Pancreatic Progenitors as Target for Islet *Neo*genesis to Manage Diabetes

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Abstract

Beta-cell replication and islet *neo*genesis are a major challenge in diabetes research. Transplantation of islets in diabetic patients has been initiated years ago; however, shortage of donor pancreas and autoimmune rejections has limited their clinical implications. Although attempts are being made to generate islets from pluripotent stem cells, their clinical applications are restricted due to ethical concerns and teratoma formation. To overcome these limitations, transdifferentiation of alpha cells and acinar cells and differentiation of ductal stem cells to beta cells are in the pipeline. The amicable substitute for islet transplantation is the islet *neo*genesis from pancreatic progenitors. The endogenous islet *neo*genesis could be accomplished with external clues employing combination of Reg protein/transcription factors/growth factors/mesenchymal stem cells to restore the lost beta cells mass. This chapter focuses on the pancreatic progenitor reservoirs within the pancreas as a target for inducing islet *neo*genesis in diabetes.

Keywords

Beta-cell mass • Diabetes mellitus • Islet neogenesis • Pancreatic regeneration • Pancreatic progenitors • Stem cells

Abbreviations

DPP4	Dipeptidyl peptidase 4
EMT	Epithelial mesenchymal transition
GABA	γ Amino butyric acid

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GLP1	Glucagon-like peptide
GLUT2	Glucose transporter-2
INGAP	ISLET neogenesis associated protein
IPCs	Insulin-producing cells
MTF1	Myelin transcription factor 1
Reg proteins	Regulatory proteins
VSEL	Very small embryonic-like stem cells

10.1 Introduction

The pancreas contains two distinct groups of cells – exocrine, portion consisting of acinar, and the duct cells – while the endocrine portion consisting of islets of Langerhans. The islet of Langerhans is the cluster of cells comprising of alpha (α), beta (β), delta (δ), epsilon (ε), and pancreatic progenitor cells which synthesize and secrete glucagon, insulin, somatostatin, ghrelin, and pancreatic polypeptide, respectively. The flaw in insulin production or secretion leads to diabetes [1]. Diabetes is a group of metabolic disorders which usually occurs with an increase in blood glucose level either due to nonfunctional β cells coupled with insulin resistance (type 2) or due to total lack of pancreatic β cells (type1). Type 2 diabetes is predominant (90% of the population) with β-cell dysfunction alarming for insulin therapy; however, proper lifestyle (diet and exercise) could manage this disorder up to 60% [2]. There has not been any successful prevention of type 2 diabetes despite of the various therapeutic strategies recommended by the clinicians. The scenario remains the same for type 1 diabetes. Type 1 diabetes in particular, which affects the 10% of the population, demands alternatives for its treatment other than insulin injection and transplantation. Transplantation of islets or pancreas faces major challenges, like (a) lack of cadaveric pancreas, (b) protection against autoimmunity and allo-rejection [3], and (c) cost intensive. Type 1 diabetes is the principal front line for replenishment of β-cell strategies for which the proof-of-concept has already been performed by islet transplantation [4]. Beta-cell replenishment can be carried out either endogenously or exogenously, hence making it major area of research. Exogenous insulin injection is one of the choices of treatment for type 1 diabetes and advanced type 2 diabetes. Transplantation of pancreatic islets from cadaveric pancreas and the insulin-producing cells generated from stem cells are the next best option for treating type 1 diabetes. However, as mentioned earlier the success rate for transplantation is not promising; alternative treatment has to be discovered.

Regenerative medicine therapies using stem cells and pancreatic progenitor cells for type 1 and type 2 diabetes are attaining lot of attentions. Another alternative could be endogenous replenishment of pancreatic β cells. Stem cells have the self-renewal capacity and also have the potential to differentiate into terminal differentiating cells, hence opening a broad spectrum for regenerative therapies. The potency of the progenitor cells depends on their development potential. For the regeneration of the diseased or injured areas, multipotent stem cells or progenitor cells are of

fundamental importance. Hence multipotent pancreatic progenitor cells would be highly beneficial for the regeneration of β cells. Next most widely used pharmacological approach is administering glucagon-like peptide (GLP1) and inhibitors of DPP4 for endogenous production of insulin [5]. Nonetheless, regulation of blood glucose remains to be challenging as fluctuation in blood glucose level leads to multiple morbidities like diabetic neuropathy, nephropathy, retinopathy, and cardiovascular complications. Therefore, regenerative therapies/stem cell-based therapies would be advantageous over pharmacological interventions. In the present chapter, we will be explaining the natural sites for islet *neo*genesis, the role of pancreatic and extra pancreatic stem cells in islet *neo*genesis, various proteins involved in islet *neo*genesis, and the new drug development to induce pancreatic stem cells to regenerate new islet.

