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PDX-1 and MafA: Key Transcription Factors in Pancreas

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Abstract: Pancreatic and duodenal homeobox factor-1 (PDX-1) plays a crucial role in pancreas development, β-cell differentiation and in maintaining normal β-cell function by regulating several β-cell-related genes including insulin. PDX-1 has potency to induce insulin-producing cells in non β-cells in various tissues such as pancreas and liver and PDX-1-VP16 fusion protein more efficiently induces insulin-producing cells, especially in the presence of NeuroD or Ngn3. MafA is a recently isolated β-cell-specific transcription factor and functions as a potent transactivator for the insulin gene. MafA markedly enhances insulin gene promoter activity and ameliorates glucose tolerance in diabetic mice, especially in the presence of PDX-1 and NeuroD. Taken together, PDX-1 and MafA play a crucial role in inducing insulin-producing cells and could be a therapeutic target for diabetes.

Key words: PDX-1, MafA, transcription factor, pancreatic β-cells, diabetes

INTRODUCTION

Decrease of functioning pancreatic β-cell number and insufficient insulin biosynthesis and/or secretion are the hallmark of both type 1 and type 2 diabetes. Type 1 diabetes results from insulin deficiency caused by the autoimmune destruction of insulin-producing β-cells and type 2 diabetes results from insufficient β-cell function as well as progression of insulin resistance. The number of patients with type 2 diabetes is markedly increasing worldwide and nowadays type 2 diabetes is recognized as the most prevalent and serious metabolic disease. Although pancreas and islet transplantation have exerted beneficial effects for diabetes, the limitation of available insulin-producing cells and requirement of life-long immunosuppressive therapy are the main obstacles. Indeed, it was shown that islet cell transplantation, together with glucocorticoid-free immunosuppression, results in excellent glycemic control with sustained freedom from exogenous insulin in type 1 diabetes[1,2]. However, the scarcity of cadaveric donors to treat millions of diabetic patients leads to a serious limitation to the widespread clinical application of this procedure. Therefore, it is very important to search for alternative sources to induce insulin-producing cells and to develop β-cell substitutes by introducing an insulin-producing cells in non-β-cells. In addition, because β-cells are destroyed by the β-cell-specific autoimmune response in type 1 diabetes, non-β-cells should be used to induce insulin production. It has been reported that various cells (tissues) such as Embryonic Stem (ES) cells, liver, pancreas, intestine and bone marrow could induce insulin-producing cells after some particular treatment. Also, many attempts have been done to replace the function of β-cells by introducing various components of the insulin secretory machinery into various non-β-cells.

Insulin gene therapy induces insulin production in non-β-cells and ameliorates glucose tolerance in diabetic model animals: It has been considered that insulin gene therapy has potential for treatment of diabetes. The goal of insulin gene therapy is to restore and control insulin production and to reduce the elevated blood glucose levels to a normal range without causing hypoglycemia. Indeed, it has been shown that direct insulin gene transfer in the liver induces hepatic insulin production and ameliorates the adverse effects of insulin deficiency in diabetic animals[3-7]. Insulin gene therapy with adenovirus-mediated delivery system ameliorated glucose tolerance in STZ-induced diabetic animals. Since the liver possesses glucose-sensing system somewhat similar to that in β-cells including glucokinase and glucose transporter 2 (GLUT2), it was thought that the liver has the potential to respond to change in blood glucose levels. However such insulin gene therapy has been hampered by the absence of enzymes that process proinsulin to insulin and a regulatory system that produces and secretes insulin in response to glucose levels. Unlike β-cells, the liver lacks the prohormone convertases PC2 and PC3 for insulin processing. Recently this limitation was somehow overcome by converting the dibasic amino acid residues to a tetrabasic sequence to allow the proinsulin synthesized in the liver to be cleaved by furin[8]. Also, although this limitation was somehow circumvented by the development of insulin single chain analog, the bioactivity of this insulin single chain was significantly reduced[9]. Since β-cells contain unique features that allow the regulation of

