Review Article

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Genes involved in pancreatic islet cell rejuvenation

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Pancreas plays an important role in maintaining the glucose homeostasis. The deterioration of β-cells in the pancreas is a crucial factor in the progression of diabetes mellitus; therefore, the restoration of β-cell mass and its function is of vital importance for effective therapeutic strategies. The precise mechanism for increase in functional β-cell mass is still unknown. This review focuses on the importance of certain genes which are involved in the rejuvenation of pancreas. These genes are divided according to their functions into three categories: participate either in proliferation (mitotic division of differentiated β-cells), neogenesis/transdifferentiation (development from precursor cells) or inhibition of β-cell apoptosis (programmed cell death). The rate of β-cell rejuvenation is the balance among the rates of β-cell proliferation, neogenesis and apoptosis. Understanding these genes and their pathways may lead to the discovery of new drugs, target based gene delivery and development of safer antidiabetic drugs.

Key words Apoptosis - pancreatic genes - proliferation - rejuvenation - transdifferentiation

Introduction

Diabetes is a major cause of health concern in the world and is growing in epidemic proportions. It is assumed that in the next ten years it will become number one disease of the world[1]. Type-1 diabetes mellitus (T1DM) is an autoimmune disease while type 2 is mostly a lifestyle disease. Majority of people suffer mainly due to type-2 diabetes and is responsible for the current diabetes explosion. The detection early markers for the disease and its prevention is an active area of research to develop target based novel drugs.

Dysfunctional pancreas in diabetes

Insulin, a key polypeptide hormone secreted by the pancreas, targets several tissues for the utilization of glucose and thus maintains the glucose homeostasis. Type 2 diabetes mellitus (T2DM) develops from a combination of genetic and acquired factors (such as changes in metabolic homeostasis) that impair β-cell function on one side, and tissue insulin sensitivity on the other[2,3]. Normally, β-cell mass can adapt to changes in metabolic homeostasis. Recurrence of these changes in metabolism creates a stress on pancreas often predating the on-set of T2DM by many years. This pancreatic stress causes β-cell mass expansion, through enhanced proliferation and neogenesis. The progression from this stress condition to a state of diabetes is inevitably associated with a decrease in the β-cell mass[2-4]. This β-cell loss arises due to an increase in β-cell apoptosis, which clearly outweighs replication and neogenesis.

The war against diabetes through the development of new drugs is an ongoing continuous process[5]. With

