STEM CELL THERAPY IN DIABETES MELLITUS: CURRENT TRENDS

Ravipati Sarath[*1], V. Leena Rani [2]

[1] School of Medical Sciences, UoH, Hyderabad – 500046, India

[2] Dept of Prosthodontics, Shadan Medical College, Hyderabad- 500046, India

***Corresponding Author:** dr.ravipati.md@gmail.com

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ABSTRACT

The pancreatic beta-cell has a pivotal role in the regulation of glucose homeostasis and its impairment leads to diabetes mellitus. The exponential increase in number of diabetics across the world poses a daunting task to the current therapeutic approach with exogenous insulin. As the insulin therapy led to long term complications, search for alternative therapies had begun. Transplantation of insulin-producing cells is the hallmark of diabetes treatment. But due to the acute shortage of pancreatic-islet donors, researchers met with a limited success. In this light, stem cell research & therapy in terms of diabetes has opened new avenues for diabetes treatment and received lot of attention recently. A stem cell with extensive proliferative capacity may provide a valuable source of islet progenitor cells. Key signaling pathways and transcription factors paved a significant role in pancreas development from progenitor cells. This review has been structured to explore the various ways and means and sources of cells responsible for generating beta cells. Hence the role of stem cells, their adverse effects in terms of therapeutic implications and their current trends in modern medicine have been discussed at length. So, this review projects the different types of sources, mechanisms, signaling pathways with transcription factors and remaining challenges regarding the limitations of cell replacement therapy.

Keywords: Stem cells; signaling; diabetes

1. INTRODUCTION

Diabetes mellitus results from inadequate mass of insulin-producing pancreatic beta cells. The most common type of diabetes is - type 2 diabetes, characterized by relative deficiency of β-Cell, impaired β-cell function with insulin resistance in peripheral organs. Type 1diabetes is characterized by absolute loss of beta cells due to autoimmune-mediated destruction affecting just 10 % of diabetic patients. So be it type 1 or type 2 diabetes, the chief problem is reduced and insufficient number of insulin producing beta cells leading to derailed glucose metabolism with life threatening complications[1].

1.1 Time for a change (shift) - insulin to islet replacement therapy: The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. The urban population in developing countries is projected to double between 2000 and 2030. These findings indicate that diabetes has grown to proportions of being called as epidemic[2]. As the number of diabetic patients in the world is increasing in recent years, the prevention of diabetes mellitus is therefore one of the urgent medical issues. The traditional method of treatment for diabetes is exogenous insulin injections. The current treatment of insulin supply is not fully capable of achieving tight control of glucose levels, leading to long-term complications. The fact is, exogenous insulin cannot maintain the optimum physiological level of glucose and is often accompanied by

hypoglycemia[3]. The alternative treatment methods are transplantation of pancreas and islet cells for beta-cell replacement therapy[4].

Over the last few decades, islet transplantation from the cadavers has been developed as a promising method to achieve strict control of blood glucose compared with insulin and a potential cure for type 1 diabetes. Islet transplantation, though proposed long back, its clinical usage was limited for many years because of its low success rate. Despite this great transition from traditional insulin to islet transplantation, it faces many challenges like limited donor availability, and a need for lifelong immune-suppression for the long-term survival and function of human islet grafts. The acute shortage of pancreatic donors needs a special mention here since only 1% per year of the total type 1 diabetics are able to receive the islet grafts[5]. So this critical shortage of pancreatic donors, led to exploration of alternative sources of beta cells from stem cells, use of porcine islets, and beta cell expansion with growth factors. Hence the focus of research has been shifted to generating beta cells via transdifferentiation from stem or progenitor cells[6].

1.2 Stem cells – future ahead: Repairing or replacing the damaged tissue in diabetes using stem cells has become the most dynamic topic of interest as stem cells are blessed with the potential of treating many debilitating & degenerative diseases. Stem cell is an unspecialized cell, which has the capacity of continuous self-renewal and can differentiate into any type to every type of specialized cell lineages of the body. There are 2 types of stem cells: 1. Human embryonic stem cells (hESC) and 2. adult stem cells. The plasticity of Pluripotent embryonic stem cells (ESCs) generates all the cells in the human body, except extra embryonic tissue like placenta[7]. The existence of adult multipotent stem cells in human beings, and their ability to maintain plasticity, has led to the differentiation into many types of cells for example, adult bone marrow stem cells can give rise to hepatocytes, cardiomyocytes, neural cells etc[8]. Despite the success in transforming and differentiation of the stem cells into insulin-producing cells, the rate of success is highly variable and this method is equally controversial. But the results from the recent studies have demonstrated promising outcome in the generation of beta-like cells from hESCs[9,10]. The important characteristics of ESCs include the capacity to self-renew and the potential to differentiate into all embryonic cell types, termed pluripotency, under in vivo and in vitro conditions**.**