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insulin biosynthesis and secretion in response to glucose levels, it is not easy to reconstruct the regulated system in non-β-cells. The major hurdle for insulin gene therapy is the lack of highly regulated biosynthesis and secretion of insulin in non-β-cells. In order to obtain glucose-responsiveness of insulin biosynthesis and secretion, liver-specific promoters, the activities of which are couples with glucose levels, have been used. The promoters of L-type Pyruvate Kinase (LPK), Phosphoenolpyruvate Carboxykinase (PEPCK), Glucose-6-Phosphatase (G6Pase) and Insulin-like Growth Factor Binding Protein (IGFBP-1) have been investigated as regulatory elements for glucose-responsive insulin production in the liver[10-12]. This approach seemed to be effective, but the regulation was not perfect.

It was also shown that K cells, which are classified as an endocrine cell type in the gut, are good target for insulin gene therapy, because the cells have glucose responsiveness and contain the necessary enzymes for proinsulin-insulin conversion. K cells function to secrete the hormone Gastric Inhibitory Peptide (GIP) in response to glucose level. In addition, K cells express glucokinase and glucose transporter 2 (GLUT2) and thereby sense the change of glucose levels. Indeed, it was shown that transgenic mice producing human proinsulin under the control of the K cell-specific GIP promoter produce mature bioactive insulin in a glucose level-dependent manner and show normal glucose tolerance even after STZ treatment[6,7,13,14]. However, since K cells are present in the crypts of the gut, viral transduction efficiency is thought to be very low. In addition, intestinal epithelial cells normally undergo very rapid turnover. Therefore, it would be necessary to deliver the insulin gene into K cell progenitor cells in order to achieve long-term insulin production and an effective gene transfer methods for K cells should be developed to use K cells for insulin gene therapy.

PDX-1 plays a crucial role in pancreatic β-cell differentiation and functions as a key transcription factor to induce insulin-producing cells: The pancreas is composed of exocrine (acini and ducts) and endocrine compartments (islets of Langerhans which contain α-,β-,δ- and PP-cells that produce glucagon, insulin, somatostatin and pancreatic polypeptide, respectively). During the pancreas development, differentiation of endocrine and exocrine compartments from pancreatic buds requires the coordinated regulation of specific genes. This process can be envisioned as a hierarchy of transcription factors that initiate and maintain various gene expression program, leading to definition of various pancreatic cell types (Fig. 1). In inducing insulin-producing cells in some source cells (tissues), it would be a useful clue to mimic and reproduce the alteration of various pancreatic transcription factors found during normal pancreas development. Also, it would be useful to induce pancreatic key (master) transcription factor(s) in some source cells (tissues) which have potency to induce various β-cell-related genes including insulin (Fig. 2). The pancreatic and duodenal homeobox factor-1 (PDX-1), a member of the homeodomain-containing transcription factor family, also known as IDX-1/STF-1/IPF1[15-17], is expressed in the pancreas and duodenum and plays a crucial role in pancreas development[18-25], β-cell differentiation[26-37] and in maintaining normal β-cell function by regulating several β-cell-related genes[38-44]. At early stages of embryonic development, PDX-1 is initially expressed in the gut region when the foregut endoderm becomes committed to common pancreatic precursor cells. During pancreas development, PDX-1 expression is maintained in precursor cells that coexpress several hormones and later it becomes restricted to β-cells (Fig. 1). Mice homozygous for a targeted mutation in the PDX-1 gene are apancreatic and develop fatal perinatal hyperglycemia[18], suggesting the crucial role of PDX-1 in pancreas development. It has been shown that the exogenous expression of PDX-1 induces the expression of various β-cell-specific genes including insulin in several cells (tissues) such as exocrine pancreas[27, 31, 32] or some cell populations in the liver[26,28,30,34-37]. Clinically, mutations in PDX-1 are known to cause Maturity-onset Diabetes of the Young (MODY)[45]. There are several other transcription factors important for pancreas development and β-cell differentiation. Neurogenin3 (Ngn3), a bHLH transcription factor, is involved in pancreas development and endocrine differentiation[46-52]. Transgenic mice overexpressing Ngn3 show a marked increase in endocrine cell formation, indicating that Ngn3 induces islet cell precursors to differentiate (Fig. 1)[47,48]. In contrast, mice with targeted disruption of Ngn3 have no endocrine cells[49]. NeuroD, a member of the basic Helix-Loop-Helix (bHLH) transcription factor family, also known as BETA2, is expressed in pancreatic and intestinal endocrine cells and neural tissue. NeuroD also plays an important role in pancreas development and in regulating insulin gene transcription[53-55]. Mice homozygous for the null mutation in NeuroD also have a striking reduction in the number of β-cells, develop severe diabetes and die perinatally[54]. Clinically, mutations in NeuroD cause Maturity-onset Diabetes of the Young (MODY)[56]. Also, it has been reported that the insulin enhancer elements, E-box and A-box, play an important role in regulating cell-specific expression of the insulin gene[57,58] and that NeuroD binding to the E-box as well as PDX-1 binding to the A-box is very important for insulin gene transcription (Fig. 3).