10.2 Beta-Cell Mass Restoration

Various methods have been employed for in vivo restoration of β-cell mass avoiding the immune rejection and surgical complications. It is logical that the restoration of islet cell mass could be achieved through the β-cell progenitors residing within the pancreas [6]. Beta-cell mass restoration comprises of β-cell mass regeneration as well as β-cell mass replacement. Beta-cell mass regeneration consists of proliferation of pancreatic β cells, transdifferentiation of α and acinar cells, and β-cell differentiation from duct cells termed as *neo*genesis. Beta-cell mass replacement mainly explores the area of regenerative therapies and generation of insulin-producing cells from embryonic stem cells, induced pluripotent stem cells, and mesenchymal stem cells. In the present article, we will be focusing on one of the above aspects that are islet *neo*genesis (Fig. 10.1).

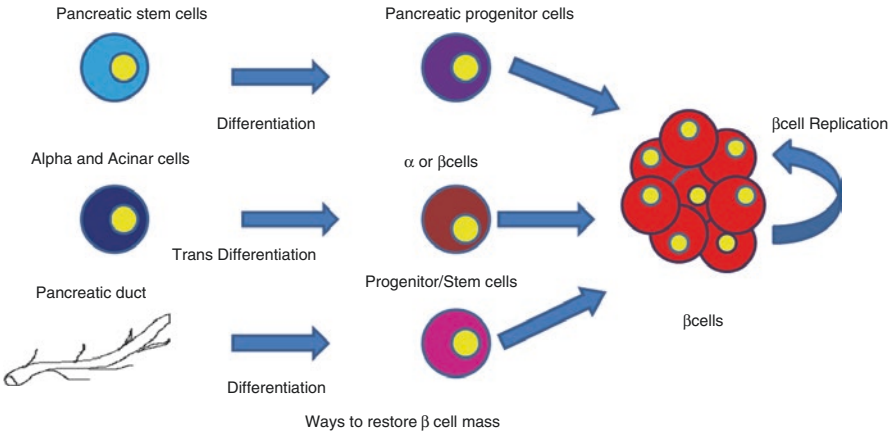

Fig. 10.1 Few aspects of β-cell mass regeneration/replication via differentiation and transdifferenetiation

10.3 Pharmacological Approaches to Islet *Neo*genesis

The term *neo*genesis defines the differentiation of islet cells from stem cell/progenitors for the growth of endocrine pancreas [3]. It has already been reported that the ideal mechanism of regeneration of injured pancreatic cells can be achieved by proliferation of β cells. A report by Dor et al. [7] suggested that new cells are generated from existing β cells as confirmed by lineage tracing technique wherein the β cells were labeled with Cre-loxP. Beta-cell mass increases considerably during pregnancy and obesity due to increased metabolic demand. Apart from in vivo islet *neo*genesis, there are various factors that contribute for islet *neo*genesis in vitro. There are studies on the application of cytokines, peptides, and proteins for the generation of insulin-producing cells from stem cells [1]. Among the various compounds, the few most instrumental molecules in islet differentiation are activin A, INGAP, glucagon-like peptide, insulin-like growth factors, keratinocyte growth factors, and hepatocyte growth factors, out of which the most explored are activin A and keratinocyte growth factors [8]. There is also a report on the application of herbal product, conophylline on the generation of insulin-producing cells [9]. This product mimicked the action of activin A on pancreatic acinar cells, differentiating them to insulin-producing cells by elevating PDX1, Ngn3, and Glut2 expression. One of the earlier reports also demonstrated the protective and regenerative effect of γ amino butyric acid (GABA) on β cells. This molecule enhances the glucose responsiveness of the islets under ultralow temperature making islet banking feasible [10]. Hence the abovementioned studies suggest that apart from transplantation and insulin therapy, there are various other approaches for combating the β-cell shortage during diabetes (Fig. 10.2).