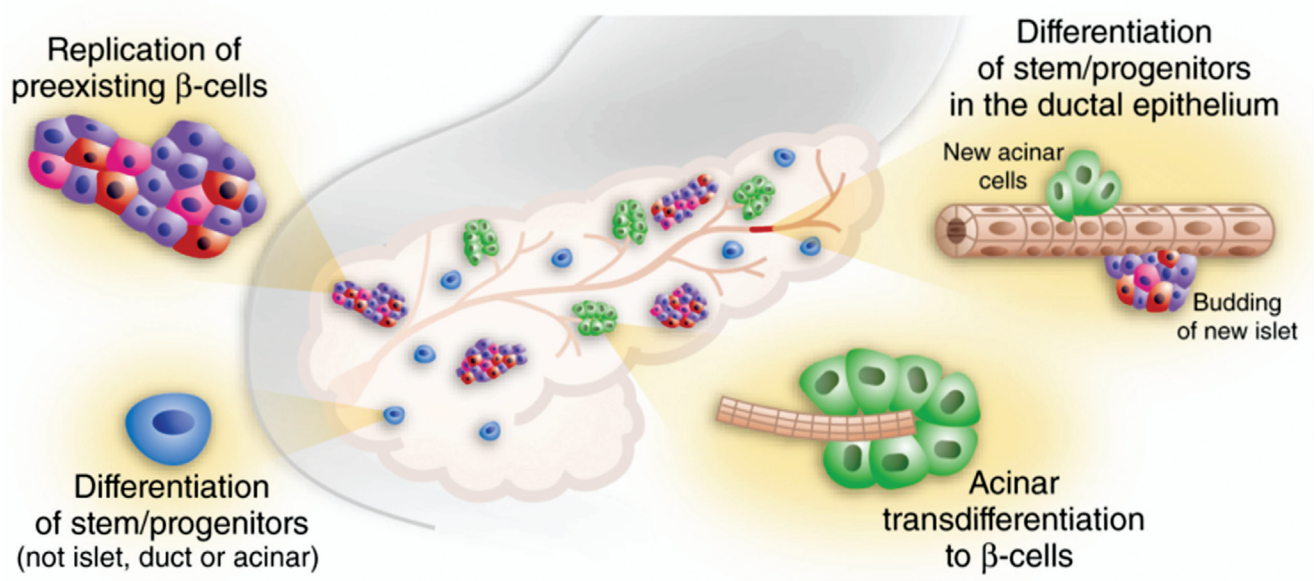

Fig. Pancreas as the source of new β-cells. New β-cells can arise from (1) Replication of pre-existing β-cells (2) Differentiaition of stem/progenitors or Transdifferentiation of acinar cells (3) Prevention of apoptosis (not shown in Figure) also contributes for regeneration of pancreas in which gene/gene products play in conjunction either with (1) or (2). All these mechanism may contribute synergistically for the regeneration of pancreas. See Tables 1, 2 and 3 for gene/gene products involved in the above processes for pancreatic rejuvenation. Reproduced with permission from Nature Publishing Group, London, UK[6].

the technological advancement, efforts are being made to rejuvenate the pancreatic cells or create artificial pancreas. Pancreatic rejuvenation can happen either due to proliferation of existing β-cells or differentiation of progenitor cells to β-cells[6] (figure), or due to decrease in β-cell apoptosis.

β-Cell proliferation

Islets regeneration refers to an increase in β-cell mass by proliferation and replication of existing islet cells. Several mouse studies[7-9] here shown that β-cells do not proliferate, however, lineage tracing studies[10-14] have confirmed that human β-cells proliferate and give rise to a population of progenitor/stem cell. Various genes and transcription factors are involved in this process *viz. Reg (Regenerating islets derived proteins)*, *Sox9, Hnf-6, NeuroD1, Neurogenin-3* and *Netrin-1* (Table I). Besides these genes, certain peptides or their analogues such as glucagon like peptide-1/exendin-4 are also involved in islet regeneration. These observations are confirmed by using dipeptidyl peptidase (DPP) IV inhibitor sitagliptin in mice[15].

So far, five REG proteins have been reported in humans that belong to *Reg* gene family. Some of the

Table I. Genes involved in beta cell proliferation

Genes proteins	Functions	References
Reg gene family *(RegI, II, IIIα, IIIβ, IIIγ)*	Increase islets cell size and density, Regeneration of pancreas	16, 22, 24, 25, 27
Sox9	Stimulates proliferation and survival of pluripotent progenitors.	28
Hnf-6	Essential for maintenance of Ngn3 expression.	30, 32
Neurog3 (Ngn-3) or Neurogenin-3	Initiates endocrine differentiation and activates NeuroD1.	34
NeuroD1/BETA-2	It is required for normal pancreatic development and glucose homeostasis.	35
Netrin-1	Involves in islets regeneration.	36

members of this family have been implicated in β-cell replication and/or neogenesis as shown in *in vivo* studies using transgenic and knockout mice[16]. These also preserve the β-cell mass in autoimmune type 1 diabetes[17]. This *Reg* family of genes are expressed in both young and old mice that were subjected to partial pancreatectomy[18]. In isolated rat islets, *Reg1* mRNA levels were significantly increased by glucose, amino acids, foetal serum or specific growth factors such as insulin, growth hormone and platelet-derived growth factors (PDGF)[19]. PDGF receptor signalling controls age-dependent β-cell proliferation in mouse and human pancreatic islet cells[20]. Disruption of *RegI* gene resulted in a significantly decreased rate of DNA synthesis and diminished β-cell hyperplasia in response to obesity, confirming the role of endogenous *RegI* in islets cell growth[21]. A study conducted by Huszarik *et al*[22] showed upregulation of *RegII* during diabetogenic process and also after adjuvant therapy in NOD mice. While all Reg family mRNAs can be detected from total pancreas, *RegII* and *RegIIIα* genes have been detected in pancreatic islet cells as confirmed by immunofluorescence[23] and RegIIIα expression was remarkably increased during pregnancy in rats[24]. Mice overexpressing *RegIIIβ* was resistant to streptozotocin induced diabetes mellitus[25]. *RegIII*γ, another member of *Reg* family of genes is also found to be involved in regeneration of pancreas. REG III protein was found to be expressed only in regenerating islets and not in normal rat pancreas[26] and its gene expression level induced 10-100 folds on day 3 of pancreatectomy[27]. These data suggest that there is a strong link between *Reg* gene family and rejuvenation of pancreatic islets.

