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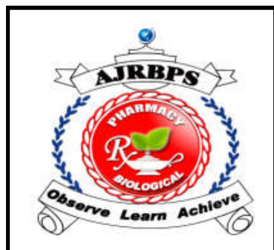

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THE MOLECULAR MECHANISM ASSOCIATED WITH STEM CELLS AND ITS PROBABILITY TO BE A CURE FOR DIABETES MELLITUS A PERSPECTIVE REVIEW

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ABSTRACT

Diabetes is a major concern among the diabetic patients due to the constant checking of glucose, planning of meals, hope for eating sugary content meals, daily monitoring of insulin levels. Stem cell plays an important role in treating patients identified with diabetes mellitus. Various researches are conducted on the ability of embryonic stem cells of humans to differentiate into islet cells for defining developmental stages and transcription factors in this process of therapy. However, clinical uses of human embryonic stem cells for treatment are minimal due to formation of teratoma. Diabetes mellitus is a growing health distress of the developing and developed countries beyond the globe. This disease is responsible for the 5% deaths around the world every year. Stem cells are unique to its function of differentiation to various types of specialized cells. The function and properties of both adult stem cells and embryonic stem cells was studied in great detail by the scientists for production of insulin secreting cells. The different type of stem cells includes induced pluripotent stem cells (iPSCs), embryonic stem cells (ESCs) and adult stem cells, which have proven to play an important role in treating diabetes with certain restrictions. This article reviews on the details of advancements, recent research and progress made in the field of stem cell research for treating diabetes, understanding the molecular mechanism of stem cells. This review also deals with the molecular in sights associated with stem cells which regenerate into pancreas, mainly its role in diabetes and also the practical hurdles associated with it.

KEYWORDS

Diabetes, Stem cells, β-Cells, Induced pluripotency, Embryonic stem cells, Induced pluripotent stem cells, Mesenchymal stem cell, Somatic cells, Blood glucose levels, Pancreatic stem cells, Progenitor stem cells.

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INTRODUCTON

Diabetes Mellitus is an alarming health problem among a large population of people in the world. It is also a major reason for more than 4 million deaths per year. The prevalence of Diabetes is more among developing countries which accounts for about 250 million every year.

Diabetes mellitus (DM) is a chronic non-communicable disease leading to various health problems. When acquired either the pancreas doesn't produce enough insulin or the produced insulin is not effectively used by the body. A number of the diabetic population round the globe is continuously increasing with a current estimation of 371 million cases in 2012 and it's expected to succeed in 552 million by 2030[1]. In 2014, 8.5% of adults aged 18 years and older had diabetes. In 2019, diabetes was the direct cause of 1.5 million deaths.

New methods which can cure Diabetes mellitus

The methods used at present don't cure the disease completely and thus there is a need for an alternative effective treatment through which diabetes is treated completely without any side effects. The unique regenerative properties of stem cells could be a vital tool that can be exploited in the treatment of diabetes. Thus, somatic cell investigation has become the middle of attraction for diabetic treatment[2].

The traditional methods of treatment such as administering exogenous insulin through injections on a daily basis are one of the most well- known treatments for Diabetes; however it is associated with glucose metabolism failure resulting in hyperglycemia. In this scenario Stem cell plays an important role in avoiding issues associated with daily injections. It also provides the therapeutic benefits of production, storage and supply insulin to maintain glucose homeostasis. Cell based therapy focuses on producing insulin-secreting β-cells to cure diabetes.

Over the years, researchers are checking out ways to transit the destroyed beta cells within the pancreas (insulin-producing cells) with healthy ones by the immune system of an individual with Diabetes Mellitus Changing the quantity of insulin is usually accompanied by a drop of blood glucose level or hypoglycemia, which may cause medical emergencies like coma. On the contrary, within the islet transplantation technique, if the transplant works properly, the results are going to be very promising; thus, glycemic control is completed by the body itself, and therefore the amount of insulin needed is given to the body with reference to the quantity of body glucose within the body; and homeostasis are completed normally.

Pancreas and Beta Cell Development

Embryonic Development

The major interest lies how embryonic pancreas develop into β-cells are generated under normal conditions. This requires not only dissecting the numerous cascades of signaling pathways and transcription factors that regulate cell autonomous differentiation, but also gathering knowledge about epithelial-mesenchymal interactions and factors secreted from surrounding tissues that instruct endocrine and β-cell development. It is the hope that once all this information has been collected, it can be combined to reconstruct the embryonic differentiation program for ex vivo generation of therapeutic β-cells.

The pancreas is a complicated organ generated from endoderm. It is made up of a variety of cell types that perform both endocrine and exocrine functions. The exocrine compartment contains acinar cells that secrete digestive enzymes like as lipases, carbohydrases, and amlyases, as well as ductal cells that transport these enzymes into the duodenum, accounting for more than 90% of the pancreatic mass[3]. Despite the fact that hormone-secreting endocrine cells account for only 1-2% of the pancreatic cell population, they are critical for maintaining glycemia in the body. The Langerhans islets in the pancreas contain five different endocrine cell types[4].

The insulin-producing Beta-cell is the most common endocrine cell type, accounting for 60–80% of the islet. The mammalian pancreatic primordium is produced from the DE during embryonic pancreas development, and it gives rise to the primitive intestine and posterior foregut[5]. Endocrine cells proliferate and gather during the development of pancreatic epithelium to form islets of Langerhans, which are aggregates of endocrine cells that also accommodate insulin-secreting Beta-cells[6].

During various stages of pancreas and β-cell development, multiple signalling pathways have been revealed to play crucial roles. Experiment developed by Kroon *et al*, and D 'Amour *et al*. hESCs can be stimulated to develop DE, primitive gut (PG), posterior foregut (PF), pancreatic

endoderm, and endocrine precursors by adding growth factors and small molecule inhibitors to the culture media (PE).After transplanting endocrine precursors into immune compromised mice, fully matured Beta-cells can be obtained. The percentage of target cells at the conclusion of each stage indicates differentiation efficiency, Figure No.1[7,8].

Recent research has discovered that the production of exocrine tissue is directly linked to the development of the endocrine compartment. Cells positive for the acinar marker carboxypeptidase a are found at the tip of the branching pancreatic epithelium and exhibit multipotent progenitors with acinar, duct, and endocrine lineage features[9]. Endocrine cells that have delaminated from the duct-like epithelium congregate into clusters scattered between acini and ducts near the end of pregnancy. Around E12.5, neural crest cells move in tandem with endocrine cells, coinciding with the vascularization process in the developing islets. The subsequent innervation of islets by sympathetic and sensory neurons originating from the neural crest, as well as astrocytes, is critical for regulating β-cell function and survival in adult islets. Islet formation and endocrine cell maturation are aided by signals from tissues surrounding the pancreatic endoderm. Developing endocrine cells detach from the duct-like pancreatic epithelium at E13 and form endocrine cell clusters. The creation of the islet of Langerhans is dependent on signalling chemicals secreted by the pancreatic mesenchyme, blood arteries, and migrating neural crest cells. In the adult islets, vascular endothelial cells and nerve fibres are also significant regulators of hormone synthesis and secretion[10,11] Figure No.2.

Molecular Mechanisms Regulating Stem Cells Fate

The ability of embryonic stem cells to differentiate into any cell type in the human body is a critical rationale for their application in biomedical research and regenerative medicine. These distinct characteristics have led to their utilization in research aimed at better understanding the molecular events that drive the stem cell regeneration process. The design of cellular or pharmacological treatment techniques to restore or modify the pool of stem cells begins with this molecular event[4,6]. The amazing flexibility of stem cells makes them very appealing for biomedical study, but it also continues to pose obstacles due to the intricacy of molecular signalling, epigenetic pathways, genetics, and other factors involved in their development control. Because stem cells might be challenging to maintain, therapeutic applications can be used to affect their surroundings. This can be a much more simple option[6,12].

Extracellular signals and transcription factors

The development of the pancreas, like that of other organs, is influenced by signals acquired from the environment. Many of these signals are secreted by neighboring germ layers as diffusible components[13,14]. Extracellular chemicals released by adjacent mesodermal structures, including as the notochord and dorsal aortas, have been demonstrated to play crucial roles in the induction of pancreatic epithelium during pancreatic primordia determination[13-15].

Cross-talk between the pancreatic mesenchyme and epithelium, mediated by soluble proteins, is also required for endocrine/exocrine lineage specification and differentiation[13]. The vascular endothelium, which is made up of cells that line the inside of blood vessels and is closely linked to the growing pancreas, is another important source of inductive chemicals[16,17]. Although the significance of various signalling pathways has been defined using knockout animal models, there is still much to learn about the interconnections and balances between these pathways.

