Pancreatic and duodenal homeobox-1 in pancreatic ductal adenocarcinoma and diabetes mellitus

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Abstract

Diabetes mellitus and pancreatic ductal adenocarcinoma are two common diseases worldwidely which are both derived from different components of pancreas. The pancreatic and duodenal homeobox-1 (PDX1) is an essential transcription factor for the early development of pancreas that is required for the differentiation of all pancreatic cell lineages. Current evidence suggests an important role of PDX1 in both the origin and progression of pancreatic diseases. In this review, we discussed recent studies of PDX1 in diabetes mellitus and pancreatic cancer, and the therapeutic strategies derived from this transcription factor.

Keywords: Pancreatic and duodenal homeobox-1; Diabetes mellitus; Pancreatic cancer

Introduction

The pancreas is a glandular organ that has both exocrine and endocrine functions and is located in the abdominal cavity behind the stomach. Around 90% of the pancreas is an exocrine gland consisting of acinar and ductal cells, whose primary role is nutrient digestion. The rest of the pancreas is its endocrine part, scattered throughout the organ, and possessing the islets of Langerhans as its functional units. Instead of secreting digestive enzymes into the digestive tract, the islets are responsible for regulating glucose homeostasis by releasing hormones into the blood stream.

The pancreatic and duodenal homeobox-1 (*PDX1*) gene is located at the chromosomal locus 13q12.1.[1] The protein encoded by this gene can transcriptionally activate several genes, such as insulin, glucokinase (GK), somatostatin, and islet amyloid polypeptide (IAPP), which are all essential for regulating glucose metabolism. It is also an essential transcription factor (TF) for pancreas development, which is required for the differentiation of all pancreatic cell lineages [Figure 1].[2-5] In mouse embryos, PDX1 is first detected at embryonic day 8.5 (E8.5) in the dorsal endoderm of the gut while it is still an open tube, and later at E9.5, its expression marks the dorsal and ventral pancreatic buds and the intervening endoderm of the presumptive duodenum.[3] Null mutation of this gene prevents the pancreatic bud from expanding, resulting in extreme hyperglycemia and perinatal death.[2,3,6] In adults, PDX1 is maintained at high levels in beta cells, where it is required for efficient insulin gene transcription, [7-9] but its low expression in exocrine cells has not been thoroughly investigated.[3,10]

Disorders that afflict the pancreas can occur in both the exocrine and endocrine glands. Diabetes mellitus ("diabetes") is the most common disease associated with the endocrine pancreas, closely related to both genetic and environmental factors. Pancreatitis is an inflammation in the exocrine gland but can secondarily affect endocrine pancreas survival and function. Another notorious disease of the exocrine pancreas is pancreatic ductal adenocarcinoma (PDA), the most common form of pancreatic cancer, which is the fourth leading cause of cancer-related mortality in the United States.[11,12] Further, diabetes is a predisposing factor of PDA; hence, they act as cause and effect, respectively. This review summarizes the current findings of the involvement of PDX1 in pancreatic diseases, with emphasis on diabetes and PDA, and discusses how PDX1 could assist the pursuit of therapeutics for the treatment of these diseases.

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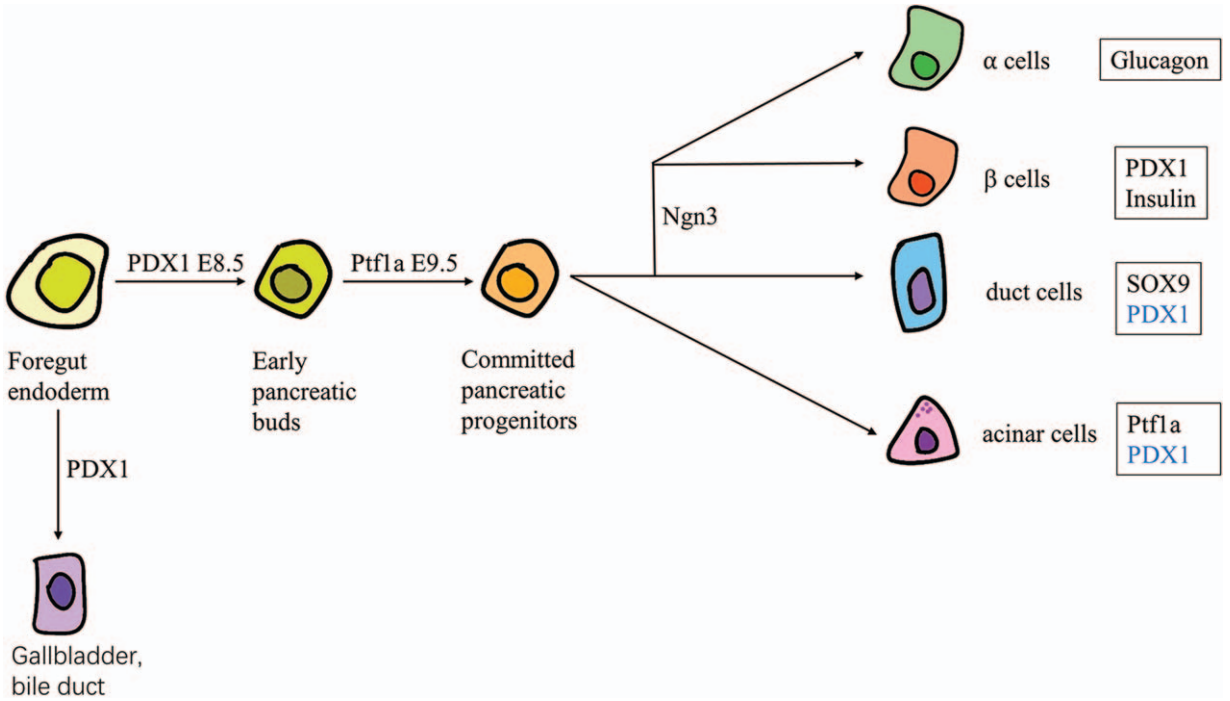