It has been reported that various cells (tissues) such as Embryonic Stem (ES) cells, liver, pancreas, intestine and bone marrow could be transdifferentiated into insulin-producing cells. It was shown that ES cells have

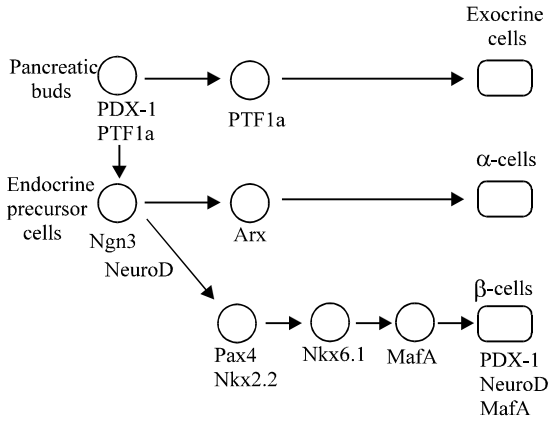

Fig. 1: Pancreatic transcription factor hierarchy during the pancreas development During the pancreas development, differentiation of endocrine and exocrine compartments from pancreatic buds requires the coordinated regulation of specific genes. This process can be envisioned as a hierarchy of transcription factors that initiate and maintain various gene expression program, leading to definition of various pancreatic cell types

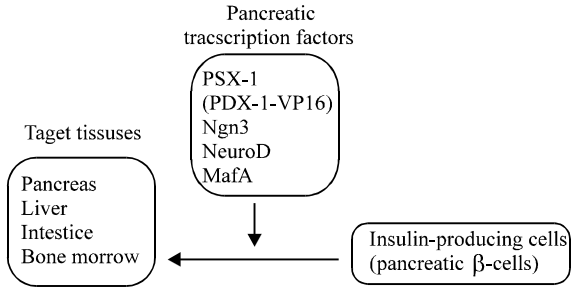

Fig. 2: Induction of insulin-producing cells by pancreatic key transcription factor expression Some pancreatic transcription factor (or together with some other pancreatic transcription factors) induces the expression of various β-cell-related genes including insulin in several cells (tissues). Thus, it seems useful to induce some pancreatic key (master) transcription factors in some source cells (tissues) that have potency to induce the expression of various β-cell-related genes

potency to differentiate into insulin-producing[59-63], but the potential use of ES cells for the treatment of diabetes might not be appropriate from the ethical point of view. Therefore, adult tissue-derived stem (progenitor) cells have been also used to induce insulin-producing cells. Stem (progenitor) cells in pancreas have been shown to be differentiated into insulin-producing cells[27,31,32,64-67]. Pancreatic ducts, acini and some populations in islets have been shown to have potency to differentiate into