Multiple extracellular signaling pathways induce pancreas development in embryogenesis

Hedgehog, Fgf, Notch, Wnt, and TGF-pathways are just a few of the embryonic signalling pathways that influence pancreas and endocrine cell cycle progression[18]. Organ boundaries are defined during early pancreas development by antagonistic effect of the Hedgehog signalling pathway found in tissues proximal to the pancreas anlage[19]. The notochord, a temporary mesodermal structure in touch with the dorsal endoderm during early stages of gut formation, provides signals that prevent Hedgehog signalling from reaching the dorsal pancreas anlage. Hedgehog signalling in the pancreas is inhibited by notochord-derived signals. Hedgehog signalling in the pancreas is inhibited by notochord-derived signals. Hedgehog inhibition

enables for pancreatic endoderm determination and the production of essential transcription factors, whereas ectopic Hedgehog ligand expression disrupts pancreas formation[15,20-22]. Hedgehog activity is found in the developing pancreas at later stages (E13.5)[21]. In addition, research in zebrafish has indicated that Hedgehog signalling is required for the activation of pancreatic β-cells within the developing endoderm[23]. As a result, variations in Hedgehog activity across time and space affect various aspects of pancreatic and endocrine production.

The TGF-superfamily is another major multifactorial signalling pathway involved in pancreatic development. TGF-signals are important for pancreatic epithelial specification and early branching morphogenesis, as previously stated[23,24]. Multiple members of the super-family are also involved in endocrine and exocrine lineage specification, including activin and growth differentiation factors[24-27].

Aortic endothelial cells become positioned close to the dorsal pancreas bud after the notochord separates from the endoderm. Ligands of members of the Fgf family, such as Fgf2 and Fgf10, are secreted by the notochord[15] and dorsal mesenchyme[28], respectively. Through the start and preservation of the expression of key transcription factors, these factors play an important role in the specification of the pancreatic primordium[28-30].

Fgf10 produced by the pancreatic mesenchyme increases the multiplication of pancreatic precursors and inhibits their differentiation into endocrine cells via activating Notch signalling in later stages[31-33]. Apart from Notch and Fgfs, Wnt signalling, an embryonic signalling pathway, has been linked to pancreatic progenitor proliferation, as well as -cell and acinar cell replication[34-38]. Signaling molecules originating from blood arteries and capillaries provide further guidance for islet development and maturation. Endocrine cells secrete vascular endothelial growth factor and insulin, which regulate endothelial cell aggregation during islet angiogenesis[39]. These co-regulatory responses keep endocrine cells and capillaries in close touch, which is essential for adult islet activity[39].

Transcription factors as different pancreatic cell populations indicators

Changes in transcription factor networks that govern pancreatic development are the result of external signalling inputs. The creation of a transcriptional regulatory cascade that promotes step-wise progression is thought to be the outcome of temporal changes in external and internal signals, according to current findings. Transcription factors as markers for distinct pancreatic cell populations.

Changes in transcription factor networks that govern pancreatic development are the result of external signalling inputs. Evidence suggests that temporal variations in external and internal signals lead to the establishment of a transcriptional regulatory cascade that promotes step-by-step progression from unengaged progenitors to specific endocrine precursors and, finally, fully committed progenitors. Furthermore, by interacting with laminin and laminin receptors, vascular endothelia regulate insulin gene expression and β-cell proliferation[40], sustaining and regulating adult islet function.

Although descriptions of these transcriptional cascades are beyond the scopes of this article. Below is a list of transcription factors that are critical for in vivo and in vitro cell development. Pancreatic and duodenal homeobox 1 (Pdx1) and pancreas transcription factor 1a are two transcription factors that are expressed early in pancreas development (Ptf1a). Both proteins are critical for pancreatic lineage specification and are expressed in pancreatic progenitor cells[41-44]. In both mice[45,46] and humans, Pdx1, a homolog of the human insulin-promoter-factor, is essential for the formation of pancreatic progenitor cells[47,48].

The description of endocrine progenitors from uncommitted pancreatic progenitor cells is the next step in pancreatic development. The contrasting effects of Notch signalling that promote expansion of indistinct progenitors as well as divergence of exocrine cells via expression of the hairy/enhancer-of-split 1 (Hes1) transcription factor and the activity of neurogenin 3 (Ngn3), a basic-helix-loop-helix family transcription factor signalling that is required for endocrine formation, regulate endocrine differentiation. In non-permissive cells, Notch signalling activates Hes1, which suppresses Ngn3

expression by binding directly to the Ngn3 promoter[49,50].

Ngn3 is transiently activated throughout embryogenesis in the developing pancreas[51,52], in cells co-expressing Hnf6 and Sox9[51-53] and its action is both essential and sufficient for the differentiation of all endocrine cell lineages[54-56.] Ngn3 activates NeuroD1, a basic-helix-loop-helix transcription factor that was first expressed in cells undergoing endocrine differentiation before being limited to mature islets, where it is important for endocrine cell viability and insulin gene transcription[57-60].

Ngn3 expression is essential for the development of all islet cells[54], but additional transcription factors, such as Pax4, Nkx2.2, Nkx6.1, and MafB[61-63], as well as sustained Pdx1 expression, are required for the specificity and maturation of insulin-secreting β-cells[64,65]. After Pdx1 expression, basic leucine zipper transcription factor MafB, which is expressed in more than 90% of insulin-secreting cells in the developing pancreas, is switched off, and another Maf factor, MafA, is turned on in the β-cell lineage (94). Many genes necessary at these late stages of β-cell differentiation are also essential for mature β -cell function, including as glucose sensing, insulin production and secretion, as well as Beta-cell population replication and survival after birth[66-69].

In conclusion, a hierarchy of secreted signals and transcription factors that guide cell development in the endoderm and pancreas has been discovered by several groups. This knowledge has been used to direct the differentiation of hESCs into β-cells using a blueprint for pancreatic β -cell differentiation.

Embryonic Stem Cells as Renewable Source of Functional β-Cells

The success of islet transplantation using the Edmonton protocol has provided a promising new therapeutic option for diabetic patients with significant -cell loss, particularly those with "brittle" type I diabetes, where hypoglycemia unawareness has severe repercussions[69,70]. The current scarcity of cadaveric islets, however, severely limits the use of this therapy strategy. To get around this issue, scientists have begun to experiment with novel ways in the search for a sustainable source of high-quality Beta -cells for transplantation.

***In vitro* differentiation of human embryonic stem cells**

Early attempts for *in vitro* β-cell differentiation: Embryo Stem cells in treatment of Diabetes

As ESCs are pluripotent, these cells have been used to treat a large number of medical conditions, the majority of it is used for the treatment of diabetes[71]. They are seen as an exceptional resource for the production of insulin secreting islet cells by developmental and differentiation pathways. ESCs are directed to differentiate into pancreatic islet cells and these cells could then be implanted in patients with diabetes, thus the β-cell deficit could be overcome.

Embryonic stem cells from mice were the major source in the production of pancreatic islet cells. Insulin-secreting clones were developed from genetically engineered and drug-selected mouse ESC line. These cells were incorporated into diabetic mice and resulted in the improvement of hyperglycemialevels in a few months[72]. D'Amour produced 100% pure, definitive endodermal cell population[73]. He and his group also demonstrated the production of pancreatic endocrine hormone-producing cells that contained both insulin and C-peptide by a five-stage in vitro differentiation process[74]. Through all these methods, researchers produced insulin from ESC cells, but failed to establish the insulin response to glucose These findings proves that ESCs have a greater role in future for cellular replacement therapy in T1DM.

Numerous ways have been explored to create insulin-secreting beta -cells from hESCs after the separation of mammalian ESCs and the development of in vitro culture conditions that allow them to remain an undifferentiated state. Although many studies have confirmed the in vitro production of fully functional Beta -cells that can secrete physiologically adequate amounts of insulin in response to glucose from mouse[75,76] monkey[77] and hESCs[78,79], none have confirmed the in vitro production of fully functional Beta cells that can secrete physiologically adequate amounts of insulin in responding to glucose.

Many early attempts to generate β-cells from ESCs were focused on the selection of cells positive for

nestin[80-82], an intermediate filament protein that served as a marker for stem/progenitor cell populations in other tissues[83] Later differentiation protocols have shown that the selection of nestin-positive cells likely leads to the generation of neuronal cell types, which agrees with the hypothesis that nestin is a marker for neural progenitors and pancreatic exocrine progenitors but does not mark endocrine progenitor cells[84-87].