Figure 1: Lineage decisions during pancreas development. Transcription factors such as PDX1, Ptf1a, and Ngn3 regulate the series of events during the development of the pancreas, leading to the transition of progenitor cells (foregut endoderm) into fully differentiated cells (α cells, β cells, duct cells, acinar cells). And the gallbladder cells and bile duct cells arise from PDX1-expressing foregut endoderm cells in early embryo. PDX1 in blue print marks its low expression in duct cells and acinar cells. E8.5: Embryonic day 8.5; E9.5: Embryonic day 9.5; PDX1: Pancreatic and duodenal homeobox-1; Ptf1a: Pancreas associated transcription factor 1a; Ngn3: Neurogenin-3; SOX9: SRY-box 9.

PDX1 and diabetes mellitus

Diabetes mellitus is a complex metabolic disorder whose main clinical and diagnostic feature is hyperglycemia, along with the progressive loss or dysfunction of the β cells in the pancreas.[13,14] It has reached epidemic proportions, affecting around 425 million people worldwide (as estimated in 2017). China has the highest number of adults with diabetes, approximately 109.6 million, ranking second in diabetes-related health expenditure among the world, followed by the United States.[15] Thus, efficient control of this disease is needed to both alleviate the patient's pain and ease the burden placed on healthcare.

There are four main types of diabetes: type 1 diabetes (T1D), type 2 diabetes (T2D), gestational diabetes, and maturity-onset diabetes of the young. Out of these, the most common forms of diabetes are T1D and T2D. T1D is a metabolic disorder caused by autoimmune-mediated destruction of pancreatic β cells, leading to a complete or near-total loss of the hormone insulin.[16] Whereas, the pathogenesis of T2D begins with lifestyle-induced insulin resistance, which gradually increases the demand on β cells to secrete insulin, resulting in the failure of β cells to deliver sufficient amount of insulin.[17,18] When considered together, the main reasons behind diabetes is loss of β cell mass, function, and identity.[19]

PDX1 maintains β cell mass, function, and identity

The major treatment methods for diabetes are focused on the pancreatic β cells, since they are the central players in glycemic homeostasis, due to their ability to produce insulin — the only hormone capable of lowering high blood glucose level. The function of β cells is controlled at the transcriptional level, in which PDX1 plays an important role. PDX1 regulates a number of genes involved in maintaining the identity and function of β cells, such as insulin, IAPP (amylin), GK, and glucose transporter 2.[20] Avrahami *et al*[21] summarized the DNA methylation patterns observed in β cells during diabetes progression, and found that major methylation regulations occurred in functional genes including *PDX1*, which further emphasized its importance in this disease. Moreover, the inactivation of insulin promoter Cre (cyclization recombination enzyme) recombinase, which utilizes PDX1, leads to pancreatic agenesis. In addition, the heterozygous *PDX1* mutant showed elevated blood glucose level at birth and decreased β cell mass later, indicating that PDX1 levels influence not only β cell function, but also their mass.[22]

Furthermore, Gao *et al*[23] generated rat insulin promoter-CreER; PDX1$^{fl/fl}$; ROSA^{YFP} mice to specifically knockdown *PDX1* in adult β cells. They found that *PDX1* deletion mice displayed overt diabetes, while heterozygous ones who still carried one allele of *PDX1* exhibited a normal basal glucose level and normal glucose tolerance rates. It suggests that mature β cells could live without PDX1 existence, and changes in glucose tolerance were not due to the incomplete insufficiency of PDX1. The complete removal of *PDX1* led to a transcriptional shift in the insulin-positive cell population to an α-like cell phenotype, attributed to the binding of *PDX1* to glucagon and MAF BZIP transcription factor B, two α cell-specific gene targets, resulting in their repression. However, this α-like phenotype was not stable; after 30 days, the proportion of

cells expressing glucagon decreased from 35% to less than 15%, indicating that losing *PDX1* alone is not enough for the shift, but more mechanisms are involved in the cell maintenance.