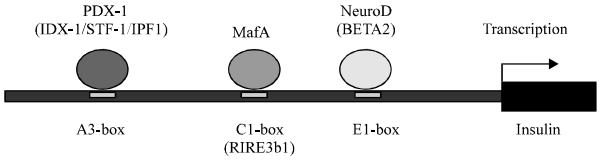

Fig. 3: Key transcription factors for insulin gene transcription The insulin enhancer elements, E-box and A-box, play an important role in regulating cell-specific expression of the insulin gene. PDX-1 binding to the A-box and NeuroD binding to the E-box are very important for insulin gene transcription. Also, MafA, a recent identified β-cell-specific transcription factor, binds to a conserved cis-regulatory element called RIPE3b in the insulin gene promoter region and functions as a potent transactivator for the insulin gene

insulin-producing cells. The pancreas and liver arise from adjacent regions of the endoderm in embryonic development and thus the liver has been thought to be one of the potential sources to induce insulin-producing cells[26,30,34-37,55,68-71]. Intestinal epithelium-derived cells were also shown to induce differentiation into insulin-producing cells[28,29]. Some populations in bone marrow cells were also shown to have potency to differentiate into insulin-producing cells[33,72,73]. In such study, various pancreatic transcription factors have been used to induce insulin-producing cells in some source cells (tissues). Indeed, it was reported that PDX-1 induces the expression of various β-cell-specific genes including insulin in several cells (tissues) such as exocrine pancreas[28,34,35] or some cell populations in the liver[26,30,34-37]. These results suggest that it is useful to induce pancreatic key (master) transcription factor(s) such as PDX-1 in some source cells (tissues) which have potency to induce various β-cell-related genes including insulin.

PDX-1-VP16 fusion protein efficiently induces insulin-producing cells and ameliorates glucose tolerance, especially in the presence of NeuroD or Ngn3: While the pancreas and liver arise from adjacent regions of the endoderm in embryonic development, the liver has been thought to be a potential target for diabetes gene therapy[26,30,34-37,55]. In addition, it has been shown recently that a modified form of XlHbox8, the Xenopus homolog of PDX-1, carrying the VP16 transcriptional activation domain from Herpes simplex virus, efficiently induces insulin gene expression in the liver of the tadpole[71]. In the study, transgenic Xenopus tadpoles carrying the contruct transthyretin promoter-Xlhbo x8-VP16 were prepared. Xlhbox8-VP16 was expressed only in the liver of the tadpoles. In the transgenic tadpoles, the liver was converted to pancreas, containing both exocrine and endocrine cells, while liver

differentiation products were lost from the regions converted to pancreas[71]. Since the timing of events is such that the liver is differentiating by the time Xlhbox8-VP16 is expressed, they consider this a transdifferentiation event rather than a reprogramming of embryonic development[71]. In contrast, conversion of liver to pancreas was not observed by Xlhbox8 alone (without VP16).

