes the strategies to manipulate in the development and progression of diabetes and its complications (138,139).

Accumulative evidence suggests that miRNAs play an important role in insulin secretion pancreatic islet development, beta-cell differentiation, and indirect control of glucose and lipid metabolism (134,140-145). Poy et al. (143,144) identified a novel islet-specific miRNA, miRNA-375, which is highly expressed in pancreatic islets. It is essential for normal glucose homeostasis, alpha- and beta-cell turnover and adaptive beta-cell expansion in response to increasing insulin demand in insulin resistance. Joglekar et al. (141) provide evidence for miRNA-mediated silencing of ngn-3, which inhibits endocrine cell development via the classical 'stem cell pathway' during mouse pancreatic regeneration, thereby favoring beta-cell regeneration. Manipulation of the miR-221-c-kit pathway may offer a novel strategy for treatment of vascular dysfunction in diabetic patients (146). High levels of miR-29 led to insulin resistance and overexpression of miR-29 caused a decrease in the levels of Insig-1 (insulin-induced gene- 1), and Cav-2 proteins (caveolin- 2). Insulin receptor substrate (IRS) proteins are important components of the insulin signaling pathway. There are three IRS proteins in humans and mice such as IRS-1 and IRS-2 and IRS-4. IRS-1 knock-out mice are insulin resistant, whereas IRS-2 deficient mice develop diabetes (147). Although IRS-2 is involved in the Type 2 diabetes, only IRS-1 has been identified to be a direct target of miR-145 (148). Recently Tang et al. (149) in a screen identified 61 glucose-regulated miRNAs including miR-124a, miR-107, and miR-30d, which were up-regulated in the presence of high glucose. However, some miRNAs including miR-296, miR-484, and miR-690 were significantly down-regulated in the presence of high glucose. Interestingly, they found that over expression of miR-30d increased insulin gene expression, while inhibition of miR-30d abolished glucose-stimulated insulin gene transcription and suggested that miR-30d may be negative regulators of insulin gene expression. Recently, it has been reported that miR-30 family miRNAs confer epithelial phenotype to human pancreatic cells (142).

Growing evidences suggest that miRNAs play an important role in insulin production, secretion and action. Diabetes leads to changes in miRNA

expression profiles in many tissues. The roles of miRNAs in diabetes are very complex as changes in miRNA levels may lead to diabetes in both early and late stages. MiRNAs provide a new class of biomarkers for various diseases including cancer, and may become a useful biomarker for diabetes in future. Furthermore, recent progress in the development and use of synthetic miRNAs such as antagomiRs to silence miRNAs *in vivo* such as miR-375 in case of diabetes (150) may provide a novel therapeutic tool for the treatment of diabetes and other diseases.

6. THERAPEUTIC CHALLENGES

Although there are several evidences to corroborate that stem cells and islet cells have tremendous capability to treat diabetic patient and maintain normoglycemic condition / insulin independency for several years (38, 57,151-153), the precise mechanism for differentiation of stem cells into IPCs is still nebulous. The genetic manipulations and micro- environmental conditions required for differentiation of stem cells into IPCs are major issues to be elucidated with concrete evidences. It will facilitate the generation of functional IPCs from mesenchymal stem cells in large scale, which is one of the major challenges ahead for the treatment of diabetes. As usual with the most of the therapy, there are several drawbacks / side effects associated with stem cell therapy, which needs to be taken seriously before going into clinical trials. Recent investigations showed the association of MSC expansion with tumor development (151, 154-156), which cautions us to understand meticulously the side effects and their remedy before using it for therapy.