Upon proliferation in a cell culture the stem cell maintains its properties; hence unlimited expansion is achieved without compromising on its differentiation capacity. As large cell numbers are required for therapeutic purposes, ESCs are superior to other cells because of this property. Hence, to generate insulin-producing β-cells, certain protocols have to be followed compounded with signaling molecules and transcription factors to facilitate undifferentiated progenitors to differentiated endocrine cells of pancreas[11].

The fundamental question is how to make terminally matured pancreatic β-Cells. Many years of research have shown different approaches for the neogenesis of β-Cells using ESCs, adult stem cells residing in the pancreas, or other nonpancreatic cell types.

Various cell types are studied along with ESCs, bone marrow stem cells etc to understand the differentiation into islet hormone secreting cells1[2]. Evidence is mounting to prove that adult pancreas might contain progenitor cells as it is exhibiting the property of self-recovery when exposed to stressful stimuli[13]. Identifying and isolating these stem cells or progenitor cells would open the door for in vitro generation of islet tissue for use in transplantation.

1.3 Sources of islet cells-pancreatic beta-cell development: The most fascinating and intriguing aspect of pancreatic development is 2 morphologically distinct types of tissues arise from one simple epithelium. The 2 different tissues, exocrine and endocrine serve 2 different functions with a different morphology [14]. The pancreas is a complex endoderm-derived organ. Several varieties of genes are required for endoderm formation; they are, Wnt/betacatenin, Nodal GATA4/6, FoxA2 and Mix and a set of Sox family including Sox17. The organ has both exocrine and endocrine parts[15,16]**.** In recent years, tremendous work has been done to unravel the molecular mechanisms that regulate pancreas organogenesis, epithelial cell differentiation, and beta-cell replacement therapy. Pancreas development is controlled by a complex interaction of signaling pathways and transcription factors that lead to differentiation of endocrine pancreas.

1.4 From ductal cells of adult pancreas: Jonsson and others proposed that ductal cells express a transcriptional factor (Pdx-1) essential for islet neogenesis[17]. Hence, it has been speculated that ductal cells are the primary source of new islet cells. It was demonstrated later that these islet-like clusters could treat diabetes in mice[18]. Several workers have confirmed the same finding with lot of authority and it was understood that human ductal cells can generate islet-like clusters by expressing Pdx-1, thereby serving as progenitor cells of adult pancreas[19]. The major drawback of these studies is the starting culture got contaminated by other pancreatic cell types. Despite these negative aspects, evidence shows that pancreatic stem/progenitor cells may be the potential sources of diabetes treatment. Numerous *in vitro* studies have demonstrated that adult pancreatic ductal tissues and acinar cells are the potential sources for differentiation into insulin-producing cells[20].

1.5 From lineage tracing-beta cells form beta cells (replication Vs neogenesis): The expansion of β-Cell population in pancreas is by 2 ways: replication or neogenesis. While replication requires the existence of an already differentiated β-Cell, neogenesis depends on the presence of active stem cells. Dor and others used a novel genetic lineage tracing method to assess and identify beta cells generated from pre-existing beta-cells and from other sources[21]. Using this unique mouse model they found that new beta cells arose through beta-cell replication not by neogenesis. This took by surprise because normally adult beta cells are in a fully differentiated stage and they lost the power of proliferation, even if such adult beta cells were made to proliferate and expand, usually they must have lost the power of differentiation and more importantly the secretory power of insulin in vitro. So this study was like a storm in the teacup. The author explains if beta-cells are the source of new beta cells, then the mandatory aspect is all of the existing beta cells in adult must dedifferentiate transiently into a progenitor state before becoming new beta cells. Dor's study portrays a significant role of beta cell replication in maintaining beta cell mass in adults and this has been ably supported by another study from Georgia and Bhushan who have found that adult mice lacking a critical gene (cyclin D2) would not be able to maintain beta cell mass in mice as this gene is responsible for beta cell replication[22]. These findings were confirmed by Teta who has used a DNA analogue- based lineage-tracing technique as well as other investigators [23,24,25.] To further support these studies, human autopsy findings serve as hallmark evidence that beta-cell replication is the primary mechanism underlying beta-cell expansion[26]. Recent evidence furthers re-iterates that all beta-cells contribute equally to islet growth and maintenance. It is understood that adult stem cell lacking tissues can be replenished equally by replication of all