Transcription factors in β-cell proliferation

Certain transcription factors (*Sox9, Hnf-6, Ngn-3* and *NeuroD1)* are also found to be involved in the proliferation of β-cells. SOX9 is the first specific marker and maintenance factor of multi-potential progenitors during pancreatic organogenesis. SOX9, in the embryonic pancreas stimulates proliferation and prevents apoptosis of pluripotent progenitor cells. It controls pancreatic progenitor cell maintenance by modulating Notch signal transduction. The phenotypic alterations in the *Sox9*-deficient pancreas shows a striking resemblance to the pancreatic defects associated with mutations in components of the Notch signalling pathway, thus establishing a possible link between *Sox9* and the notch signal transduction pathway for stem cell maintenance[28]. The *hepatocyte nuclear factor 6 (Hnf-6),* homeodomain-containing transcription factor, is an important regulator of endocrine development. *HNF6* is expressed in early pancreatogenesis in all endodermally derived cells, but is not detected in differentiated endocrine cells at late-gestation[29]. *Hnf-6* null mice embryos showed impaired endocrine differentiation and perturbed duct morphogenesis during embryogenesis[30]. In addition to defects in endocrine development, *Hnf-6* null embryos showed defects in duct development[31]. Loss of *Hnf-6* from *Ngn-3* expressing cells did not affect β-cell function or glucose homeostasis suggesting that *Hnf-6* is dispensable for later events of endocrine differentiation. These data confirm that HNF6 has both early and late functions in the developing pancreas and is essential for maintenance of *Ngn-3* expression and proper pancreatic duct morphology[32]. *NeuroD1,* a downstream target of *Ngn-3,* carries on the endocrine differentiation programme initiated by *Ngn3* and participates in the maintenance of the differentiated phenotype of the mature islet cells[33]. During pancreatic endocrine development, *Ngn-3* acts early to determine endocrine cell fate, while *NeuroD1* directs endocrine cell differentiation[34]. At early stage of life, mice lacking a functional *NeuroD1* (also called as *BETA2*) gene exhibit a striking reduction in the number of insulin-producing β-cells and failed to develop mature islets with a marked hyperglycaemia. Attempts to rescue the diabetic phenotype by administration of insulin were unsuccessful, suggesting that the mutant animals were unable to respond to insulin, have become insulin resistant, or perhaps contained additional defects[35]. Thus *BETA-2* is required for the expansion of the pancreatic β-cells population, as well as other islet cell types which are involved in the development of endocrine cells into islets of Langerhans[34].

Netrins are laminin-like diffusible chemotactic proteins involved in pancreatic morphogenesis and play a role in the regulation of duct-cell and foetal islet cell migration. In adult rat pancreas, *Netrin-1* mRNA was practically undetectable. After duct ligation, its expression was very low in the head part of the pancreas whereas it was strongly upregulated in the tail part at 3rd, 5th and 7th day of post-ligation with the maximum expression on day 5[36]. *Netrin-1* mRNA was found to be expressed by islet cells and exocrine cells with ductal characteristics. These observations suggest that *Netrin-1* plays a role in pancreatic morphogenesis, both prenatally and in the regenerating adult rat pancreas.