Furthermore, early investigations were complicated by the use of insulin immune histochemistry as a standard marker to identify β-cells, an approach that proved to be misleading because differentiating ESCs and apoptotic cells can take up insulin from the culture media, causing false results[88,89]. To determine the mature beta-cell phenotype, more stringent criteria were implemented. To confirm the differentiation of ESCs into definitive endoderm (DE) and then pancreatic cell types, lineage-specific gene markers are now routinely used[90,91]. C-peptide, a gene-derived cleavage product, is used to assess insulin synthesis and release in terminally differentiated cells.

The failure of attempts to create β-cells has emphasizes the importance of recognizing biological problems in their physiological context. As a result, techniques were created that more precisely matched the differentiation phases observed during the embryogenesis of beta-cells.

Directed differentiation of hESCs: lessons from *in vivo* pancreatic development

Assady *et al*[92], showed in 2001 that hESCs can be used to generate insulin-secreting cells through spontaneous differentiation. Despite the limited number of insulin-producing cells and insulin content in these cells, it was the first proof-of-concept experiment demonstrating that hESCs could be used to generate beta-like cells. However, subsequent experiments that employed the signals that regulate embryonic endoderm and pancreatic formation provided the actual accomplishment in our efforts to make completely differentiated β-cells.

These signals have previously been discovered, a multistep approach that promotes ESC differentiation through the typical embryonic phases might be used to imitate the in vivo orchestra of events. Many groups have reported the creation of β-like cells utilising a variety of procedures in recent years. The recent studies by D'Amour *et al*[93] and Kroon *et al*[94]. Constitute the most effective attempts at pancreatic lineage differentiation in vitro, and will be reviewed in detail below.

The identification of DE is the initial and most important step in the differentiation of hESCs toward the pancreatic lineage. Because hESC differentiation is irreversible under normal culture conditions, effective DE creation is critical for proceeding pancreatic cell induction. hESCs that do not develop into endoderm are unlikely to become pancreatic progenitors and, as a result, hormone-producing cells. Furthermore, non-endodermal cells may produce signals that prevent DE cells from progressing to the pancreas. TGF- and Wnt signals have previously been implicated as key signals that drive DE formation *in vivo*[95-98].

Thus, activin A, a TGF-family member, was employed in combination with Wnt3a at high concentrations to efficiently induce DE *in vitro*[98-100]. Further research revealed that serum components required for ESC survival and self-renewal contain phosphatidylinositol 3-kinase pathway activators that prevent DE induction[101]. As a result, newer procedures demand that serum be removed during the initial differentiation process[100,102,103].

It's worth noting that the efficiency of DE formation can be determined by comparing transcription factor quantitative PCR and immunohistochemistry staining sequences to those discovered in a growing embryo. For example, hESCs activate markers of mesendoderm, a transitory tissue that leads to both the mesodermal and endodermal germ layers, in response to the initial activin treatment. After activin treatment, expression of the gene BRACHYURY/(T), a hallmark of this cell lineage, surges immediately and subsequently drops swiftly when cells transition to endodermal characteristics. Testing for upregulation of endodermal markers such SOX17, FOXA2, and GATA4) can verify the establishment of DE[100,103-105].

Importantly, the absence of genes like SOX7 expression in primitive endoderm, parietal, and visceral endoderm, tissues that are comparable to DE but do not give rise to gastrointestinal organs during development, must be taken into account[106].

Because DE cells express the cytokine receptor CXCR4, fluorescent-activated cell sorting gives new tools for DE cell purification[106,107]. Thus, by combining activin and Wnt3a treatment and monitoring the expression profile of a variety of cell lineage-specific markers, governed specialization of ESCs into DE can be achieved.

DE is partitioned into defined sections along the anterior-posterior axis during development, eventually giving rise to epithelial-derived organs such as the liver, lung, gut, and pancreas (reviewed in Ref. 17). After activin A was removed from the differentiation media for the induction of foregut endoderm from DE, Fgf proteins were introduced to the differentiation media to govern the specification of gut-derived organs[100,108]. Fgf therapy causes the expression of FOXA2, a DE marker, to remain constant, as well as the induction of transcription factors HNF4 and HNF1ß, which are commonly expressed in the developing gut tube[109-112].

The stimulation of pancreatic epithelium is the next step after the creation of primitive gut endoderm. The activity of Shh, a member of the Hedgehog signalling system, must be suppressed in order for pancreatic buds to be defined *in vivo*[113]. Treatment with cyclopamine, a cholesterol analogue known to impede the activity of Smoothened[114,115] a key component of the Hedgehog signalling cascade, can imitate Hedgehog inhibition in cell culture.

When cyclopamine is added to the differentiation media at this stage, it facilitates the growth of pancreatic endoderm, which is characterised by the expression of PDX1, the ancient marker of pancreatic epithelium, as well as a number of other transcription factors involved in pancreas organogenesis[93,104], Retinoic acid is used to encourage PDX1-positive pancreas progenitors to adhere to the endocrine lineage rather than pancreatic exocrine cells[93,104,116] NGN3, a transcription factor required for endocrine cell development, is highly up-regulated at the end of this phase.

NGN3-positive endocrine progenitors emerge to five different endocrine cell types in the islets of Langerhans during development. Despite the fact that cells producing endocrine hormones were created in cell culture, recent investigation shows that these cells have not achieved the full maturation phase found in adult human islets. Insulin-producing cells co-express additional hormones including glucagon and somatostatin, according to immunostaining with antibodies against several hormones.

The proportions of insulin-positive cells were also modest (approximately 7% of the overall cell population), presumably due to the absence of the second wave of PDX1 expression seen in embryonic endocrine cells during the final stage of differentiation. PDX1 is essential for optimum β-cell function as well as differentiation, and its absence could explain at least part of the limited response to glucose stimulation. Furthermore, there is no evidence of MAFA expression, a transcription factor that is generally elevated during the latter phases of beta-cell creation.

Interestingly, when given the right stimuli, ESC-derived endocrine precursors that give rise to multihormone positive cells in culture have the ability to become functional endocrine cells, including β-cells. When endocrine precursors were transplanted into immunocompromised severe combined immunodeficiency mice, final differentiation of β-cells and other hormone-secreting cells was achieved, implying that yet unidentified signals or cell-cell interactions are required to promote final endocrine differentiation[108].

Importantly, implantation of hESC-derived endocrine precursors resulted in insulin-positive cells capable of reversing hyperglycemia produced by streptozotocin, a chemical that causes apoptosis in murine but not human cells. The diabetic phenotype returned after the transplanted cells were removed, further confirming the theory that hESC-derived cells were required to restore normoglycemia[108]. These findings support the idea that hESCs have the ability to differentiate into fully differentiated cells, including pancreatic endocrine cells.

Generation of insulin-secreting cells through nuclear reprogramming

As mentioned in the preceding section, Beta-cell specification and differentiation in the developing pancreatic is dependent on a battery of transcription factors that work in an extremely coordinated temporal and spatial manner. This procedure can be

reproduced in differentiating ESCs by adding growth factors that stimulate expression of the proper combination of transcription factors. Nuclear reprogramming, a more direct genetic engineering technique, has also been investigated by constitutively expressing key transcription factors in ESCs[117-120].

Although no functional β-cells were created from these techniques, early reports showed that ESCs expressing high quantities of exogenous Pdx1, Ngn3, and Pax4 might accomplish more robust pancreatic differentiation[117-120]. Although direct production of transcription factors can guide cell differentiation, it is worth noting that during in vivo pancreatic differentiation, the quantity and duration of these transcriptional signals are finely controlled. The forced expression of these factors at an inopportune moment or at non-physiological amounts causes abnormalities in typical ESC characteristics or cell fate decision during differentiation.

For example, despite increased differentiation toward pancreatic lineages in general, continuous overexpression of Pdx1 failed to trigger the specification of insulin-secreting cells[121] (140). Similarly, early Ngn3 expression during pancreatic development favours the production of β-cells at the expense of other endocrine cell types[122,123]. Furthermore, several transcription contributing factors in pancreatic development also play a role in neural differentiation.

Furthermore, several transcription factors involved in pancreatic development also play a role in neural differentiation. Overexpression of certain elements, such as Ngn3 and Pax4, at inopportune times may result in the formation of nonpancreatic neuronal cell types that share some but not all pancreatic cell features. In this regard, it's uncertain if the insulin-secreting cells generated by this approach are endodermal or ectoderm cell types.