7. FUTURE DIRECTIONS

Stem cells have been identified in many of the adult organs and across the animal and plant kingdom (157-165). They are maintained in a specialized microenvironment known as the stem-cell niche. Two fundamental questions in stem cell research are what controls stem cell number and which signaling pathways regulate its self-renewal (157-165). Accumulative evidences suggest that the niche maintain the stem cell number and multiple signals are required to maintain a balanced / control of stem cell self-renewal (157-165). An interesting method for generating beta-cells in bulk is to understand the signaling pathway that promotes differentiation of any stem cell into beta-cells. Technological advancement is required for proper transplantation of beta-cells into suitable niches for maximum success of treatment. Recent progress on pancreatic stem cell research has revealed that the putative multipotent pancreatic stem cells and / or beta-cell precursors may reside in the pancreatic gland in the adults. The presence of undifferentiated pancreatic cells with stem cell-like properties opens the possibility of stimulating their expansion and differentiation for beta-cells replacement-based therapies for Type 1 or Type 2 diabetes. In addition, the transplantation of either insulin-producing beta-cell from embryonic, fetal and other tissue-resident adult stem / progenitor cells or genetically modified adult stem / progenitor cells may also be a promising alternative therapy for treating diabetes and associated diseases including diabetic cardiomyopathy. The most important issue is to understand the side effects associated with transplantation of beta-cells and how to regulate it. The precise regulatory role of microRNAs in several pathological conditions (132,133,137) tempted us to speculate that they may provide an impetus in investigating the regulatory mechanisms underlying differentiation of stem cells into beta-cells.

Diabetes mellitus (DM) is a well known and important risk factor for cardiac disease (166-174). Although the most common cardiac manifestation in diabetic patients is coronary artery disease, DM is also strongly linked to heart failure (HF). Approximately 15 to 25% of patients with HF are diabetic. It has been known that hyperglycemia and hyperinsulinemia increase the risk of death due to premature and accelerated coronary artery disease. Hyperglycemia, over time can lead to increased deposits of fatty materials on the insides of the blood vessel walls that affect blood flow, increasing the chance of clogging and hardening of blood vessels resulting into diabetic cardiomyopathy and heart failure (166-174). Diabetic cardiomyopathy is a clinically condition diagnosed when ventricular dysfunction develops in patients with diabetes in the absence of coronary atherosclerosis and hypertension (Figure 3). It has been demonstrated that following an ischemic insult to the heart, the neural stem cells participated in sympathetic fiber innervations of the peri-infarct / infarct region, de novo blood vessel formation and maladaptive healing (166-174). The cardiac function can be improved following MSCs transplantation, which significantly increased myocardial arteriolar density and decreased the collagen volume in diabetic myocardium. MSCs transplantation increased MMP-2 activity and decreased transcriptional level of MMP-9 (173). Zhang *et al.* (173) suggests that MSCs transplantation improved cardiac function, possibly through angiogenesis and attenuation of cardiac remodeling. The growing evidence suggest that the heart acquire a compartment of multipotent progenitor cells (MPCs) that differentiate into myocytes, endothelial cells and smooth muscle cells. The heart cells continuously self-renew and any alteration between cell death and regeneration following diabetes could be mediated by defects in growth and survival of MPCs leading to an excessive number of old, dying and poorly contracting myocytes that eventually results into heart failure. A recent study also suggests that diabetes promotes cardiac stem cell aging and heart failure (174). However, this can be prevented by deletion of the *p66shc* gene (174). These studies suggest that stem cells can be a potential therapeutic target for the diabetic