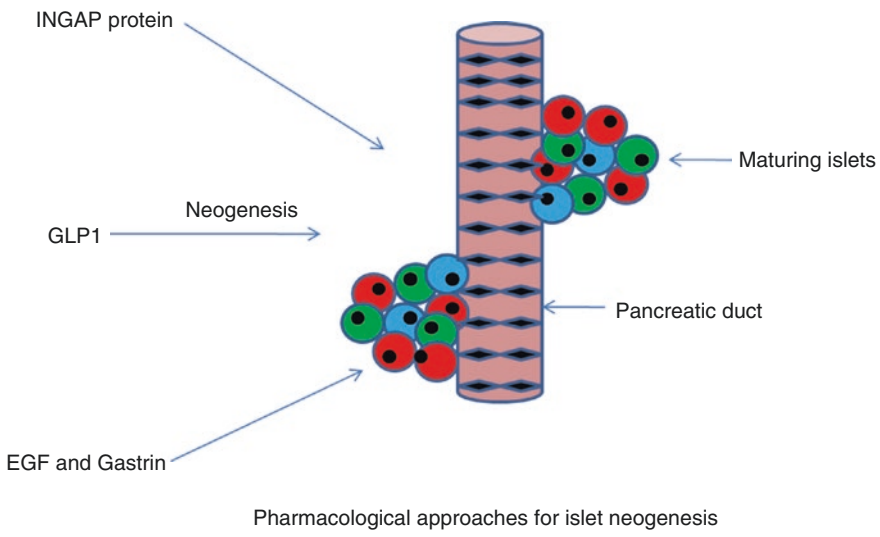

Fig. 10.2 A group of assorted molecules or factors which helps in pancreatic islet neogenesis

10.4 Sites of Precursor Cell Pools Within Pancreas

10.4.1 Beta-Cell Replication

Pancreatic β cells are the solitary source of insulin in the vertebrates. Under certain normal physiological conditions, the pancreatic β-cell mass increases mainly during obesity, aging, pregnancy, and type 1 diabetes. Beta-cell replication is the major source for increase in β-cell mass in neonatal mice and is the predominant mechanism having equal replicative capacity throughout the different subpopulations [11]. Beta cells have the inherent capacity to regenerate unless constrained by autoimmune attack or persistent hyperglycemia [12]. However, the underlying mechanism is not clearly understood till date. There are reports on the proliferative action of insulin, free fatty acids (FFA), and incretin during insulin resistant state; nonetheless, other mitogens for β-cell replication are yet to be identified [13]. Beta cell adjusts their proliferation rate according to the glycolysis rate maintaining the normoglycemia, hence adjusting according to the organism's need. There are evidences which demonstrate that human islets cultured in vitro on a culture dish attach and expand to epithelial cells expressing PDX1, and they also undergo epithelial to mesenchymal transition forming fibroblastoid cells [14]. These EMT-derived cells express stem cell properties; however, this report was later contradicted by another study wherein they described the cells expanded from human islet cultures as human islet precursor cells (hIPC) [15]. These cells were generated from islet mesenchymal stem cells and expressed all the MSC markers and could differentiate in vitro into mesodermal osteocytes and chondrocytes. There is also a report demonstrating that human islet cells expanded in vitro could be re-differentiated to normal β cells in the presence of β cellulin in the differentiation medium [16]. Age-related decrease in the expression of PDX1 in human islets indicates decreased plasticity and reduced insulin formation and secretion. Maximum β-cell replication occurs in premature and developing tissue rather than postnatal tissues. Hence there are different factors that could lead to β-cell replication either in vitro or in vivo; however, precaution should be taken to avoid neoplastic alteration [17].