Bernardo *et al*[124]. Recently used a more complex approach to selectively regulate Pdx1 expression in differentiating mouse and hESCs, mimicking the biphasic expression of Pdx1 during normal pancreas development.An increase in -cell counts was attained with the activation of the second wave of Pdx1 expression in late differentiation stages.These insulin-secreting cells, unlike Beta cells in mature islets, express MafB rather than MafA.Furthermore, the cells did not respond to high glucose stimulation, implying that full activation of the Beta-cell state has yet to be reached and that exogenous expression of other components may be required[124].

ROLES OF OTHER STEM CELLS IN THE DIABETES MELLITUS

Adult stem cells in the treatment of Diabetes

Pancreatic stem cells

Over the last decade, technological advances have enabled scientists to extract stem cells from a variety of tissue sources, including bone marrow, umbilical cord blood, adipose tissue, skin, periosteum, and dental pulp. The pancreas is the first organ to be examined for potential stem cells. Limited amounts of pancreatic tissue have been shown to recover full pancreatic-cell mass in animal studies[125,126].

This is due to the replication of differentiated β-cells in the pancreatic ducts, as well as their differentiation into pluripotent cells, which develop more β-cells. This population of ductal cells could be cultivated and guided to form insulin-producing clusters in vitro, according to further research[127-129] created a clonal population of adult pancreatic precursor cells that can produce both C-peptide and insulin from ductal cells. Multipotent stem cells have been discovered in both rodent and human islets, as per research[130,131].

Despite the success and potential of pancreatic stem cells, several researchers have speculated about the existence of pancreatic adult stem cells[132]. Later[133] found clear evidence for multipotent progenitor cells in the pancreatic ducts of mice that can give rise to new ß-cells. These and many other studies conclusively show two facts: first, that pancreatic stem cells exist, and second, that non-cells can be transformed into ß-cells.

However, further research is needed to find and activate pancreatic stem cells in diabetic patients in order to facilitate the development of ß-cells. More research is needed to establish appropriate methods to resolve the issue of stem cell isolation and ex vivo expansion for transplantation[134].

Progenitor stem cells

The identification of progenitor cells within the adult pancreas has paved the way for various

advances. The adult stem cells of the haemopoietic system can differentiate into a number of other cell lineages, including lung, liver, brain and even gastrointestinal tract cells[135-138]. The bone marrow cells of the mouse were differentiated into functionally efficient beta cells in an *in vivo* experiment[139]. Pancreatic progenitor cells were differentiated into islets in vitro and then were transplanted into STZ-induced mice, progenitor cells were seen to migrate into the injured pancreas, resulting in rapid differentiation into IPCs which resulted in reduced glucose levels towards normoglycemia. Experiments with the mice was performed which indicated that the bone marrow cells could be targeted to the pancreas and reversal of hyperglycaemia could be achieved[140]. Stem cell-like cells with the power to be expanded and form clone's ex vivo have also been reported. These cells have the power to proliferate and form cellular aggregates that display the capacity for endocrine and exocrine differentiation. These results suggest that stem/progenitor cells exist within the pancreas which these cells could be a source for brand spanking new islets.

Induced pluripotent cells in treatment of Diabetes

Induced pluripotency is the process of production of pluripotent stem cells from nonpluripotent source. Induced pluripotent stem cells are produced from somatic cells which are reorganized to produce pluripotent stem cells under appropriate conditions. Specific transcription factors are expressed directly to achieve induced pluripotency[141]. These iPSCs are preferred for cell-based therapy for diabetes management due to its property of being patient specific and removes the possibility of rejection. The fibroblast cells are induced to produce iPSCs which are converted to pancreatic beta cells by a three staged differentiation process. The fibroblast derived beta like cells were transplanted in diabetic mouse model which controlled hyperglycaemia for long term[142]. The fibroblastic cells from type 1 diabetic patient were induced to produce pluripotent stem cells by the lentiviral transfection method which was identified as insulin secreting cells[143]. With these advancements induced pluripotent stem cells were used in cell therapy for diabetes.

TREATMENT OF TYPE 1 DIABETES WITH MESENCHYMAL STEM CELLS

Molecular and repair mechanism of mesenchymal stem cells in treatment of diabetes

In vitro technique of inducing and differentiation to convert stem cells to insulin producing cells is carried out as one of the methods to treat diabetes using mesenchymal stem cells which in carried out in presence of appropriate growth factors and suitable in vitro environment[144]. The functions of stem cells are under the control of intrinsic genetic program and signals from extracellular sources[145,146]. MSCs would the perfect source for β cell generation due to its advantage of microenvironment which is tissue specific and plays an important role in pluripotent cell differentiation.

Many articles and reports have explained the presence of tissue specific pancreatic mesenchymal stem cells which share similar characteristics of the ability to differentiate into osteocytes, adipocytes as similar to MSC under specific induction media[147]. Findings from one of the reports showed that proliferating mesenchymal cells are obtained from transition of epithelial mesenchymal type of pancreatic cells which is then converted to IPC under in vitro condition[148].

Administration of Mesenchymal Stem Cells through systematic method enhances β-cell and reverses the condition of hyperglycemia in diabetic mice induced with streptozotocin[149]. Suggestion from Dor and his co members showed new β-cells could be generated only from which are in pre-existing forms and his studies was concluded with the finding that MSCcould act only as Btropic mediator for functioning of islet and for promoting angiogenesis[150]. IPCs could also be obtained from regeneration of transdifferentiated cells which includes pancreatic duct cellsand acinar cells[151-153].

Chen and his fellow researchers[154] demonstrated trans differentiation of MSCs from rats under *in vitro* conditions which was converted to insulin-producing islet-like cells which was functional andresulted in active control of levels of blood glucose in diabetic rats.Pdx-1is the important factor responsible for trans differentiationto happen in MSCs which would convert to β-cells[155,156]. Neurogenin 3 (Ngn3), paired box gene 4 (Pax4)and epidermal growth factor (EGF) are among the other

transcription factors involved in regeneration of β-cells[157-159]. Glucagon- and somatostatin-expressing cells can also be transdifferentiated from MSCs[160].

Gao and his fellow researchers explained the MSCs paracrine mechanism[161] by validating the effects of cytoprotective medium from MSCs cultured medium which was exposed to STZ under in vitro conditions. The results showed that positive transplantation of MSCs results in regeneration of β-cells which reduces hyperglycemia in diabetic mice. β-cell regeneration is accomplished by replication of beta cells and differentiation of islet progenitor. Transplantation results in an Enhanced phosphorylation of Akt and Erk is achieved by transplantation of MSC islets under *in vivo* which also promotes proliferation of islet cell followed by increased phosphorylation of Akt and Erk under *in vitro* condition. The MSC conditioned media induces β-cell proliferation is completely restricted by treating it with PI3K/Akt inhibitor LY294002.It could be drawn that PI3K/Akt signal pathway plays a major role in proliferation of β-cell after transplantation of transplantation[161].

MECHANISM INVOLVED IN TRANSPLANTATION OF PANCREATIC ISLET BY MESENCHYMAL STEM CELLS

In one of the major study and report[162], Figliuzii and fellow research workers had interpreted an approach in which MSC can play a major role in pancreatic islet transplantation and its associated functions. If transplanted with pancreatic islets, MSCs have probable chance of exerting properties associated with pro-angiogenic and immunomodulatory effects which are released from angiogenic factors of MSCs. these in turn improve vascularization of islet cells and helps in functioning of graft in transplantation of islet cells. Drastic reduction in damage caused to islets by inflammatory effects can be brought by the immunomodulatory effects. These also are involved in slumping of autoimmunity by its ability of inhibiting proliferation of T cells[162].

Sakata and her co researchers have addressed as to how angiogenesis can be promoted by bone marrow which is followed by co-transplantation along with islets. They analysed and found that level of blood glucose was lesser thanthe levels of serum insulin in mice when both islet cells are administered along with the MSCs of bone marrow. Expression of VEFG was at increasing levels mice with bone marrow MSCs implanted than islet implanted mice. Positive regions of PDX-1 were found in bone marrow cells with enhanced stain over period of time. With this we could conclude that when bone marrow cells are co-transplanted with cells of islets it enhances vascularization of graft of islet and its function[163].

Bone marrow cells would be the perfect choice as they have the ability to perform differentiation to produce pancreatic β-cells along with which they are effective at boosting β-cell regeneration in injured regions of pancreatic tissue. Even without differentiating into β-cells it could be seen that transplantation of bone marrow stem cell decreases plasma glucose levels and enhances systemic insulin. Bone-marrow stem cells offer best treatment as MSCs blocks T cell-mediated immune responses against β-cells which are newly formed[164]. MSCs showbest and enhanced properties of immunosuppression and angiogenic effects due to which many are examining methods of co-transplantation of MSC into islet for studing its function and recovery[165-167].