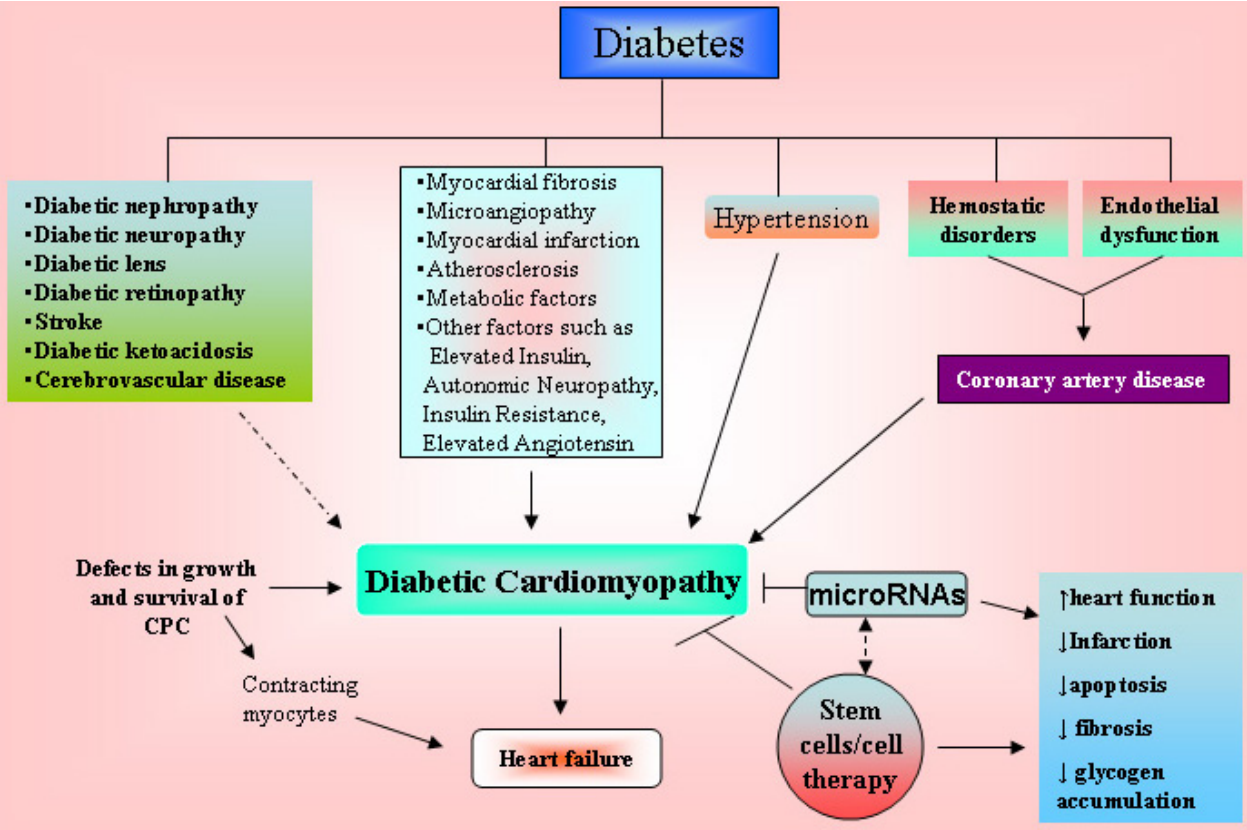

Figure 3. Effect of diabetes on cardiomyopathy. Diabetic cardiomyopathy resulted due to diabetes is caused by defects in growth and survival of cardiac progenitor cells (CPC) and / or myocardial fibrosis, abnormal myocardial metabolism, hypertension and coronary artery disease (CAD). These pathophysiological remodeling in the heart may be reverted by transplantation of the stem cells as well as miRNAs. Details are provided in the text.

cardiomyopathy that eventually restores cardiac function (Figure 3). Furthermore, since miRNAs play important roles in myocardial dysfunction associated with insulin resistance, it may provide novel therapeutic approaches for the management of diabetes-induced cardiomyopathy.

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Abbreviations: BM: bone marrow, CAD: coronary artery disease, CPC: cardiac progenitor cells; DM: Diabetes mellitus, GDF: growth differentiation factors, HSC: hematopoietic stem cell, HF: heart failure, hMSCs: human bone marrow-derived mesenchymal stem cells, hESCs: human embryonic stem cells; MSCs: mesenchymal stem cells, MPCs: multipotent progenitor cells, UCB-MSCs: mesenchymal stem cells derived from human umbilical cord blood, IPCs: insulin producing cells, IRS: Insulin receptor substrate, iPS: induced pluripotent stem cells, IHBECs: intra-hepatic biliary epithelial cells, miRNAs: MicroRNAs, Nkx2.2.: NK2 homeobox 2, Nkx6.1.: NK6 homeobox 1, NeuroD: neurogenic differentiation factor, Ngn3: Neurogenin3, Pax-4: paired box gene 4, PaSCs: pancreatic stellate cells, VEGF: Vascular endothelial growth factor.

Key Words: Diabetes mellitus, Mesenchymal stem cells, adult stem cells, insulin producing cells, induced pluripotent stem cells, human embryonic stem cells, nuclear reprogramming**,** MicroRNAs, stem cell therapy, pancreas development, beta-cells regeneration, Review

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