In humans, PDX1 expression is detected around E28 of embryonic development.[24] Similar to rodents, humans with homozygous mutations in the *PDX1* gene were born with pancreatic agenesis. These individuals had permanent neonatal diabetes, with exocrine pancreas insufficiency. A case in 2019 reported an (Persian) 65-day-old Iranian patient with *PDX1* homozygous mutation was diagnosed with neonatal diabetes.[25] Additionally, their parents, possessing heterozygous *PDX1* mutations, had high susceptibility to diabetes with diagnosis reported as early as 2.5 years old.[26] Later, Guo *et al*[28] found the levels of PDX1 and a few other key islet-enriched TFs were dramatically reduced in human T2D islet β cells.[27] The Kyoto Encyclopedia of Genes and Genomes database also shows that PDX1 is highly disordered and inactivated in human T2D islets β cells and mouse models of T2D.

In summary, PDX1 is not only an essential TF for adult β cell survival, but also a "master factor" in regulating genes that maintain β cell function and identity, closely related to the progress of diabetes. Knowing more about the epigenetic mechanisms of PDX1 in β cells could provide a valuable approach for the treatment of diabetes.

PDX1 is essential in β cell reprogramming

At present, exogenous insulin and ancillary drugs are the major treatments for diabetes. But the burden on patients, with constant treatment and disease monitoring, leads us to pursue alternate therapies. The most definitive treatment for the disease is allogeneic pancreas/islet transplantation, which provides a self-regulating insulin source.[29] However, like any organ transplantations, all the recipients need to receive immunosuppressive drug therapy to prevent rejection after operations, which increases the risk of infection and certain types of cancer. In addition, 5-year follow-up data revealed that only a minority of the recipients still maintained insulin independence[30]; though more than half of them were free from exogenous insulin after 1 year from the operation. Therefore, the limitations of pancreatic islet transplantation lead researchers to investigate autologous insulin-producing cells for β cell replacement therapy.

Potentially, the two general approaches to generate β cell replacement are the differentiation of pluripotent stem cells (PSCs)[31-35] and the reprogramming of adult cells from endoderm-derived tissues.[36-39] To produce functional and mature β cells for cell-replacement therapy of diabetes, Wang *et al*[40] used PDX1 and H3K27AC chromatin immunoprecipitation (ChIP-seq) to further study *PDX1* target genes. They produced a novel induced pluripotent stem cell (iPSC) line with human pancreatic progenitors (PPs), and compared the PDX1 binding profiles obtained from them. They found that PDX1 regions include important pancreatic TFs, such as PDX1 itself, regulatory factor X6, hepatocyte nuclear factor 1 homeobox B, and Meis homeobox 1, which were activated and required for the differentiation and function of mature β cells. Additionally, they proved that the molecular programs that the PDX1 target sites activated were changed during the developmental stage. Recent studies in developing β cells differentiation protocols have shown that human PSCs (hPSCs)-derived β-like cells are functionally immature, and the efficiencies of differentiation can be variable depending on the hPSC lines used. Wang *et al*[41] derived a Pdx1-monomeric red fluorescent protein/insulin-humanized renilla reniformis green fluorescent protein (Pdx1-mRFP/insulin-hrGFP) dual-reporter cell line from MRC5-iPSCs and monitored the expression of PDX1 and insulin, to provide a better method for the development and refinement of β cell differentiation protocols from hPSCs. Though significant progress has been made in the differentiation of hPSCs to mature β cells, in clinical applications the practicality of this is hampered by the risk of teratoma formation, immunogenicity, and epigenetic abnormalities.[42,43] Thus, due to the important role of PDX1 in pancreatic fate, studies have attempted to use ectopic *PDX1* to reprogram "permissive" tissues and assorted cell lines into functional β cells.[44]

Pancreatic α and β cells are closely lineage-related cells. When ablating β cells, large fractions of regenerated β cells are derived from α cells, which implies that the two types of cells might be easily transformed into one another. However, exogenous *PDX1* in glucagon (Gcg)-positive embryonic or adult α cells could suppress Gcg expression but did not induce α/β switching. On the contrary, the neurogenin-3 (Ngn3)-positive endocrine progenitor cells enforced by PDX1 readily transformed to cells indistinguishable from normal β cells, suggesting that the plausibility of exogenous PDX1 alone inducing β cells depends on differentiation evaluation stage of endocrine maturation. Finally, transgenic mice were generated to express both PDX1 and MAF BZIP transcription factor A (MafA) in embryonic endocrine Ngn3-positive and committed glucagon-positive progenitors, and data showed that MafA could potentiate the ability of PDX1 in both Ngn3-positive and glucagon-positive progenitor reprogramming activities.[19]