Transcriptional factors involved in causing the differentiation of bone marrow mesenchymal cells (BM-MSC) to insulin producing cells

Surrogate β-cells were obtained by trans differentiating, dedifferentiating, or differentiating target cells to surrogate β-cells in the conventional way by expressing some critical transcription factors involved in pancreatic development and β-cell gene expression[168,169.]

In this study, combination of 3 transcriptional factors PDX-1, NeuroD1, and MafA were delivered in into the BM-MSC on adenoviral vector for insulin producing cells. In mMSCs, overexpression of PDX-1, NeuroD1, and MafA significantly increased insulin gene expression and triggered insulin production and secretion. When compared to a single or double infection, the triple infection has a far greater impact.Any single component or combinations of two factors were able to trigger insulin expression in this investigation, however the effect in mMSCs was too weak in comparison to the specific combination of these three factors.In gene

detection assays, genetic transformation of PDX-1 stimulated endogenous NeuroD1 expression, and exogenous NeuroD1 or MafA could activate endogenous PDX-1. The results of the experiment suggested that each transcription factor may have an adjustment or interaction. However, PDX-1 and MafA, when combined with endogenous NeuroD1, were unable to have the same effect on insulin gene expression as a combination of the three transcription factors. Due to the low expression levels of induced components, we believe that fine synergism could not be accomplished.

The MSCs' intracellular Green Fluorescence protein was then started to express 3 days after gene delivery, close along with the factors. However, with the breakdown of partial mitochondrial DNA, the intensity of the fluorescence diminished one week later. As a result, induced efficiency was drastically reduced without the need for a second infection. To confirm the function of generated IPCs *in vivo*, researchers transplanted cells into the liver parenchyma. The findings of an Intraperitoneal glucose tolerance test showed that these implanted cells could handle a glucose load, and their glucose tolerance was comparable to that of normal mice. It should be mentioned, however, that after additional 7 days, impaired glucose tolerance was discovered. It's possible that the induced IPCs that were implanted did not proliferate.

Finally, this study concludes that genetic alteration resulting in infection of mMSCs with a combination of PDX-1 NeuroD1 and MafA, as well as their subsequent expression, greatly improved insulin-producing ability. However, research is being conducted to determine the differentiation of mMSCs that express a mixture of the three factors *in vivo* and their long-term stability in order to maintain stringent blood glucose levels[170].

Approaches to increase β-cell mass

The loss of beta-cell mass and function occurs in both type 1 (T1D) and type 2 (T2D) diabetes. T1D is characterised by autoimmune destruction of beta cells, which results in an absolute reliance on exogenous insulin, whereas T2D is characterised by peripheral insulin resistance, which can lead to beta-cell decompensation and failure. Apoptosis, differentiation, neogenesis, and proliferation are all processes that control overall beta-cell mass[170].

Cell cycle protein

Overexpression of cdk-4 and cyclin D1 in islets was one of the first pieces of evidence of induction in human beta cells. Furthermore, overexpression of the cyclin-dependent kinase cdk-6, with or without cyclin D1, increases human beta-cell proliferation in both intact and dissociated islet cells[171].

GSK-3

The Wnt pathway appears to be involved in beta-cell survival and proliferation, according to multiple lines of evidence[172,173]. Wnt signalling activation has been demonstrated to increase beta-cell proliferation in beta-cell lines and primary mouse islets, as well as over expression of cell-cycle genes such as cyclins D1 and D2, and cdk-4[173,174]. Furthermore, upregulating axin expression, a negative regulator of Wnt signalling, blunts Wnt-stimulated gene expression and inhibits beta-cell proliferation in islets, but upregulating beta-catenin expression induces an expansion of beta-cell mass *in vivo*[174].

Glucokinase

In the first phase of glycolysis, glucokinase (GCK) phosphorylates glucose to glucose-6-phosphate, as previously stated. It is the most common hexose kinase in beta cells and serves as a glucose sensor in these cells. Wild-type mice showed considerable beta-cell hyperplasia when fed a high-fat diet, however GCK heterozygous animals were unable to increase beta-cell replication adequately, leading to the conclusion that glucokinase plays a vital role in promoting beta-cell replication[175]. GCK+/ animals were fed a high-fat diet in the presence or absence of a glucokinase activator (GKA) for 20 weeks to investigate this[175]. After chronic treatment, there were no significant variations in beta-cell proliferation, while beta-cell proliferation was considerably enhanced after an acute 3-day treatment with GKA[176].

GLP-1

A recent study found that human beta cells transplanted into mice treated with exendin-4 replicated more efficiently, but only when the islets were from youthful donors[177]. Such findings raise questions concerning the differences between mouse and human models in terms of molecular machinery and islet physiology. The fact that GLP-1 appears to activate Wnt in a TCF7L2-dependent

way, on the other hand, is positive for a translation to human biology, as TCF7L2 single-nucleotide polymorphisms have the strongest genetic connections with T2D[178]. It's possible that the benefits of GLP-1 activation are only visible when given to an entire organism, rather than just cells[179].

GPR119

Another promising target for T2D therapy is G protein-coupled receptor 119 (GPR119)[180]. GPR119 was discovered as an orphan GPCR in a variety of mammalian species[181,18]. Beta cells and GLP-1-secreting intestinal L-cells are the main targets of this receptor[181]. GPR119 is activated by the phospholipids lysophosphatidylcholine and oleoylethanolamide (OEA), which increase intracellular cAMP levels and cause glucose-dependent insulin production[182,183] Gao *et al*,[183,184] looked at the effects of OEA and synthetic agonists (PSN632408 and AR231453) on murine beta-cell replication and discovered that they all increased the amount of replicated beta cells when compared to control mice[185,186].

Chemical Screening and Beta-Cell Proliferation

One of the first research looked at the growth of the reversibly immortalised mouse cell line R7T1 using a heterocyclic library of 850,000 chemicals[187]. This beta-cell line was immortalisedutilising the SV40 T antigen and the Tet-On method, which causes the cells to proliferate in the presence of tetracycline but stop growing when it is removed[188]. In streptozotocin-treated animals, these cells display distinctive beta-cell markers (insulin 1, insulin 2, and Pdx1), produce insulin, and restore euglycemia[188].

Phosphorbol esters, dihydropyridines (DHP), and thiophene pyrimidines are among the structurally varied active chemical classes discovered. Thiophene pyrimidines, in particular, appeared to promote beta-cell proliferation through activating the Wntsignalling pathway. With an IC50 of roughly 1.1 M, a piperazinyl derivative induced R7T1 proliferation in a dose-dependent manner. In MIN6 and HIT-T15 beta-cell lines, as well as primary rat beta cells, the same chemical was active. Surprisingly, this chemical turned out to be a strong inhibitor of GSK-3[170].

Calcium channels regulate glucose-stimulated insulin secretion and insulin synthesis in beta cells, and polymorphisms in calcium channel-encoding genes have been linked to both T1D and T2D[189,190]. However, it's also plausible that these mutations cause beta-cell proliferative abnormalities. Indeed, knocking out the LTCCa1D subunit resulted in a considerable decrease in postnatal beta-cell proliferation, implying that calcium channel signalling is required for beta-cell replication.

Molecular Insights into pancreatic regeneration: Moving beyond conventional diabetes mellitus treatments through stem cell therapy[191]

The major organ that consistently regulates glucose homeostasis is Pancreas.

Factors which influence the stem cells to develop into pancreas-: Stem cell differentiation into pancreatic progenitor cells are influenced by the expressions of certain factors. Most of the protocols which help in the stem cell differentiation focuses on the generation of mature, single hormone-expressing, human β responsive to glucose, using information from studies of pancreatic development[192,193].

In the programming of insulin –producing β cells specific signals are involved, The transcription factors which are involved in the formation of the endoderm during gatrulation are SRY (sex determing region Y)-box (Sox)17 and home box gene HB9 (H1xb9) are involved which induces the development of pancreas.

Certain foregut formation which induces the development of the pancreas are fibroblast growth factor (FGF)-10, retinoic acid, SOX9, and hedgehog signaling pathways.

According to the current research the specification for the stem cells to develop into pancreas and its budding occur through pancreas-specific transcription factor-1a (Ptf-1a), pancreatic andNK6 homeobox 1 (Nkx6.1), neurogenin-3 (Ngn-3,duodenal homeobox 1 (PDX-1),and mafA[194], enabling endocrine formation.

+ The consequent stimulation of ISL LIM homeobox 1 (Isl-1), neurogenic differentiation factor (NeuroD), paired box gene (Pax)4, NK2 homeobox 2 (Nkx2.2), and Pax6 signaling plays an important role in the formation of the islets of Langerhans.