Transdifferentiation of pancreas

Islet neogenesis specifically refers to an increase in β-cell mass via transdifferentiation of adult pancreatic stem cells, putatively found in the ductal epithelium

or acinar tissue. Trans-differentiation involves in the conversion of alpha or delta cells of the pancreas into insulin producing β-cells. Various genes/proteins contribute to this process. These include *INGAP, Gastrin, MafA, Pdx-1, Foxa2, Nkx2.2, Nkx6.1, Pax4, etc.* (Table II).

INGAP (islets neogenesis associated protein) is a member of the C-lectin protein family that serves as the initiator of a cascade of events that culminates in islet neogenesis and can reverse diabetes in streptozotocin-induced diabetic C57BL/6J mice[37]. These studies were further confirmed in beagle dogs[38]. There was also a significant increase in insulin gene expression in the *INGAP* treated animals. *INGAP* is also found in human pancreas during pathological states involving islet neogenesis which further suggests that *INGAP* is of primary importance in the process of islet neogenesis[39]. Gastrin, a classical gut hormone secreted by G cells in the stomach lining is found to stimulate pancreatic β-cell neogenesis. Intravenous infusion of gastrin into the ligated duct cells, resulted in a doubling of the β-cell mass in rats[40], due to high expression of gastrin/cholecystokinin (CCK) B receptors in duct ligated cells[41]. These observations were confirmed using EGF plus gastrin combinatorial therapy, which showed an increase in insulin positive cells in human islet[42]. In another study using *GLP-1* and gastrin in NOD mice, there was a significant reduction in blood glucose due to increase in pancreatic β-cell mass and insulin content[43]. *MafA* a member of *Maf* subfamily of leucine zippers is capable of strongly activating the insulin promoter. Maf factors play important roles in cellular differentiation of exocrine cells to β-cells[44]. *MafA* is restricted to β-cells and known to be important in the embryonic development of pancreas[45]. It has been observed that *MafA* expression is decreased during the diabetic condition[46].

Transcription factors in pancreatic neogenesis

There are several transcription factors involved both in neogenesis and replication. However, it is not clear whether these work alone or in combination with other transcription factors in a coordinated manner. The list of the transcription factors and their role in transdifferentiation is summarized in Table II. *Pancreatic duodenal homeobox-1* (*Pdx-1*), a homeobox transcription factor, besides being involved as a regulator of pancreatic development (the differentiation and gene expression in the β-cell)[47], Pdx1 also turns out to be a major player in the maintenance of an adequate pool of healthy β-cells in adults[48,49]. It maintains the homeostasis between β-cell neogenesis and apoptosis. In mice with a 50 per cent reduction in *Pdx1*, the isolated islets showed more susceptibility to apoptosis at basal glucose concentrations along with impaired ability to maintain β-cell mass with age. Its expression is shown to be down-regulated during hyperglycaemic condition[50]. The survival functions of Pdx1 may be mediated by insulin/IGF signaling acting through the forkhead transcription factor *Foxo1* (*forkhead/winged helix transcription factor).* Foxa2 (formerly known as *Hnf-3*) is a key regulator of foregut development that plays an essential role in the cell type-specific transcription of the *Pdx-1* gene in the pancreas[51]. On deletion of *Foxa2* in mice, there is a significant down-regulation of *Pdx-1* mRNA[52]. This shows that *Foxa2*

Table II. Genes involved in pancreatic neogenesis

Genes	Functions	References
INGAP	An initiator of islet neogenesis.	39
Gastrin	Induces islet β-cell neogenesis from pancreatic exocrine duct cells.	40
MafA.	Reprogrammes differentiated pancreatic exocrine cells into cells that closely resemble β-cells.	44
Pdx-1	Initiates endocrine neogenesis.	47
Foxa2 (HNF-3 Beta)	A major upstream regulator of Pdx-1.	52
Nkx2.2	Regulator of pancreatic endocrine differentiation.	53
Nkx6.1	Maintaining & expanding population of β-cell precursors as these progress from precursors to differentiated β-cell.	58
Pax4	Expressed in endocrine cell progenitors and directs formation of β and delta cells.	60

is an essential upstream factor that regulates *Pdx-1* mRNA levels in β-cells.