Based on the findings in tadpoles[71], effects of PDX-VP16 fusion protein (PDX-1-VP16) on differentiation to insulin-producing cells have been examined in murine. Indeed, it was reported recently that PDX-1-VP16 rather than wild PDX-1 efficiently induces insulin-producing cells in the liver and ameliorates glucose tolerance in diabetic mice[35-37]. Also, it was shown that PDX-1-VP16 efficiently induces insulin gene expression in the liver especially in the presence of NeuroD or Ngn3[35]. Although PDX-1-VP16 exerted only a little more obvious effect on the insulin promoter compared to wild type PDX-1, PDX-1-VP16, together with NeuroD (or Ngn3), dramatically increased insulin promoter activity. To examine the additional effect of VP16 on PDX-1-mediated induction of insulin gene expression, adenovirus expressing the PDX-1-VP16 fusion protein (Ad-PDX-1-VP16) was delivered into the cervical vein of C57BL6 mice. After Ad-PDX-1-VP16 injection, both insulin 1 and 2 was detected in the liver, although insulin 1 was not detected by expression of wild type PDX-1 (without VP-16)[35]. In addition, larger amounts of insulin gene expression were detected by PDX-1-VP16 in the presence of NeuroD (or Ngn3). After treatment with Ad-PDX-1-VP16 plus Ad-NeuroD (or Ad-Ngn3), several insulin-positive cells and insulin secretory granules were observed in the liver in immunostaining and electron microscopy, respectively[38]. Various endocrine pancreas-related factors such as islet-type glucokinase, glucagon and somatostatin were also induced after treatment with Ad-PDX-1-VP16 plus Ad-NeuroD (or Ad-Ngn3). Furthermore, in STZ-induced diabetic mice, blood glucose levels were decreased by PDX-1-VP16 alone, which was more pronounced compared to the effects of wild type PDX-1 and were further decreased by overexpression of PDX-1-VP16 plus NeuroD (or Ngn3)[38]. The marked effects of PDX-1-VP16 expression, together with NeuroD (or Ngn3), on insulin production and glucose tolerance indicate that such combination is useful and efficient for replacing the reduced insulin biosynthesis found in diabetes and that PDX-1 requires the recruitment of coordinately functioning transcription factors or cofactors in order to fully exert its function (Fig. 2). In addition, these results suggest that synergistic activation of the E-box by bHLH transcription factors such as NeuroD or Ngn3 would be important to induce insulin-producing cells in non-β-cells for the achievement of β-cell regeneration therapy in the future.

It was also shown recently that PDX-1-VP16 expressing hepatic cells converts into functional insulin-producing cells in the presence of high glucose[36]. In the study, they generated a stably transfected rat hepatic cell line named WB-1 that expresses PDX-1-VP16. Expression of several genes related to endocrine pancreas development and islet function was induced by PDX-1-VP16 in the liver cells, although some pancreatic transcription factors were missing. Also, theses cells failed to secrete insulin upon the glucose challenge. However, when WB-1 cells were transplanted into diabetic NOD-scid mice, almost all β-cell-related transcription factors were induced and hyperglycemia was normalized[36]. In addition, *in vitro* culture in high glucose medium was sufficient to induce completely maturation of WB-1 cells into functional insulin-producing cells[36]. These results suggest that PDX-1-VP16 should be very efficient and useful for replacing the reduced insulin biosynthesis and for amelioration of glucose tolerance but that PDX-1-VP16 alone is not enough to induce complete transdifferentiation to functional insulin-producing cells (Fig. 2).

MafA, a recently isolated β-cell-specific transcription factor, functions as a potent transactivator for the insulin gene and in the presence of PDX-1, efficiently induces insulin-producing cells and markedly ameliorates glucose tolerance: It was previously shown that an unidentified β-cell-specific nuclear factor binds to a conserved cis-regulatory element called RIPE3b1 in the insulin gene enhancer region and is likely to function as an important transactivator for the insulin gene[74]. Recently, this important transactivator for the insulin gene was identified as MafA, a basic-leucine zipper transcription factor (Fig. 3)[75-77]. The C1/RIPE3b1 activator from mouse βTC-3 cell nuclear extracts was purified by DNA affinity chromatography and two-dimensional gel electrophoresis[75]. C1/RIPE3b1 binding activity was found in the roughly 46-kDa fraction at pH 7.0 and pH 4.5. MafA was detected in the C1/RIPE3b1 binding complex by using MafA peptide-specific antisera. In addition, MafA was shown to bind within the enhancer region of the endogenous insulin gene in βTC-3 cells in the chromatin immunoprecipitation assay[75]. These results strongly suggest that MafA is the β-cell-enriched component of the RIPE3b1 activator. MafA controls β-cell-specific expression of the insulin gene through a cis-regulatory element called RIPE3b1 and functions as a potent transactivator for the insulin gene[75-80]. During pancreas development, MafA expression is first detected at the beginning of the principal phase of insulin-producing cell production[78] while other important transcription factors such as PDX-1 and NeuroD are expressed from the early stage of pancreas development (Fig. 1). In addition,