The transcription factors whichare expressed throughout pancreatic development are Sox17,

hepatocyte nuclear factor (HNF)-6, and HNF-3beta (also known as forkhead box A2, Foxa2). Finally, the stimulation for neogenesis to create β cells occurs via FGF-10 and notch signaling-induced stem cell and pancreatic progenitor cell differentiation[195,196].

Recent research has helped to obtain β cells, either by inducing the expression of pancreatic-related transcription factors in distinct types of stem cells or by supplementation of soluble factors during culture. (Figure No.3).

Embryonic stem cells

The ESCs (Embryonic stem cells) have been proven as the best model for pancreatic regeneration. To induce the differentiation of ESCs into endocrine cells that are positive for insulin, somatostatin, and glucagon expression, transgenic expression of PDX-1 and Nkx6 were seen[197]. The formation of ESC-derived C-peptide/insulin-positive islet-like cell clusters that release insulin upon glucose stimulation are regulated by some of the growth and extracellular matrix factors, including laminin, nicotinamide and insulin. It also releases Pax4[198].

Retinoic acid (RA) plays a major role in the differentiation of ESCs and hence it is crucial for pancreatic development. Activin A-induced human ESCs expressing CXCR4 becomes 95% positive for pancreatic marker PDX-1 when RA is added to it[199]. Mature β cells encapsulated in alginate and transplanted into a streptozotocin (STZ)-induced diabetic mouse model result in effective glycemic control are seen in some of animal model studies that have human ESC-derived glucose-responsive cells[200]. However, clinical applications of ESCs are limited due to the ethical implications. Therefore, induced pluripotent stem cells have been proposed as a suitable alternative cell source with the same pluripotent characteristics as ESCs. (Figure No.4).

Induced pluripotent stem cells

When human somatic cells are reprogrammed for the generation of stem cells which have pluripotent properties the (iPSC) are formed. New studies showed that they were successful for producing glucose responsive β-like cells[201-204]. As it is difficult, to obtain an efficient and replicable β cell differentiation due to complex processes involved in β cell development protocol. A potential solution has been proposed to initiate differentiation in those cells which have a prolonged proliferation potential and the ability to produce C-peptide positive β cells. Those cells are human iPSC-derived PDX-1 and SOX9-expressing pancreatic progenitor cells which are involved in the production of β cells[205].

Other coherent protocol which triggers the differentiation in the stem cells are the supplement factors which are epidermal growth factor (EGF), thyroid hormone, and RA signaling ,transforming growth factor β (TGF-β), as well as ɤ-secretase inhibition[202]. It also induces the β cells with the ability to initiates Ca^{2+} flux in response to glucose, package insulin into secretory granules, and secrete insulin. Sodium cromoglicate in combination with a previously described protocol causes the induction rate of insulin-positive cells to increase[206]. The problems associated is the when transplanted they may induce autoimmune response and sometimes cells which are not differentiated may give rise to tumours, but use of mesenchymal cells can solve this. (Figure No.5)

Mesenchymal stem cells

Mesenchymal stem cells are considered one of the best option for regenerative medicine as they have certain properties like multi-potentialities, low antigenicity, reduced toxicity, self-renewal ability, pluripotency and ease of culture and expansion *in vitro* to obtain sufficient cells for treatment[207,208].

MSC are defined by certain criteria as explained by the International Society for Cellular Therapy. The criteria includes adherence to the plastic in culture, express the cell surface markersCD73, CD105, and CD90[209], lack expression of CD45, CD14 or CD11b ,CD34,or CD19, CD79a, and HLADR surface molecules[210] and also have the capacity to differentiate intochondrocytes, adipocytes, osteoblasts[208]. MSCs are located at different parts of our body i.e the bone marrow, placenta, adipose tissue, umbilical cord and amniotic fluid, blood. MSCs have also been shown to be able to differentiate into insulin-producing cells (IPCs)[211]. MSCs have feasibility for islet transplantation[212,213], through the suppression of inflammatory damage and immune-mediated rejection they demonstrates improved engraftment of pancreatic islets.

The immunodulatory properties of MSCs are mediated through cell–cell interactions and/or

secretion of soluble factors[214]. T cell activation and leukocyte recruitment to the inflammatory site through CD106 are the cell mediated responses seen in MSCs. These $CD106^+$ cells which shows unique immunoregulatory properties and help in activating the T helper cells are derived from the PDMSCs which are isolated from the chorionic villi .$CD106^+$ also induce tumor necrosis factor (TNF)-α/interleukin (IL)-1b-mediated MSC expansion[215]. When hepatoma cell line is cocultures with the Umbilical cord derived MSCs they effectively alleviates palmitic acid and lipopolysaccharide-which further induce insulin resistance by blocking the activation of NLRP3 inflammasome and inflammatory agents[216,217].

Recent research showed that when the type 2 diabetic rats were infused with MSCs hyperglycemia was notably ameliorated, and reduction in the inflammatory was seen. Therefore it results into improved insulin sensitivity in insulin target tissues.

The problems associated with the transplantation like graft rejection of stem cells can be solved by using bone marrow-derived MSCs(BM MSCs). It has the immunomodulatory properties such as reduced inflammatory markers and increased immune tolerance markers, demonstrating it has the ability to solve the issues. The 2 ways are as follows-:

Diabetic NOD mice when induced with adipose-derived MSCs(AD-MSCs) it reverses hyperglycemia through inducing high serum amylin, insulin and glucagon like peptide 1 levels compared to untreated controls. The reduction of inflammatory cell infiltration, CD^{4+} T helper (Th) 1 cells, interferon-γ ,and also expanded Tregs present in a cell contact-dependent manner *in vitro* and within the pancreas is done by AD-MSC[218].

The type 1 diabetes can be controlled by administration of bone marrow-MSC-derived extracellular vesicles which was infused into ice resulted in the inhibition of antigen-presenting cell activation and suppression of Th1and Th17 cell development[219].

Interstingly, a recent study in a UC-MSC model demonstrated that MSC-derived IPCs exhibited hypo-immunogenic characteristics *in vitro* but became immunogenic after transplantation to the host, possibly due to activation from the immune microenvironment[220].

The current studies showed that MSC-derived IPCs in vitro exhibited hypo-immunogenic characteristics, but as they are transferred to the host they became immunogenic because of the activation from the immune microenvironment

MSCs also have an additional effects beside immunomodulatory effects, that provide a supportive micro-environment niche by secreting paracrine factors and depositing extracellular matrix[221]. They also have another role in the regeneration of endogenous β cells. Recent research showed that BM-MSCs from mice can differentiate *in vitro* into IPCs and these IPCs also express marker genes which are pancreas-specific[222].

In BM-MSCs the mutation occurs and PDX-1 in human over expression occurs and it results into differentiation of IPCs[223]. The 3 genes plays a major role, when BM-MSCs are transfected with PDX-1, Neuro D, and Ngn-3, they starts to differentiate into insulin-expressing cells *in vitro*. The BM-MSCs lack glucose-responsive insulin expression.

However, when the BM-MSCs are transplanted inside the mice they are seen to reduce the level blood glucose level in diabetic mice. Recent studies showed that when the IPCs which are differentiated are infused in the patients they results into the fall of a 30–50% in their insulin requirement, but showed a 4- to 26-fold uprise in serum C-peptide levels[224]. Specific antigen 4 (SSEA4) and octamer 4 (Oct4) which is expressed in umbilical cord blood can differentiate into insulin-producing islet-like cells that express insulin and C-peptide protein[225], (Figure No.7).

Remaining challenges

The encouraging results of recent human islet transplantation attempts have paved the way for new diabetic therapy alternatives. Cell-based medicines, on the other hand, are still in the experimental phase, with a long way to go before they can be used as regular treatments. Some of the risks and side effects are linked to the islet implantation methodology itself, such as ischemic and enzymatic destruction caused by the islet isolation and purification protocol, as well as concerns about thrombosis and portal hypertension

caused by transplanting islets into the liver portal vein[226,227].

Risk of cancer formation upon transplantation of hESC-derived cells

Aside from the contentious ethical problems that impede the production of hESCs, stem cell-based therapies must be thoroughly validated in animal models before being used in humans. After cell transplantation, one of the primary issues is the persistence of undifferentiated ESCs capable of causing tumour growth[228,229]. When a group of cells enriched in pancreatic progenitors was implanted into mice, teratomas, the most likely tumour type predicted to form after hESC-derived cell transplantation, were detected[230].