10.4.2 Transdifferentiation of α Cell to β Cell

Among the various strategies for generating new β cell, one of the most advanced choices is reprogramming or transdifferentiating a differentiated cell to pluripotent cells using various genetic factors and later reprogramming them to other differentiated cells [18]. A recent report claimed that α cells possess both active and repressive histone markers showing a bivalent chromatin signature at the active genes of β cells such as *Pdx1* and *MafA*. This report also suggested that α cells could be reactivated by treating the islets with histone methyltransferase inhibitors. Ectopic expression of *Pax4* is also sufficient for conversion of α cells to β cells in vivo; nonetheless, loss of *Pax4* leads to loss of β cells leading to an increase in number of α cells [19]. Another in vitro study demonstrated that ectopic expression of *HNF4*

in mouse αTC1 clone 9 cells changed the morphology, reduced the glucagon expression, and enhanced the expression of insulin, *Pax4*, C-peptide, glucose transporter-2 (GLUT2), and glucokinase, hence reprogramming them to β-like cells [20]. Alpha cells can also be reprogrammed to β cells by the inhibitory action of *Nkx6.1* on glucagon gene in turn activating the pancreatic β-cell gene. With respect to the above findings, a report suggested that glucagon-Cre transgenics in α cells induces its conversion to β cells. Alpha and β cells share common functional machinery like they metabolize glucose and secrete hormones, express glucokinase, and share a number of transcription factors (ISL1 and Pax6), making α cell an appropriate candidate for β-cell reprogramming.

10.4.3 Intra-islet Precursor

Intra-islet precursor cells are present in diabetic mice and, upon proper stimulation, generate *neo* islets [21]. There are reports on the presence of islet precursor cells or stem cells in adult pancreas near the duct and have the capacity to differentiate into endocrine cells upon stimuli [22]. One of the earlier reports suggested the administration of sodium tungstate helped in the restoration of β-cell mass in the pancreas and maintained the normoglycemic state [23]. It is also known from earlier studies that pancreatectomy, duct ligation, chemical toxins, and viruses could induce regeneration of diabetic pancreas [24]. There are postulations that cells expressing *Pdx1* and somatostatin serve as precursor for β cells in streptozotocin-injected mice [25]. This gives scope to consider intra-islet precursor cells for inducing endogenous islet *neo*genesis.

10.4.4 Exocrine Pancreas

10.4.4.1 Acinar Cells

An alternative population for exocrine to endocrine transdifferentiation is acinar cells, owing to their plasticity along with ectopic expression of various transcription factors. Acinar cells from rodents are highly plastic enough to transdifferentiate into duct cells, hepatocytes, and islet-like pancreatic β cells [7]. However, there are no such reports for human pancreatic acinar cells; nonetheless, they do undergo spontaneous metaplasia in vitro to duct cells Earlier reports suggest that the direct reprogramming of acinar cells to beta cells could be achieved by the combinations of three transcription factors like *Ngn3*, *Mafa*, and *Pdx1* [26]. A very recent approach describes the nongenetic manipulation for reprogramming acinar cells to β cells by inducing with bone morphogenetic protein 7 (BMP7) in the acinar cells leading to the formation of clusters which are insulin positive and respond to glucose both in vitro and in vivo [24]. The nongenetic reprogramming of the exocrine cells would be a novel strategy for the *neo*genesis of the pancreatic β cells. One of the key transcription factors for the differentiation and development of the pancreatic β cells is *Ngn3*. It is expressed in the adult rodent pancreas upon certain injury in the exocrine

cells and allows the conversion of exocrine cells to the endocrine ones. However, their exact role and mechanism of action toward the conversion is unclear. There are various reports that describe the conversion of exocrine cells to pancreatic β cells following partial duct ligation, adenoviral expression, 90% pancreatectomy, in vivo delivery of EGF, and ciliary neurotrophic factor (CNTF). Several cytokines like EGF, HGF, and CNTF have been used in combinations to regenerate pancreatic β cells in vitro from the acinar cells without genetic modulation [27]. Hence various genetic and nongenetic manipulations using various transcription and growth factors can successfully lead to the conversion of exocrine acinar cells to the endocrine pancreatic β cells in hyperglycemic mice model both in vitro and in vivo. However, it remains to be seen whether this approach would work in case of human pancreas.