In fact, multiple other adult cell types have been reprogrammed to undergo same the same fate as β cells. Among them, one promising attempt is to convert the antral stomach cells into functional insulin-secreting cells by expressing the hallmark pancreatic endocrine TFsPDX1, Ngn3, and MafA (NPM). Previously, the transformation of intestine cells to insulin-positive cells by providing NPM factors has been reported.[45,46] However, this results in incomplete β cells conversion, due to blocking of the intestine-specific caudal type homeobox 2 gene. In comparison, antral stomach endocrine cells and β cells have closer transcriptional similarity, and antral stomach endocrine cells have been proven to reprogram more effectively under NPM condition, with steady expression of key β cell genes and substantially improved glucose responsiveness. Besides, based on the published protocols of bioengineering stomach, researchers embedded insulin-positive cells into "stomach mini-organ" and transplanted it into diabetes mice. This bioengineering technique proved to be effective, regulating blood glucose

by resealing insulin directly into the circulation.[47] With this approach, patients might get long-term treatment with fewer side effects. Furthermore, besides NPM, paired box 4 was introduced by Zhang *et al*[48] as another TF that synergistically acts to promote functional β cells transformation process, which may better serve for further investigation.

Murine gallbladder can be another choice for β cell replacement. It is a dispensable organ easily acquired by invasive laparoscopic procedure without serious sequelae. Also, it shows close developmental proximity to β cells – they are both derived from pancreatobiliary progenitors. Grompe *et al*[49] found that overexpressing NPM factors in mouse gallbladder cells *in vitro* led to limited amount of insulin production and immature cell types. Moreover, the reprogrammed cells did not display glucose-responsiveness. But when they improved the protocol by adding paired box 6 (Pax6), another key TF for pancreatic endocrine progenitors, the reprogramming was seen to become more efficient in increasing not only β-like cells numbers, but also insulin expression. This second-generation reprogrammed β-like cells (rGBC2) could synthesize, process, and secret insulin in response to glucose stimulation, and survived for at least 5 months *in vivo*.[39] Later, the team combined adenoviral-mediated transduction of NPM factors and Pax6 with small molecule-dependent transdifferentiation into the human GBCs and achieved insulin-producing pancreatic β-like cells which could be transiently engrafted in immunodeficient mice.[50]

If future studies can uncover how to apply patient-derived GBCs to the patients themselves, it will essentially solve the problems raised by reprogramming PSCs mentioned above. However, other complications related to the reliability and robustness of the reprogrammed GBCs, the risk of transducing TFs like PDX1 into individuals, and the efficiency of reprogramming will need to be further evaluated.

Utilizing human cells for the treatment of diseases is not an unfamiliar concept. Hematopoietic stem cell transplantation for treating hematologic malignancies is a successful and well-developed medical procedure. Prevalent focus is still on multifunctional cells, such as PSCs and mesenchymal stromal cells. If different types of mature cells derived from the same progenitors can be successfully reprogrammed into each other, under stable and well-functioning conditions, it may provide us more resources for the development of improved therapeutic approaches.

PDX1 and PDA

PDA accounts for >85% of all pancreatic cancers and is one of the most lethal malignancies worldwide. Despite immense progress in the diagnosis and treatment of pancreatic cancer, its 5-year overall survival is only 8.2% in the United States.[51] Kirsten rat sarcoma gene (*KRAS*) mutations are found in >90% of PDA patients, and are required for tumor initiation and maintenance.[52] Studies have shown that mouse models with oncogenic *KRAS* throughout the pancreatic parenchyma mimic the human PDA progression to maximum extent.[53-55] PDA got its name from its histological similarity with ductal structures. However, studies reveal that ductal cells are actually refractory to oncogenic transformation. On the contrary, acinar cells can easily form acinar-to-ductal metaplasia (ADM), the PDA precursor lesions with ductal features, indicating the cellular origin of PDA.[56]

PDX1 in pancreatic injury and ADM formation

During the development of the pancreas, PDX1 and other TFs regulate the cascade of events which leads the transition of progenitor cells into fully differentiated cells. For instance, the increased expression of SRY-box 9 and PDX1 in PPs induces the differentiation into pancreatic lineages, at the same time inhibiting the formation of intestinal and hepatic lineages. Later, the TFs required for the active maintenance of adult acinar cell differentiation, such as pancreas associated transcription factor 1a (Ptf1a) and nuclear receptor subfamily 5 group a member 2 (Nr5a2), are maintained at high levels but the expression of PDX1 conversely becomes low. However, PDX1 is found to be upregulated in ADM induced by the constitutive overexpression of transforming growth factor alpha (TGFα).[57] Likewise, the abnormally increased expression of PDX1 is also detected in mouse PDA models with mutant *KRAS*.