Nkx2.2 is another essential pancreatic transcription factor that affects the expression of ghrelin during pancreatic development[53]. *Nkx2.2*-null mice lose all β-cells and the majority of the α-cells; and the islet is predominantly populated by gherlin expressing cells. The discovery that ghrelin cells often replace the other endocrine populations in the pancreas[54] suggests a lineage relationship between the ghrelin producing epsilon cells and other hormone-producing populations[55]. In another study, disruption of the *Nkx2.2* gene has shown to lead to the accumulation of incomplete differentiated β-cells that express some β-cell markers, but not insulin[56]. This illustrates the role of Nkx2.2 in pancreatic endocrine cell differentiation. The phenotypic effects of *Nkx2.2* mutant mice may in part result from the loss of other homeodomain transcription factor *Nkx6.1*. In pancreas, *Nkx6.1* also follows an expression pattern similar to that of *Nkx2.2*; but restricted to the β-cells alone[57]. Deletion of the *Nkx6.1* gene in mice caused a marked reduction of β-cells. In the same study, with the double knockout animal model (*Nkx2.2*/*Nkx6.1*) showed the same phenotype as of *Nkx2.2* single mutant[58]. This shows that *Nkx6.1* functions downstream of *Nkx2.2* in pancreatic development. The paired homeobox transcription factor Pax4 appears to be a strong candidate for specifying β-cell lineages. Mice deficient in *Pax4* fail to develop beta and delta cells within the pancreas suggesting that *Pax4* expression commits selected endocrine precursors towards the beta and delta cell lineage[59]. The *Pax4* genes are expressed during the embryonic pancreatic development, but later on these are restricted to β-cells alone. Inactivation of *Pax4* in mice results in the improper maturation of alpha- and β-cells[60]. This indicates the role of PAX4 in the maintenance of progenitors and in maturation of β-cells.

Inhibition of β-cell apoptosis

Under normal circumstances, apoptosis is highly regulated to maintain normal physiological function of the cells. In diabetes, during excess stress, the pancreatic cells not only undergo apoptosis but also become necrotic and are unable to secrete insulin. A study conducted by Butler *et al*[61] indicated that increased apoptosis rather than decreased neogenesis/proliferation might be the main mechanism leading to reduced β-cell mass in T2DM. Thus, decrease in the rate of apoptosis itself, may increase the β-cell mass via proliferation. Several genes/proteins are involved in pancreatic apoptosis and their functions are summarized in Table III.

Perforin (pore forming protein) is a cytolytic protein, initiating apoptosis by inducing minimal cell membrane damage while effectively releasing

Table III. Genes involved in inhibition of beta cell apoptosis

Genes/proteins	Functions	References
Pdx-1	Possesses anti-apoptotic activity that helps facilitate the maintenance of β-cell mass.	48
Granzymes	A serine protease, that activates Bid (a pro-apoptotic factor) essential for death-receptor induced apoptosis of islets.	66
Caspase-3	Principle executioner of apoptosis.	69
BIRC5 (Survivin)	Bifunctional role, controlling both proliferation and inhibition of apoptosis	73
Bcl-2	Survival genes that suppresses apoptosis.	75
C-Myc	Induces extensive apoptosis in pancreatic β-cells.	76
Thioredoxin-interacting protein (TXNIP)	It is a pro-apoptotic factor that plays an essential role in glucose toxicity-induced β-cell apoptosis.	82
Huntingtin-interacting protein 14 (HIP-14)	Prevents apoptosis and regulates insulin secretion in β-cells.	83
Bace-2	Negative regulator of β-cell mass.	84