while both PDX-1 and NeuroD are expressed in various cell types in islets, MafA is the only β-cell-specific transactivator for the insulin gene. Thus, the potency of MafA as an insulin gene activator, together with its unique expression in β-cells, raises the likelihood that MafA is a principal factor of β-cell formation and function. Also, it is likely that MafA is a useful factor to generate insulin-producing cells.

It was shown recently that MafA, together with PDX-1 and NeuroD, efficiently induces insulin gene expression in the liver[79]. In HepG2 cells, basal insulin promoter activity was increased by MafA alone which was much more significant after overexpression of PDX-1 or NeuroD alone. Furthermore, MafA, together with PDX-1 plus NeuroD, drastically increased insulin promoter activity[79]. These results clearly show that MafA, PDX-1 and NeuroD exert strong synergistic effect on insulin promoter activity. Large amounts of insulin 1 and 2 mRNA and insulin content were clearly observed in the liver after the triple infection (Ad-MafA, Ad-PDX-1, plus Ad-NeuroD). Also, in immunostaining for insulin, many insulin-positive cells were clearly observed in the liver after the triple infection[79]. In STZ-induced diabetic mice, serum insulin levels were markedly increased after the triple infection. Furthermore, in the diabetic mice, blood glucose levels were decreased by MafA plus PDX-1 (or NeuroD)[79], although no such effect was observed after infection of Ad-MafA alone. Furthermore, blood glucose levels were dramatically decreased by the triple infection (Ad-MafA, Ad-PDX-1 and Ad-NeuroD). Theses results suggest a crucial role of MafA as a novel therapeutic target for diabetes and imply that such combination should be very efficient and useful for replacing the reduced insulin biosynthesis found in diabetes (Fig. 2).

Chronic hyperglycemia reduces expression and/or DNA binding activities of PDX-1 and MafA by provoking oxidative stress, leading to suppression of insulin biosynthesis and secretion found in diabetes: Chronic hyperglycemia is a cause of impairment of insulin biosynthesis and secretion; once hyperglycemia becomes apparent, β-cell function gradually deteriorates and insulin resistance aggravates. This process is called "β-cell glucose toxicity"[74,81-84]. In the diabetic state, hyperglycemia *per se* and subsequent production of reactive oxygen species decrease insulin gene expression and secretion[85-99]. It was previously shown that loss of insulin gene expression is accompanied by decreased expression and/or DNA binding activity of two important pancreatic transcription factors, PDX-1[82,85,87-91] and RIPE3b1 activation (which was recently identified as MafA)[74,81,97]. After chronic exposure to a high glucose concentration, expression and/or DNA binding activity of PDX-1 and MafA are reduced (Fig. 4).

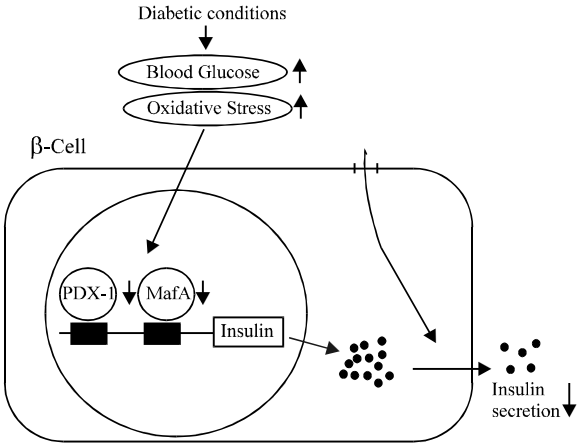

Fig. 4: Role of PDX-1 and MafA in β-cell glucose toxicity Chronic hyperglycemia suppresses insulin biosynthesis and secretion by provoking oxidative stress, accompanied by reduction of expression and/or DNA binding activities of PDX-1 and MafA. It is likely that the alteration of these two transcription factors leads to suppression of insulin biosynthesis and thus plays a crucial role in β-cell glucose toxicity