In effect, elevated PDX1 expression in adult exocrine pancreas has been observed in many pathological conditions, including pancreatic injury and pancreatitis. Cerulean is a cholecystokinin analog that stimulates transient ADM in wild-type pancreatitis. Studies have reported abnormally high PDX1 expression in non-islet pancreas, after pancreatectomy or cerulean treatment.[58] Well-differentiated cells tend to dedifferentiate to an earlier stage when they get injured, so that they have more potential to recover from the impairment. Since all pancreatic lineages come from PDX1-positive progenitors, the elevation of it is reasonable in these damages. In addition, when the wild-type and PDX1 ablation pancreas ($Ptf1a^{CreERTM/+}$; $PDX1^{f/f}$) are treated with cerulean, the wild-type cells recovered from the stimulation around 7 days later, but acinar cells with null PDX1 displayed severe reduction in the pancreas-to-body-mass ratio and did not recover, indicating that ADM derived from acinar cells requires PDX1 for regenerating the damaged organ.[59] However, PDX1 overexpression in exocrine precursors marked dysmorphogenesis in the exocrine pancreas.[60] Additionally, its overexpression in benign HEK293 cells and human pancreatic duct epithelial cells results in increased cell proliferation, invasion, and colony formation *in vitro*.[61] Collectively, PDX1 takes the responsibility to maintain and re-establish acinar differentiation when damage happens to the pancreas. Its expression needs to be tightly controlled to properly maintain adult acinar tissue functionality.

In accordance with injury, PDX1 deficiency in *KRAS*-induced PDA mouse model ($Kras^{LSL-G12D/+}$; $Ptf1a^{CreERTM/+}$; $PDX1^{f/f}$) displays a widespread loss of acinar cells, accompanied by extensive formation of ductal lesions. This is due to erosion of acinar differentiation, rather than its expansion through increased cell proliferation, based on 5-bromo-2-deoxyuridine incorporation. Genome-wide

transcriptome profiling on those animals showed that genes related to the mature acinar cell state, such as amylase alpha 2B (Amy2b), carboxypeptidase A1, carboxypeptidase B1, carboxyl ester lipase, and chymotrypsin like elastase 2a (Cela2a), were down-regulated in PDX1-negative acinar cells, explaining the extensive erosion of acinar cells.[59] Overall, these data explain how PDX1 maintains acinar cells identity, and its important function in tissue regeneration and ADM formation.

Multiple roles of PDX1 in tumorigenesis

Unlike the role of PDX1 in diabetes, this master gene behaves differently at different tumor stages. PDX1 function as a tumor suppressor in maintaining acinar cell identity and preventing ADM formation. Whereas, further data shows that knocking down *PDX1* in mouse PDA-derived cell lines can block cell growth and increase apoptosis, which is consistent with human PDA cell lines,[61] and this is not affected by the different malignant cell origins.[62] Hence, PDX1 changes its role from tumor suppressor to oncogene during the process of tumorigenicity.

Interestingly, recent next-generation sequencing analysis has revealed that PDA is a mixture of four different sub-types with distinct histopathological features and prognosis.[63,64] Among them, the *PDX1* promoter is highly methylated in the worst sub-type, the squamous one, indicating epigenetic silencing of *PDX1*. The squamous sub-type displays higher metastatic potential compared to others, which is the reason PDA patients usually die from distant metastasis. Considering this, later studies suggest that epithelial cells need to reduce PDX1 expression to undergo the epithelial-to-mesenchymal transition (EMT) process, where epithelial cells lose their epithelial marks to undergo easier dissemination into a vascular or lymphatic system, thereby enhancing metastasis. Furthermore, Kondratyeva *et al*[65] proposed the possibility of PDX1 delivering into this aggressive PDAC cells to stop cancer metastasis. Thus, in this stage, PDX1 reduction appears to enhance tumor aggressiveness, hence increasing PDX1 levels may be a novel method for gene therapy.[66]

According to *PDX1* ChIP-seq (chromatin immunoprecipitation combined with high-throughput sequencing) done on primary mouse acinar cells and mouse PDA-derived cell lines, there is minimal overlap of *PDX1* binding regions between acinar and PDA cells, indicating the shift of gene sets that *PDX1* regulates in primary and transformed cells. Further investigation shows that in acinar cells, *PDX1* interacts with genes maintaining acinar differentiation state (Cela1, Cela2, and Amy1), embryonic development, and epithelial cell differentiation (Nr5a2, FoxA2, and Onecut1). On the contrary, in tumor cells, *PDX1* binds to genes important for tumorigenesis, including EMT and cellular response to TGFβ signal. Its enrichment of different regions affects active transcription of associated genes, which is also thoroughly proved in the RNA-seq data. *PDX1* tends to bind acinar-specifying genome regions in acinar cells (Hnf4a, Nkx6.1, Hnf1α, and Hnf1β), but binds to the motifs of oncogenic TFs in PDA cells (Gsc2, Prrx2, Stat5, c-Jun, and c-Fos).[59] The dynamic shift of PDX1 explains its multi-functionality in tumorigenesis, but detailed analysis about this process is needed to further elucidate how PDX1 regulates tumor formation by changing targets.