granzymes from the endosomal compartment into the cytosol[62]. Of the granzyme family, granzyme A and B are the most common in human and mouse[63]. Granzyme A induces single-strand DNA breaks, while granzyme B cleaves specific substrates including caspases and the pro-apoptotic molecule Bid[64]. Activated Bid is targeted to the mitochondria where it sequesters anti-apoptotic members of the Bcl-2 family, allowing the oligomerization of Bax and/or Bak which mediates loss of mitochondrial outer membrane potential, release of cytochrome C and causes irreversible apoptosis[65]. During hyperglycaemic condition its expression is elevated in β-cells, thus increasing the rate of apoptosis[51]. Deficiency in Bid prevents β-cells from undergoing mitochondrial apoptotic pathway[66,67]. Caspase-3 has been extensively studied in various tissues due to its role as the principal executioner of apoptosis[68]. As such, Caspase-3 is an attractive target to inhibit apoptosis in diseased conditions including diabetes[69]. *Caspase-3* null (*Casp3*-/-) mice were found to be protected from developing diabetes in a multiple-low-dose streptozotocin autoimmune diabetic model[70]. This illustrates the importance of Caspase-3 in β-cell death and its activity is found to be increased during the diabetic conditions. An attractive regulator of β-cell replication and survival after birth is *Survivin*. This protein blocks the functions of Caspases in the mitochondria-dependent cell death pathway, protecting cells from apoptosis[71]. Deletion of *Survivin* within the mice endocrine pancreas results in diabetes manifested by hyperglycaemia and polyuria. Exogenous expression of *Survivin* in streptozotocin -induced diabetic model protects the β-cells from apoptosis[72]. Thus, it may play a role in the replication and/or survival of matured β-cells[73]. Surprisingly it is modestly upregulated in diabetic patients[74] which may help to assuage diabetes.

Beside *survivin*, there are other proteins present in pancreas which prevent apoptosis. BCL-xL, an anti-apoptotic protein coded by the "survival gene", is involved in the inhibition of apoptosis. Marked overexpression of *Bcl*-xL, resulted in a severe defect in insulin secretion and hyperglycaemia in transgenic mice[75]. Under conditions of stress, β-cells require BCL-xL to maintain their survival *in vivo*[48]. *Myc* is a potent inducer of both β-cell proliferation and apoptosis *in vitro*[76]. Myc sensitizes cells to a variety of apoptotic triggers rather than directly inducing apoptosis by itself[77]. Sustained *Myc* activation leads to initial hyperplasia and increased apoptosis later on, suggesting that apoptosis ultimately predominates over proliferation[78]. In chronic hyperglycemia, an increase in the expression of *Myc* was observed in pancreas[79]. A thioredoxin-interacting protein (TXNIP), a regulatory protein, is involved in the inhibition of thioredoxin and thereby modulates the cellular redox state and promotes oxidative stress[80]. Its overexpression in β-cells is found to induce apoptosis[81], and the process involves the activation of intrinsic mitochondrial pathway, while the Endoplasmic reticulum (ER)-mediated cell death remains unaffected. It was demonstrated that their mRNA expression levels are elevated during the diabetic conditions[82]. In a recent study, huntingtin-interacting protein 14 (HIP-14) is found to posses anti-apoptotic property of β-cells. This also plays an important role in glucose-stimulated insulin secretion[83]. Bace2 (Beta site amyloid precursor protein cleaving enzyme 2), a proteolytic enzyme acts as a negative regulator of β-cell mass via inhibiting *Tmem27* expression. Overexpression of *Tmem27* or ablation of *Bace2* leads to an increased β-cell mass by inhibiting apoptosis[84].

Future prespective

Several lifestyle diseases such as T2DM, obesity are increasing significantly. It has been predicted that in near future diabetes will overtake all the current infectious and non-infectious diseases. In spite of rapid progress in our understanding regarding the pathophysiology of diabetes, challenges remain high due to increased complexity of the disease contributed by genetic and environmental factors. In future, there need to be more emphasis on the prognosis and better treatment for diabetes associated diseases, and on the discovery of reliable biomarker(s) for its early detection. Development of new technologies *i.e.* rejuvenation of the pancreas by small molecules or gene targeted drug delivery, pancreatic transplantation, stem cell therapy and creating artificial pancreas, *etc*. will be the mainstay of the diabetes research. Thus, it becomes essential to have better understanding of the various pathways at the molecular level that are involved in pancreatic islet cell rejuvenation.

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