differentiated cells[27]. According to Bonner-Weir, even though beta-cell replication can alone maintain the required pancreatic mass, growing amount of evidence is emerging with the view that new beta-cells are generated by neogenesis from pancreatic ductal stem cell[28].

The debate between beta cell replication versus neogenesis is at stake since there are many limitations and constraints involved with Dor's model. The reasons for debate are the study does not prove if the beta cell neogenesis has not occurred from other sources, how accurate and effective the model is and as this study has been done in mouse-can all the findings observed in mouse is extrapolated into human beings who are suffering from long standing type1 disease is still a huge question to ponder[19]. Hence further studies are required to take into account the study done by Dor and his colleagues, until this is answered the importance of replication versus neogenesis of beta-cell mass continues.

1.6 From PMPs-renewable beta cells: Pancreas-derived multipotent precursors (PMPs) are special variety of cells which can differentiate into many other varieties of pancreas cell types because they express marker Pdx-1 and also can generate neural-like cells. These cells were isolated from both ductal and islet cells obtained by special technique called clonal dilution[29]. Again the biggest constraint for this study is the percentage of PMP colony forming cells is so less (0.03%) that its role is underplayed. Nevertheless, this novel finding triggers the possibility of coming to a conclusion that islet cell progenitors do exist beyond doubt in pancreas and they can induce differentiation into potential beta-like cells. This holds promise and does require more supportive studies which can identify and isolate progenitor cells so that beta-cell replacement therapy in diabetes can be taken to next level.

There are few other evidences from various studies which show generation of beta-cells. The differentiation of liver cells can generate beta cells. Bone marrow can do the same and neural progenitor cells from the brain also have the same potential to generate beta cells[30,31]. So, there are different cells in various areas of human body which can differentiate and generate beta cells. But the role of pluripotent stem cells which arise from the inner mass of blastocyst has a special mention here since they can differentiate into the 3 germ layers.

1.7 MSC-immunomodulation: The mesenchymal stromal cells derived from the bone marrow are a rare, heterogeneous, multipotent stromal population of non-haematopoietic progenitor cells with the capacity to differentiate into multiple mesenchymal lineages like bone, fat and cartilage. These are considered as the most promising therapeutic tool for tissue regeneration and repair. Because of immunosuppressive actions, it can be an ideal treatment choice for type 1 diabetes[3]. There are 2 ways MSCs can come in handy- Firstly, Human insulin gene is expressed in MSCs which is unique property. Secondly, MSCs from tissues like pancreas, bone marrow etc. have the potential to differentiate into IPCs under specific culture conditions compounded with genetic modification[32]. Another study demonstrated that MSCs derived from bone marrow can be transplanted to generate IPCs as they possess the T-cell suppressor activity, could be a novel therapeutic strategy to prevent type1 diabetes due to autoimmunity[4,33]. Cord blood MSCs *in-vitro* requires notch signaling mechanism, as it plays a crucial role in regulation of IPCs differentiation and the study done by Hu also shown that notch signaling inhibition is a novel method of increasing IPCs and hence this could be a potential treatment for type 1 diabetes because a solution for shortage of islet cells is found[34].

1.8 iPSCs & Nuclear reprogramming-generate IPCs: IPCs generated from induced pluripotent stem cells (iPSCs) are derived from human fibroblasts have