As for the role of PDX1 in metastasis, it is hard to give an accurate explanation. A hypothesis is that tumor cells use mechanisms to compensate for PDX1 loss. Due to the fact that *MYC* is up-regulated in PDX1-absent PDA cells,[63] and well-differentiated tumors are *PDX1*-high and *MYC*-low, while undifferentiated invasive tumors are *MYC*-high and *PDX1*-low, the *MYC* regulatory network becomes a potential candidate.[59] However, more evidence and a deeper understanding about this replacement mechanism are needed.

Tons of discovered cancer genes are classified into oncogenes or tumor suppressor genes, according to their function in a cancer cell. However, PDX1 sheds light on another group of genes whose characteristics rely on the genetic network, in context of the whole genome. The multi-functionality of those "chameleons" may elucidate the mechanisms of tumor initiation and progression, highlighting the reasons of chemotherapy failure.

PDX1 is also closely associated with other pancreatic diseases, such as insulinoma, a rare tumor derived from islet β cells within the pancreas. The incidence of this disease is approximately 3 to 4 per 1,000,000 in general population.[61] These patients suffer from uncontrolled hypoglycemia due to the expanded β cell mass continues to secret insulin. Studies have found that PDX1 is significantly overexpressed in human insulinoma specimens, as well as in mouse insulinoma cell lines. Further, using RNA interference therapy to knock down *PDX1* exhibits decrease in insulin expression and secretion; this may be harnessed as a novel therapeutic strategy in alleviating the patient's pain in the future.

Summary

In summary, knowing how PDX1, as an essential TF in pancreas, functions in pancreatic diseases could give us important insights into the epigenetic mechanisms of disease occurrence and development, and also provide a chance to develop new therapeutic strategies.

Conflicts of interest

None.

References

1. Brooke NM, Garcia-Fernandez J, Holland PW. The ParaHox gene cluster is an evolutionary sister of the Hox gene cluster. Nature 1998;392:920–922. doi: 10.1038/31933.
2. Holland AM, Hale MA, Kagami H, Hammer RE, MacDonald RJ. Experimental control of pancreatic development and maintenance. Proc Natl Acad Sci U S A 2002;99:12236–12241. doi: 10.1073/pnas.192255099.
3. Guz Y, Montminy MR, Stein R, Leonard J, Gamer LW, Wright CV, *et al*. Expression of murine STF-1, a putative insulin gene transcription factor, in beta cells of pancreas, duodenal epithelium and pancreatic exocrine and endocrine progenitors during ontogeny. Development 1995;121:11–18. doi: 10.1016/S0070-2153(08)60569-4.