Under diabetic conditions, Reactive Oxygen Species (ROS) are increased in pancreatic β-cells and are involved in "β-cell glucose toxicity" found in diabetes[85-99]. There are several sources of ROS productions in cells such as the electron transport chain in mitochondria[94] and the non-enzymatic glycosylation reaction[85]. It was shown that when β-cell-derived HIT cells or rat isolated islets were exposed to oxidative stress, insulin gene promoter activity and mRNA expression were suppressed[85,87-91]. Also, the DNA-binding activity of PDX-1 is rather sensitive to oxidative stress; when HIT cells or rat isolated islets were exposed to oxidative stress, PDX-1 binding to the insulin gene was markedly reduced. Also, it was shown that the decrease of insulin gene expression after chronic exposure to high glucose concentration is prevented by treatment with antioxidants[87,88,97]. Furthermore, reduction of expression and/or DNA binding activities of PDX-1 and MafA by chronic exposure to high glucose was prevented by an antioxidant treatment. These results suggest that chronic hyperglycemia suppresses insulin biosynthesis and secretion by provoking oxidative stress, accompanied by reduction of expression and/or DNA binding activity of two important pancreatic transcription factors, PDX-1 and MafA. Therefore, we assume that the alteration of these two transcription factors explains, at least in part, the suppression of insulin biosynthesis and secretion and thus play a role in "β-cell glucose toxicity" found in diabetes (Fig. 4).

CONCLUSIONS

Pancreatic transcription factor PDX-1 plays a crucial role in pancreas development, β-cell differentiation and in maintaining normal β-cell function by regulating several β-cell-related genes including insulin. MafA is a recently isolated β-cell-specific transcription factor and functions as a potent transactivator for the insulin gene. In inducing insulin-producing cells in some source cells (tissues), it would be a useful clue to mimic and reproduce the alteration of various pancreatic transcription factors found during normal pancreas development. Also, it would be useful to induce pancreatic key (master) transcription factor(s) in some source cells (tissues) which have potency to induce various β-cell-related genes including insulin. Induction of such transcription factors induces insulin-producing cells in some source cells (tissues) and combination of such transcription factors is very efficient and useful for replacing the reduced biosynthesis found in diabetes. Also, these two transcription factors PDX-1 and MafA play a crucial role in insulin gene transcription and in maintaining normal β-cell function. Under diabetic conditions, however, chronic hyperglycemia reduces expression and/or DNA binding activities of PDX-1 and MafA by provoking oxidative stress, which leads to suppression of insulin biosynthesis and secretion and thus plays a role in "β-cell glucose toxicity" found in diabetes. So far many studies have been performed to overexpress such transcription factors using adenovirus, but it would be very difficult to apply the adenovirus-mediated approach to clinical medicine. Some new strategies would be necessary to safely overexpress such transcription factors. Protein Transduction Domains (PTDs) such as the small PTD from the TAT protein of Human Immunodeficiency Virus (HIV-1), the VP22 protein of Herpes simplex virus and the third □-helix of the homeodomain of Antennapedia, a Drosophila transcription factor, are known to allow various proteins and peptides to be efficiently delivered into cells through the plasma membrane and thus there has been increasing interest in their potential usefulness for the delivery of bioactive proteins and peptides into cells[31,52,100-102]. Thus, it would be promising to induce pancreatic key (master) transcription factor(s) in some source cells (tissues) by using protein delivery system.

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