10.4.4.2 Duct Cells

The reprogramming factors for the acinar as well as the duct cells remain almost the same. As discussed earlier, the combination of three major transcription factors such as *Ngn3*, *Pdx1*, and *MafA* via adenoviral expression could lead to the *neo*genesis of pancreatic insulin-producing cells from the duct cells in vitro. However, the direct conversion of duct cells to pancreatic β cells via *Ngn3* remains to be controversial due to lack of evidences [28]. There are also reports that describe either the ectopic expression of gastrin and transforming growth factor alpha (TGFα) in the pancreas or the induction of gastrin in combination with EGF or GLP1 analogs can lead to an increase in β-cell mass and can also improve glucose tolerance in diabetic mice [29]. Since the duct cells of the human pancreas are highly plastic, they could easily be reprogrammed toward the differentiation to β cells. Genetic reprogramming of the human adult pancreatic duct cells with cardinal islet development regulators like *Pdx1*, *Pax6*, *MafA*, and *Ngn3* led to the conversion of islet exocrine cells to endocrine progenies having the properties of pancreatic β cells. There is also a report which focuses on the adenoviral transduction of *NGN3* in the human adult duct cells for their differentiation to pancreatic β cells, although the neuroendocrine shift was found to be incomplete [30]. The same study also suggested that conversion could be enhanced with the co-expression of myelin transcription factor 1 (*MYT1*) but not *PDX1* and *MAFA*. There is also a report which highlights the isolation and expansion of stem cells derived from pancreatic ducts as an alternate source to generate large number of islets for β-cell replacement [31]. Although various factors have been employed toward the transdifferentiation of duct cells to pancreatic β cells in rodents and humans, the percentage generation of *neo* β cells is quite minimal, and hence various other effective strategies should be developed for the generation of large number of the β cells.

10.4.4.3 Pancreatic MSC

Apart from various sources, pancreatic mesenchymal stromal cells are abundantly available for the *neo*genesis of the β cells. These multipotent precursor cells present within the pancreas can be successfully isolated from rodents and converted into islet-like cell aggregates in vitro [32]. These pancreatic MSCs could be an attractive

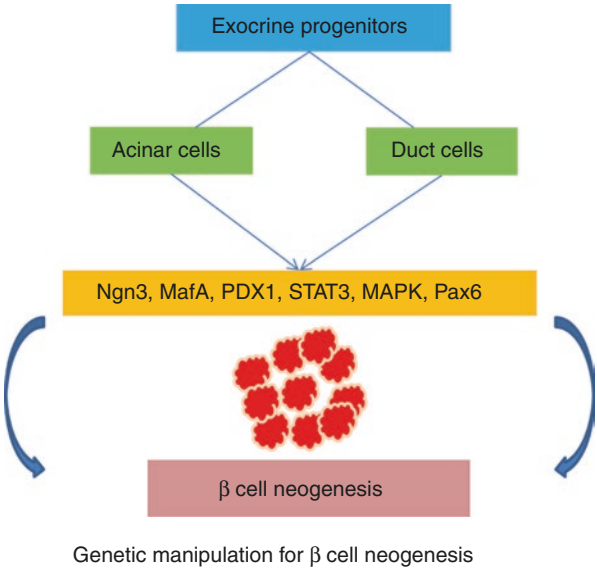

Fig. 10.3 Pancreatic MSCs can be directed to β-cell regeneration via genetic manipulations with different factors

target for stem cell therapy in diabetes (Fig. 10.3). Although controversial, there is a report which demonstrates the presence of very small embryonic-like stem cells (VSEL) in adult mouse pancreas which help in the regeneration of the diabetic pancreas [33].