4. Jonsson J, Carlsson L, Edlund T, Edlund H. Insulin-promoter-factor 1 is required for pancreas development in mice. Nature 1994;371:606–609. doi: 10.1038/371606a0.
5. Offield MF, Jetton TL, Labosky PA, Ray M, Stein RW, Magnuson MA, *et al*. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. Development 1996;122:983–995. doi: 10.1073/pnas.95.22.13036.
6. Ahlgren U, Jonsson J, Edlund H. The morphogenesis of the pancreatic mesenchyme is uncoupled from that of the pancreatic epithelium in IPF1/PDX1-deficient mice. Development 1996;122:1409–1416. doi: 10.1101/gad.10.10.1284.
7. Ahlgren U, Jonsson J, Jonsson L, Simu K, Edlund H. Beta-cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. Genes Dev 1998;12:1763–1768. doi: 10.1101/gad.12.12.1763.
8. Ohlsson H, Karlsson K, Edlund T. IPF1, a homeodomain-containing transactivator of the insulin gene. EMBO J 1993;12:4251–4259. doi: 10.1002/j.1460-2075.1993.tb06109.x.
9. Melloul D, Marshak S, Cerasi E. Regulation of pdx-1 gene expression. Diabetes 2002;51 (Suppl 3):S320–S325. doi: 10.2337/diabetes.51.2007.s320.
10. Wu KL, Gannon M, Peshavaria M, Offield MF, Henderson E, Ray M, *et al*. Hepatocyte nuclear factor 3beta is involved in pancreatic beta-cell-specific transcription of the pdx-1 gene. Mol Cell Biol 1997;17:6002–6013. doi: 10.1128/mcb.17.10.6002.
11. Shih HP, Wang A, Sander M. Pancreas organogenesis: from lineage determination to morphogenesis. Annu Rev Cell Dev Biol 2013;29:81–105. doi: 10.1146/annurev-cellbio-101512-122405.
12. Roy N, Hebrok M. Regulation of cellular identity in cancer. Dev Cell 2015;35:674–684. doi: 10.1016/j.devcel.2015.12.001.
13. Chen Y. Diagnosis of diabetes. N Engl J Med 2013;368:192. doi: 10.1056/NEJMc1212738.
14. Inzucchi SE. Diagnosis of diabetes. N Engl J Med 2013;368:193. doi: 10.1056/NEJMc1212738.
15. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, *et al*. IDF diabetes atlas: global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pract 2017;128:40–50. doi: 10.1016/j.diabres.2017.03.024.
16. Zaccardi F, Webb DR, Yates T, Davies MJ. Pathophysiology of type 1 and type 2 diabetes mellitus: a 90-year perspective. Postgrad Med J 2016;92:63–69. doi: 10.1136/postgradmedj-2015-133281.
17. Mokdad AH, Bowman BA, Ford ES, Vinicor F, Marks JS, Koplan JP. The continuing epidemics of obesity and diabetes in the United States. JAMA 2001;286:1195–1200. doi: 10.1001/jama.286.10.1195.
18. Alejandro EU, Gregg B, Blandino-Rosano M, Cras-Meneur C, Bernal-Mizrachi E. Natural history of beta-cell adaptation and failure in type 2 diabetes. Mol Aspects Med 2015;42:19–41. doi: 10.1016/j.mam.2014.12.002.
19. Swisa A, Glaser B, Dor Y. Metabolic stress and compromised identity of pancreatic beta cells. Front Genet 2017;8:21. doi: 10.3389/fgene.2017.00021.
20. Ashizawa S, Brunicardi FC, Wang XP. PDX-1 and the pancreas. Pancreas 2004;28:109–120. doi: 101097/00006676-200403000-00001.
21. Avrahami D, Kaestner KH. The dynamic methylome of islets in health and disease. Mol Metab 2019;27S:S25–S32. doi: 10.1016/j.molmet.2019.06.007.
22. Gannon M, Ables ET, Crawford L, Lowe D, Offield MF, Magnuson MA, *et al*. Pdx-1 function is specifically required in embryonic beta cells to generate appropriate numbers of endocrine cell types and maintain glucose homeostasis. Dev Biol 2008;314:406–417. doi: 10.1016/j.ydbio.2007.10.038.
23. Gao T, McKenna B, Li C, Reichert M, Nguyen J, Singh T, *et al*. Pdx1 maintains beta cell identity and function by repressing an alpha cell program. Cell Metab 2014;19:259–271. doi: 10.1016/j.cmet.2013.12.002.
24. Jennings RE, Berry AA, Kirkwood-Wilson R, Roberts NA, Hearn T, Salisbury RJ, *et al*. Development of the human pancreas from foregut to endocrine commitment. Diabetes 2013;62:3514–3522. doi: 10.2337/db12-1479.
25. Sahebi L, Niknafs N, Dalili H, Amini E, Esmaeilnia T, Amoli M, *et al*. Iranian neonatal diabetes mellitus due to mutation in PDX1 gene: a case report. J Med Case Rep 2019;13:258. doi: 10.1186/s13256-019-2149-x.
26. Fajans SS, Bell GI, Paz VP, Below JE, Cox NJ, Martin C, *et al*. Obesity and hyperinsulinemia in a family with pancreatic agenesis and MODY caused by the IPF1 mutation Pro63fsX60. Transl Res 2010;156:7–14. doi: 10.1016/j.trsl.2010.03.003.
27. Guo S, Dai C, Guo M, Taylor B, Harmon JS, Sander M, *et al*. Inactivation of specific beta cell transcription factors in type 2 diabetes. J Clin Invest 2013;123:3305–3316. doi: 10.1172/JCI65390.
28. Du Z, Uversky VN. A comprehensive survey of the roles of highly disordered proteins in type 2 diabetes. Int J Mol Sci 2017;18:E2010. doi: 10.3390/ijms18102010.
29. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, *et al*. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 2000;343:230–238. doi: 10.1056/NEJM200007273430401.
30. Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, *et al*. Five-year follow-up after clinical islet transplantation. Diabetes 2005;54:2060–2069. doi: 10.2337/diabetes.54.7.2060.
31. Macri-Pellizzeri L, De-Juan-Pardo EM, Prosper F, Pelacho B. Role of substrate biomechanics in controlling (stem) cell fate: implications in regenerative medicine. J Tissue Eng Regen Med 2018;12:1012–1019. doi: 10.1002/term.2586.
32. Pagliuca FW, Millman JR, Gurtler M, Segel M, Van Dervort A, Ryu JH, *et al*. Generation of functional human pancreatic beta cells in vitro. Cell 2014;159:428–439. doi: 10.1016/j.cell.2014.09.040.
33. Rezania A, Bruin JE, Riedel MJ, Mojibian M, Asadi A, Xu J, *et al*. Maturation of human embryonic stem cell-derived pancreatic progenitors into functional islets capable of treating pre-existing diabetes in mice. Diabetes 2012;61:2016–2029. doi: 10.2337/db11-1711.
34. Rezania A, Bruin JE, Xu J, Narayan K, Fox JK, O'Neil JJ, *et al*. Enrichment of human embryonic stem cell-derived NKX6.1-expressing pancreatic progenitor cells accelerates the maturation of insulin-secreting cells in vivo. Stem Cells 2013;31:2432–2442. doi: 10.1002/stem.1489.
35. Russ HA, Parent AV, Ringler JJ, Hennings TG, Nair GG, Shveygert M, *et al*. Controlled induction of human pancreatic progenitors produces functional beta-like cells in vitro. EMBO J 2015;34:1759–1772. doi: 10.15252/embj.201591058.
36. Banga A, Greder LV, Dutton JR, Slack JM. Stable insulin-secreting ducts formed by reprogramming of cells in the liver using a three-gene cocktail and a PPAR agonist. Gene Ther 2014;21:19–27. doi: 10.1038/gt.2013.50.
37. Li W, Nakanishi M, Zumsteg A, Shear M, Wright C, Melton DA, *et al*. In vivo reprogramming of pancreatic acinar cells to three islet endocrine subtypes. Elife 2014;3:e01846. doi: 10.7554/eLife.01846.
38. Thorel F, Nepote V, Avril I, Kohno K, Desgraz R, Chera S, *et al*. Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. Nature 2010;464:1149–1154. doi: 10.1038/nature08894.
39. Wang Y, Galivo F, Pelz C, Haft A, Lee J, Kim SK, *et al*. Efficient generation of pancreatic beta-like cells from the mouse gallbladder. Stem Cell Res 2016;17:587–596. doi: 10.1016/j.scr.2016.10.009.
40. Wang X, Sterr M, Burtscher I, Chen S, Hieronimus A, Machicao F, *et al*. Genome-wide analysis of PDX1 target genes in human pancreatic progenitors. Mol Metab 2018;9:57–68. doi: 10.1016/j.molmet.2018.01.011.
41. Wang Q, Donelan W, Ye H, Jin Y, Lin Y, Wu X, *et al*. Real-time observation of pancreatic beta cell differentiation from human induced pluripotent stem cells. Am J Transl Res 2019;11:3490–3504.
42. Holditch SJ, Terzic A, Ikeda Y. Concise review: pluripotent stem cell-based regenerative applications for failing beta-cell function. Stem Cells Transl Med 2014;3:653–661. doi: 10.5966/sctm.2013-0184.
43. Kim K, Zhao R, Doi A, Ng K, Unternaehrer J, Cahan P, *et al*. Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. Nat Biotechnol 2011;29:1117–1119. doi: 10.1038/nbt.2052.
44. Teo AK, Tsuneyoshi N, Hoon S, Tan EK, Stanton LW, Wright CV, *et al*. PDX1 binds and represses hepatic genes to ensure robust pancreatic commitment in differentiating human embryonic stem cells. Stem Cell Reports 2015;4:578–590. doi: 10.1016/j.stemcr.2015.02.015.
45. Chen YJ, Finkbeiner SR, Weinblatt D, Emmett MJ, Tameire F, Yousefi M, *et al*. De novo formation of insulin-producing "neo-beta cell islets" from intestinal crypts. Cell Rep 2014;6:1046–1058. doi: 10.1016/j.celrep.2014.02.013.