opened the doors of success in designing patient specific treatment from patient's own somatic cells by process of nuclear reprogramming with the aid of factors. iPSCs belong to a type of pluripotent stem cell artificially derived by reprogramming a somatic cell. iPSCs are morphologically similar to embryonic stem cells and are capable of differentiating into a variety of different somatic cell types; thereby offering a new cell source for type I diabetes, so this kind of cell therapy reduces the risk of immunologic rejection[35,36]. Stadtfeld et al. studies provided 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[37]. Recently, Zhang et al. reported a procedure where induced hESCs and iPSCs differentiate into mature IPCs in a culture system with the expression of defined beta-cell transcription markers[38]. As an add on, derivation of iPSCs from patients helps in autologous cell replacement therapy, disease modeling and drug screening for both types of diabetes [39]. These studies have confirmed that IPCs can be generated from skin fibroblasts, promising a new horizon and possibility that patient-specific iPSCs could potentially provide a treatment for diabetes in the future. Populations such as MSCs can be expanded to a much lesser extent than ESCs or iPSCs[40]. Nuclear reprogramming, which is more of a direct genetic engineering process wherein right doses at right duration of critical transcription factors if expressed in right manner in ESCs can generate IPCs[41]. A novel strategy for β-cell generation is inspired by the success of nuclear reprogramming. Introducing critical transcription factors into acinar cells can convert exocrine cells into insulin-secreting cells[42].

1.9 MicroRNAs (miRNAs) – next generation regulators: MicroRNAs (miRNAs) are a unique and novel group of non-coding RNAs with their potential therapeutic ability are responsible for precise regulation of biological functions by negatively modulating the gene expression either through promotion of mRNA degradation or through translational repression of proteins[43,44.] The potential of these tiny regulators has been recently documented in many cellular pathways including development, cell differentiation, proliferation and apoptosis, and in diverse diseases including cardiovascular, different types of cancer as well as diabetes[45,46.] Recent evidence suggests that miRNAs play an important role in insulin secretion pancreatic islet development, beta-cell differentiation, and indirectly control glucose and lipid metabolism[47,48.]

Poy et al. identified a novel islet-specific miRNA, miRNA-375, which is highly expressed in pancreatic islets, essential for normal glucose homeostasis, alpha- and beta-cell turnover and adaptive beta-cell expansion in response to increasing insulin demand in insulin resistance[49]. Joglekar et al. provided evidence for miRNA-mediated silencing of ngn3, which inhibits endocrine cell development via the classical 'stem cell pathway' during mouse pancreatic regeneration, thereby favoring beta-cell regeneration[48.]. Growing evidence suggests that miRNAs play an important role in insulin production, secretion and action. The roles of miRNAs in diabetes are very complex as changes in miRNA levels may lead to diabetes in both early and late stages. Dicer is a special type of enzyme which can generate Micro RNAs (miRNAs), has been reported to have many important roles in the developmental processes. In this context, Dicer1 has ciritical functions in maintaining the adult pancreas[50]. Exploring the molecular mechanisms of miRNAs regulation in insulin secretion and glucose homeostasis may lead to new horizons in analyzing and understanding the pancreatic cell biology and pathophysiology of diabetes. This is the

future strategy of therapeutic intervention as these appear to the mainstay regulators for the treatment of diabetes and abrogate the complications[51].

1.10 Signaling pathways-growth, development, differentiation &specification of islet cells: The expression of pro-endocrine gene called neurogenin3 (Ngn3) is essential for the endocrine fate, in turn determined by notch signaling as it paves way for expansion and differentiation of pancreatic progenitor cells[52,53]. There are number of signal pathways like Hedgehog, Fgf, Notch, Wnt, and TGF-beta regulate the endocrine pancreatic cell development, proliferation and differentiation where as activin and growth differentiation factor (GDF) specifically regulate endocrine cell lineage[54]. It is important to understand the mechanisms how endogenous beta cells develop and differentiate, hence the extrapolation of these mechanisms in *in-vitro* can develop pancreas from progenitor cells. Hedgehog signaling pathway plays an important role in differentiation of pancreas development and in disease condition[55]. Pancreas development, differentiation and specification are controlled by Hedgehog signaling and this pathway crosses with other signaling pathways[56]. Fibroblast growth factor (Fgf) and its receptorsby virtue of Fgf signaling, act as key signaling molecules regulating pancreatic growth and development during the emergence of mature islet cells[57]. Wnt gene was identified in the year 1982, ever since its discovery, Wnt signaling regulate both embryonic stem cells and adult stem cells besides homeostasis[58]. Active and its receptors are one of the growth factors of super family of TGF-β, are expressed early in pancreatic epithelium and later in islets[59]. Vascular endothelial growth factor (VEGF) regulates insulin gene expression and beta-cell proliferation through laminin and maintains adult islet function[52].