10.5 Islet *Neo*genesis from Extra Pancreatic Sources

10.5.1 The Role of MSCs in Inducing Islet *Neo*genesis in Diabetes

One of the most promising therapeutics for curing diabetes is involvement of stem cells. Cell-based therapies are employed for the treatment of diabetes due to various lacunae in transplantation as well as insulin therapies. Stem cells can be easily isolated and grown in laboratory serving as a better candidate for β-cell replacement/regeneration in treating type 1 diabetes. Immunomodulatory properties and regenerative capacities of the MSCs are the major driving force for their therapeutic benefits. MSCs have the potential to transdifferentiate into mesodermal as well as non-mesodermal lineages including insulin-producing cells (IPCs). MSCs can also be committed to transdifferentiate into a particular lineage by genetic reprogramming or by altering the culture conditions in vitro. MSCs have marked their potential in tissue regeneration as they have the potential to migrate to the site of injury [34]. They have also proved to be effective in treating autoimmune diseases. It has been shown that adipose-derived MSCs have the potential to produce anti-inflammatory cytokines and angiogenic factors that could help in rescuing the diabetic patients with inflammatory and ischemic conditions. One of the hypotheses suggests that transplantation of MSC in diabetic animals prevented apoptosis of injured

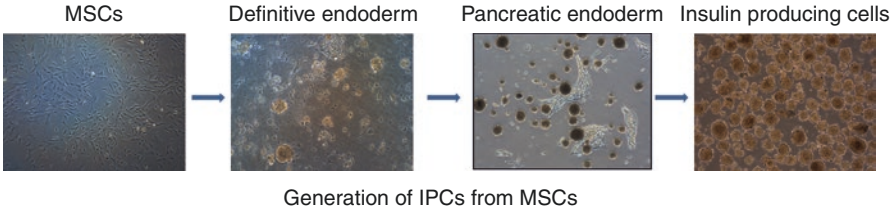

Fig. 10.4 Generation of insulin-producing cells from different sources of MSCs by altering the culture medium

β cells and enhanced the regeneration of endogenous precursor cells by various paracrine secretions [35]. MSCs can be differentiated into insulin-producing cells following various protocols, by altering their culture conditions [36]. Addition and removal of various extrinsic insulin-promoting factors are essential for the generation of IPCs. Among the various factors, HGF, FGF, β cellulin, activin A, and nicotinammide are important for the generation of IPCs from MSCs (Fig. 10.4). Thus MSC-derived IPCs could be a good substitute for cell-based treatment in type 1 diabetes.

10.5.1.1 Methods to Induce Islet *Neo*genesis

INGAP

Islet *neo*genesis-associated protein (INGAP) is a peptide found in the duct and non-β cells from normal hamsters [37], pancreatic fetus of normal mice [38], adult rats, and human beings. The bioactive portion of INGAP, pentadecapeptide 104–118 (INGAP-P), reverses diabetes in animal models and also improves glucose tolerance in patients with diabetes. It has been reported that the duct cells isolated from human pancreas and differentiated in four-step protocol using nicotinamide, exendin-4, TGFβ1, and INGAP-PP generate islet-like clusters [39]. There is also a report which suggests that PDX1 negatively regulates the stimulation of INGAP by shifting the Neuro-D with Pan-1 at the DNA binding site, hence making it a non-DNA binding site [40]. INGAP peptide improves the insulin production in type 1 diabetic patients and also maintains the glycemia in type 2 diabetics. Thus far studies showed positive result for the islet *neo*genesis using INGAP peptide; however, oral administration of this peptide could be of beneficial effect to the patients suffering from β-cell loss.

Reg Proteins

The regenerating protein family (Reg protein) is the group of secretory proteins which are involved in proliferation and differentiation [41]. They serve as growth factors for pancreatic cells, neural cells, and epithelial cells in the digestive system. The expression of this protein is associated with islet *neo*genesis in the pancreas. The Reg proteins in mouse, especially Reg1, Reg2, and Reg3δ, help in β-cell regeneration by activating cyclinD1 and support their development [42].

Conclusion

Thus we can conclude that there are various possibilities for β-cell *neo*genesis. Although the approaches are varied, the target and the result obtained remain unchanged. Despite various reports, the regeneration of the adult pancreas remains debatable. The mechanism underlying the pancreatic regeneration has to be addressed to widen the scope of research. Alternatives have been identified for β-cell regeneration either through MSCs that are present in pancreas or by the exogenous sources; however, exogenous sources have their own limitations. We have attempted to show here the ways to trigger endogenous pancreatic regeneration employing external agents like small molecules, growth factors, and MSCs to enhance the pancreatic regeneration. The article provides new dimension to islet *neo*genesis in diabetic pancreas.

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