Teratomas are cancerous tumours that develop from pluripotent stem cells that have stayed in the layers[230]. Transplanting a pure, homogeneous population of partially or fully differentiated cells derived from hESCs is one clear strategy to avoid the risk of tumorigenesis. The development of cell type-specific cell surface markers present on endocrine progenitor or fully differentiated β-cells would be required for such an approach. Then, fluorescence-activated cell sorting and magnetic-activated cell sorting could be employed to purify high-efficiency cell populations for transplanting.

Full maturation of β-cells diverse population and contain cells that have differentiated into tissues spanning various germ *in vitro*.

Many studies have shown that hESCs can produce insulin-secreting cells in vitro; nevertheless, these cells are most likely immature Beta-cells because they have low insulin concentrations, express many hormones in the same cell, and have a weak glucose response[231-233]. One reason why totally differentiated Beta-cells have yet to be generated in cell culture is the paucity of cell-cell interactions between mesenchymal and epithelial cells that occur throughout embryonic pancreas development *in vivo*.

Cells are cultivated in two-dimensional cultures as monolayers in the most recent in vitro differentiation methodology. As a result, there are no three-dimensional connections between epithelial cells during islet development *in vivo*. Furthermore, hESCs efficiently differentiate into DE and posterior gut cells[230,233] and this relatively pure cell population may absence the signals secreted by mesodermal and ectodermal cells that are required for successful pancreatic lineage differentiation during the final stages of endocrine cell differentiation. As a result, coculture with pancreatic mesenchyme or endothelial cells may enhance beta-cell specification and maturation efficiency. Screening for genes that are differentially expressed in the pancreatic mesenchyme could also lead to the addition of additional components to the differentiation media.

The distinct environment in which beta -cells in the pancreas and beta-cells grown in vitro live is a significant difference. Different hormone-secreting cells, neuronal cells, and vascular endothelial cells make up pancreatic islets, which are complex structures made up of many cell types. This three-dimensional structure appears to be crucial for insulin sensing and secretion, as it allows interactions between β-cells and endothelial cells, as well as contacts between β-cells and cells from other endocrine lineages[234,235]. Providing *in vitro*-generated β-cells with a three-dimensional scaffold laden with extracellular matrix that promotes β -cell aggregation and angiogenesis may help β-cell maturation and function after transplantation into patients. The effectiveness of nuclear reprogramming has inspired a unique technique for β-cell creation. Exocrine cells can be converted to insulin-secreting cells by introducing key transcription factors into acinar cells[236]. Pancreatic progenitor and endocrine precursor cells, both of which may be created through guided *in vitro* differentiation of hESCs, should also be studied since they may be more susceptible to gene transfer and provide a better genetic "context" for β-cell maturation than hESCs.

In the absence of lasting genetic alterations, tiny chemical substances have been found to influence cell differentiation[237]. Given the growing amount of libraries comprising a wide range of chemical compounds and technological advancements that enable quick screening of these libraries, finding molecules that guide specific phases during hESC to -cell differentiation has become feasible.

Transplantation-related immune rejection

When widespread transplantation of hESC-derived endocrine cells can become a reality, one final

worry must be addressed: how to avoid the recipient's immunological response. When it comes to islet transplantation, there are two sorts of immune reactions that must be addressed. An autoimmune reaction destroys β-cells in type I diabetic patients, and suppressing autoimmunity is critical for the effectiveness of islet transplantation-based therapy. As reported in a recent study by Chatenoud and Bluestone, much progress has been made in this area, notably in the subject of immunological tolerance[238].

The second immune response against grafted tissues occurs when the recipient's immune system detects the transplanted cells as foreign entities, triggering a host vs. graft immunological response. Despite the fact that superior immunosuppressive regimes are being explored and regularly modified in animal models and clinical studies[239,240], current developments in cellular reprogramming may offer new options for reducing or eliminating the allograft reaction.

New research has shown that reprogramming somatic cells into induced pluripotent stem (iPS) cells requires only a cocktail of four transcription factors. Because iPS cells are comparable to hESCs and may develop into cells from all three germ layers, patient-specific stem cell populations may become a reality in the future[241-244]. iPS cells have recently been produced from cohorts of human people who suffer from a range of diseases, including type 1 diabetes, to validate this theory[245]. Even though the first iPS cells were created by transfecting cells with lentiviruses or retroviruses and some of the transcription factors used for reprogramming are known oncogenes, recent research suggests that some of the factors can be replaced with small chemicals or histone deacetylase inhibitors[243], implying the possibility of creating iPS lines that are safe for human transplantation. Most recently, a virus-free reprogramming technique was established, marking yet another step toward clinical trials of iPS cell-based therapeutics[246,247]. Several laboratories are currently conducting research to see if iPS cells can respond to the current hESC development process and if iPS-derived-cells will be completely functioning.

Figure No.1 the mammalian pancreatic primordium is produced from the DE during embryonic pancreas development, and it gives rise to the primitive intestine and posterior foregut. Endocrine cells proliferate and gather during the development of pancreatic epithelium to form islets of Langerhans, which are aggregates of endocrine cells that also house insulin-secreting β-cells. Only the endoderm-derived pancreatic epithelium is depicted in the picture, with the surrounding mesenchymal tissues excluded for clarity. During various stages of pancreas and β-cell development, multiple signalling pathways have been shown to play critical roles. D'Amour *et al*. established a step-by-step differentiation technique for producing β-cells from hESCs based on this understanding. hESCs can be stimulated to develop DE, primitive gut (PG), posterior foregut (PF), pancreatic endoderm, and endocrine precursors by adding growth factors and small molecule inhibitors to the culture media (PE). After transplanting endocrine precursors into immuno compromised mice, fully matured β-cells can be obtained. The percentage of target cells at the conclusion of each stage indicates differentiation efficiency. Green represents activating pathways and chemicals, whereas red represents inhibiting pathways and compounds.

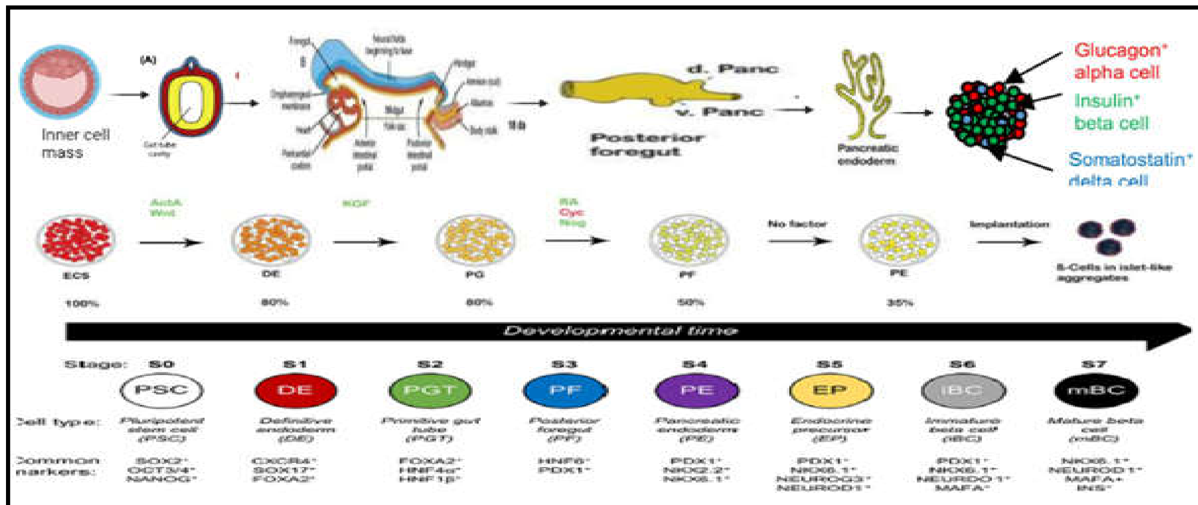

Figure No.1: Pancreatic organogenesis in mice and step-wise hESCs to β-cell differentiation are compared. RA stands for retinoic acid; Cyc stands for cyclopamine; Nog stands for noggin; Hh stands for hedgehog; VEGF stands for vascular endothelial growth factor; d. Panc stands for dorsal pancreas; v. Panc stands for ventral pancreas; KGF stands for keratinocyte growth factor