1.11 Transcription factors - markers for distinct pancreatic cell populations: Transcription factors form a regulatory cascade which promotes sequential transformation of uncommitted progenitors to specific endocrine precursors and finally completely differentiated β-cells. Transcriptional factors are the culmination of external and internal signals. Early stages of pancreatic development critically need two important transcription factors such as pdx1 and pancreas transcription factor 1a (Ptf1a). These are responsible for pancreatic growth, specification and maturation of β-cells[60]. As described in this review, Pdx-1 is an important marker and has a crucial role to play in exocrine and endocrine parts of pancreatic generation. More recently, Sox4 has been implicated in the regulation of insulin secretion in adult β cells[61]. Sox9 plays a critical role in the maintenance of the pancreatic progenitor pool and Sox9 positively regulates neurogenin3[62,63]**.** Transcriptional regulator Islet-1 (Isl-1) is essential for the maturation, proliferation and survival of the endocrine pancreas[64]. Pdx1, Ptf1 and Sox constitute pancreatic progenitors.

HNF1β has a role in endoderm differentiation, overall pancreas development, in the precursor population for Ngn3-expressing islet progenitor cells[65]. Ngn3 can generate different endocrine cell lineages at different stages of development[66]. Lineage tracing reveals that $Pax4^{+}$ cells contribute to all endocrine lineages. Pax-4 acts as a beta cell determining factor[67]. HNF1β, Ngn3 and Pax4 constitute islet progenitors. Pax-6 is essential for islet cell proliferation, morphology and beta cell function[52].

The transcription factors responsible for lineage specification and β-cell differentiation are Nkx2.2, Nkx6.1, Mafs, Forkheads and HNF4α. MafA is a recent and new transcription factor discovered, controls insulin gene expressed on beta cell, development of beta cells and pathogenesis of diabetes[68]. Nkx2.2 is meant for final differentiation of beta cells leading to insulin production, whereas Nkx6.1 acts as beta cell determining

factor[52]. Hence great progress has been made in this regard in recent years regarding the transcription factors, regulation and pancreatic cell lineage specification.

1.12 Therapeutic limitations-challenges to be met: Cell-based therapeutics is still in the experimental phase, and many hurdles remain before they can be used as standard treatments. Some of the risks and side effects associated with cell therapy by transplanting islets into hepatic portal vein are met with challenges which had to be overcome. When ESC after differentiation are transplanted into humans certain negative effects are observed[69,70].

Risk of tumorigenesis-Stem cell therapies must be first tested in animal models before implementing in humans, as it can cause tumors like teratomas. The best solution is to transplant purified, fully differentiated cells generated from hESCs[71].

Disadvantage of immature β-cells- *In vitro* generation of IPCs from hECSs are immature and glucose homeostasis is incomplete which does not serve the real purpose. In vitro conditions usually show absence of cell to cell interactions in comparison of in vivo. This is a setback which needs to be corrected[72]. A major difference between β-cells in the pancreas and the β-cells generated in vitro lies in the different environment they reside in.

Transplant rejection-Two kinds of challenge are to be met when islet tissue is transplanted- 1. In type 1 diabetes patients, autoimmunity destroys β-cells, so the suppression of automunity is crucial for success of the cell replacement therapy. 2. The immune system recognizes grafted tissue as foreign initiating a host vs graft, alloimmune reaction[73]. In spite of improved immunosuppression regimens there are still problems of rejection, the only solution is cellular programming which can effectively fight against the allograft reactions.

CONCLUSION

Adult progenitor cells with the ability to regenerate tissue during normal turnover or after damage are of considerable interest, even though their abilities to self-renew and to generate various cell types are greatly restricted. Key is signal pathways and transcription factors which transform progenitor cells to insulin producing beta cells. Though beta cell replacement by islet transplantation has provided new hope for a cure of type 1 diabetes and to certain extent type 2 diabetes patients, the unending transplant rejection problems restrict its role in permanent cure for diabetics. So, the research, therapeutic consideration and technology to identify, isolate, purify and characterize the adult cells have paved novel treatment option for diabetes in the future. However, significant challenges are to be met and overcome with ES before they can be used extensively in a clinical setting. Today, cell replacement therapy for both type I and type II diabetic patients has become a promising scenario that could be achieved in the near future. Although progress is encouraging, major gaps in our understanding of developmental biology of the pancreas and adult beta-cell dynamics remain to be bridged before a therapeutic application is made possible.

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