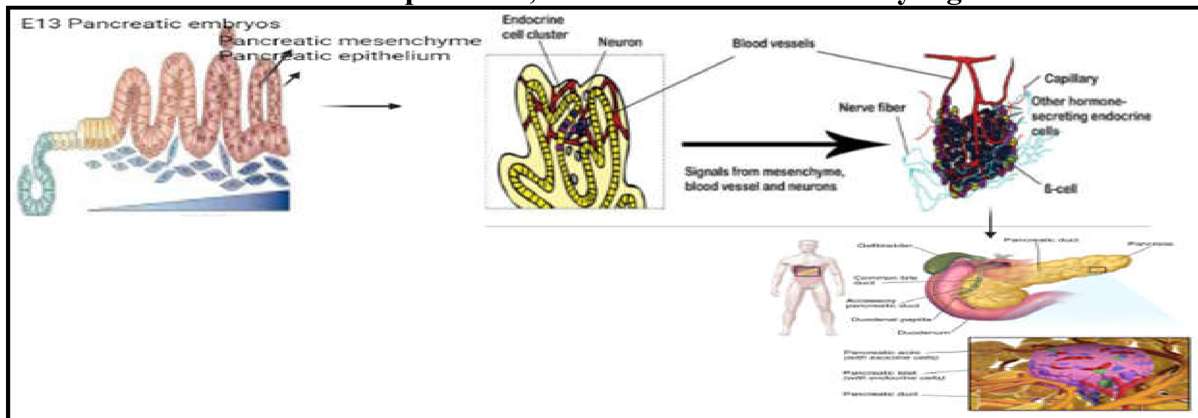

Figure No.2: Islet formation and endocrine cell maturation are aided by signals from tissues surrounding the pancreatic endoderm. Developing endocrine cells detach from the duct-like pancreatic epithelium at E13 and form endocrine cell clusters. The creation of the islet of Langerhans is dependent on signalling chemicals produced by the pancreatic mesenchyme, blood arteries, and migrating neural crest cells. In the adult islets, vascular endothelial cells and nerve fibres are also significant regulators of hormone synthesis and secretion

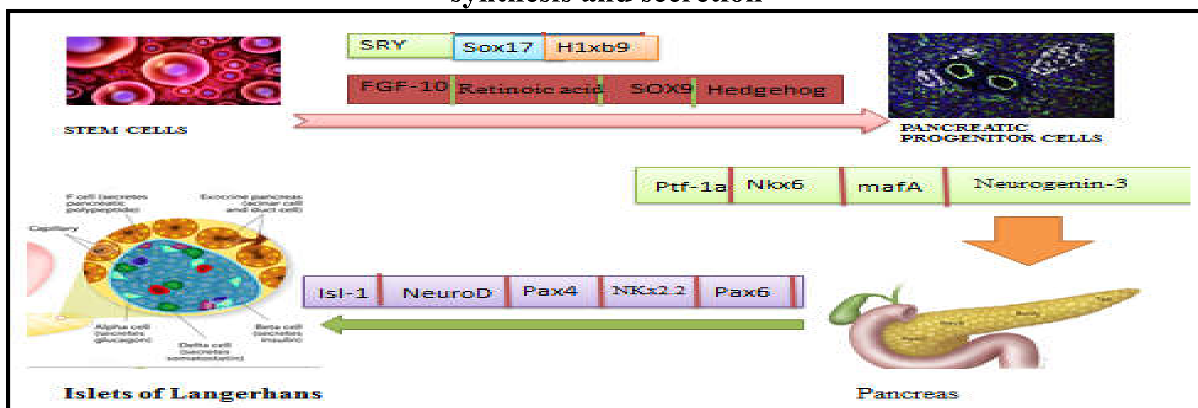

Figure No.3: Molecular events which leads to the formation of pancreas and Islets of Langerhans from stem cells

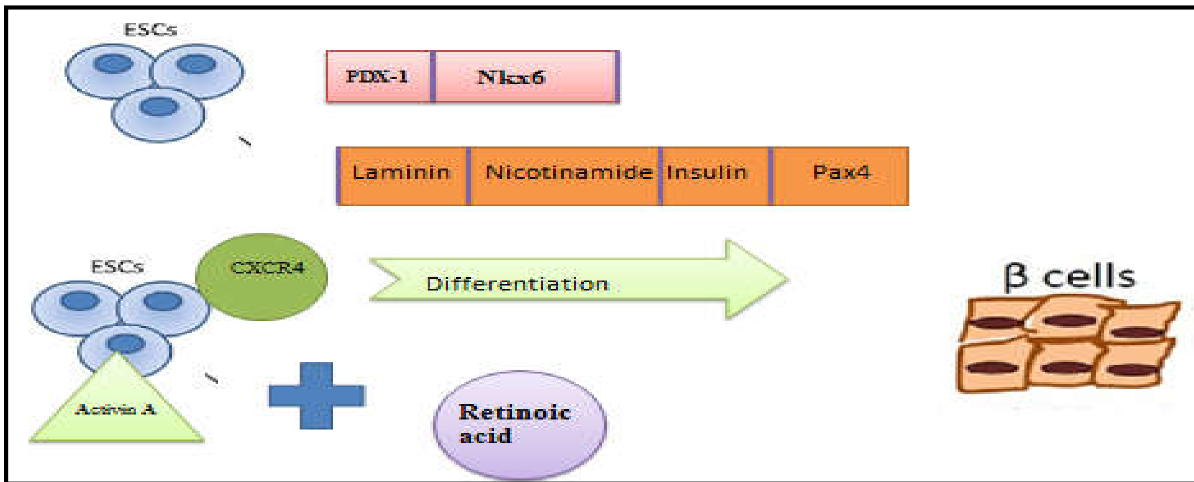

Figure No.4: Molecular events which leads to the formation of β cells from Embryonic stem cells

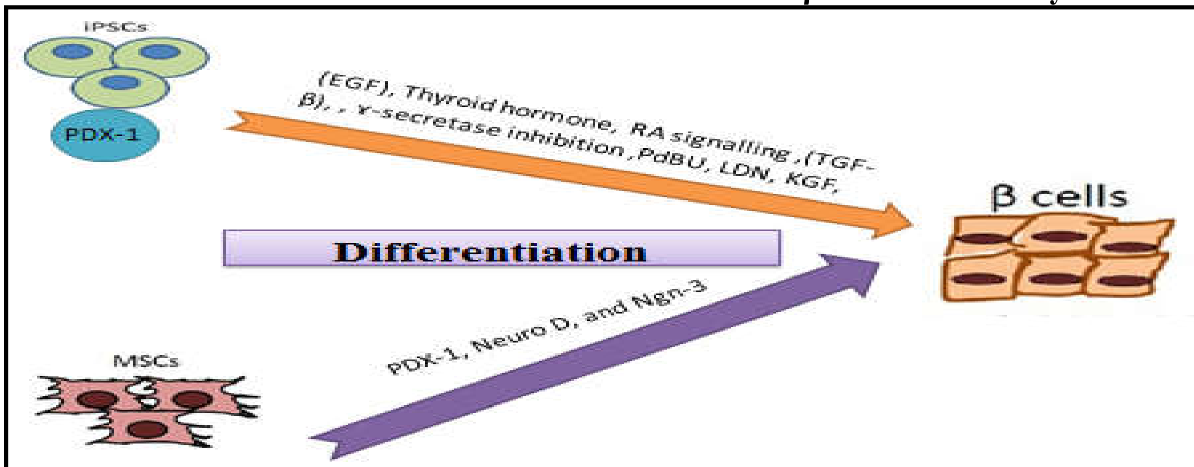

Figure No.5: Molecular events which leads to the formation of β cells from iPSCs; MSCs

CONCLUSION

The major aim of this article focusses on treatment of Type 1 Diabetes Mellitus using molecular mechanism of stem cells. We have concluded that function of paracrine of MSC's which are transplanted has an important role on comparison with cell trans-differentiation. With the help of this function the transplanted MSCs would effectively help in treating type 1 diabetes condition. Excellent results are obtained when MSC are co-transplanted with cells of islet. MSC's would show best results due to its ability of fibrosis mitigation, enhancing vascular growth and management of inflammation along with its abundant availability from various organs and cells. There is still a need to enhance stem cell therapy mediated by MSC in the process of targeting of tissues, engraftment and enhancing production of cells. Facing these challenges successfully would have enhanced usage of these techniques in clinical aspects of treating MSC for Type 1 Diabetes Mellitus. Hence, techniques of regenerative medicine would be novel in the field of research in treatment of patients with diseases.

The report also indicated that genetic alteration resulting in infection with a combination of PDX-1 NeuroD1 and MafA, as well as their subsequent expression, dramatically improved the insulin-producing function of mMSCs, as well as the chemical that stimulates beta cell proliferation.

ABBREVIATIONS

ESC - Embryonic Stem Cells
iPSCs - Induced pluripotent stem cells,
DM - Diabetes mellitus,
Hb1Ac - Glycated Haemoglobin,
CD - Cell Divison

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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