46. Zhu Y, Liu Q, Zhou Z, Ikeda Y. PDX1, neurogenin-3, and MAFA: critical transcription regulators for beta cell development and regeneration. Stem Cell Res Ther 2017;8:240. doi: 10.1186/s13287-017-0694-z.
47. Ariyachet C, Tovaglieri A, Xiang G, Lu J, Shah MS, Richmond CA, *et al*. Reprogrammed stomach tissue as a renewable source of functional beta cells for blood glucose regulation. Cell Stem Cell 2016;18:410–421. doi: 10.1016/j.stem.2016.01.003.
48. Zhang T, Wang H, Wang T, Wei C, Jiang H, Jiang S, *et al*. Pax4 synergistically acts with Pdx1, Ngn3 and MafA to induce HuMSCs to differentiate into functional pancreatic beta-cells. Exp Ther Med 2019;18:2592–2598. doi: 10.3892/etm.2019.7854.
49. Hickey RD, Galivo F, Schug J, Brehm MA, Haft A, Wang Y, *et al*. Generation of islet-like cells from mouse gall bladder by direct ex vivo reprogramming. Stem Cell Res 2013;11:503–515. doi: 10.1016/j.scr.2013.02.005.
50. Galivo F, Benedetti E, Wang Y, Pelz C, Schug J, Kaestner KH, *et al*. Reprogramming human gallbladder cells into insulin-producing beta-like cells. PLoS One 2017;12:e0181812. doi: 10.1371/journal.pone.0181812.
51. Miller KD, Nogueira L, Mariotto AB, Rowland JH, Yabroff KR, Alfano CM, *et al*. Cancer treatment and survivorship statistics, 2019. CA Cancer J Clin 2019;69:363–385. doi: 10.3322/caac.21565.
52. Ying H, Dey P, Yao W, Kimmelman AC, Draetta GF, Maitra A, *et al*. Genetics and biology of pancreatic ductal adenocarcinoma. Genes Dev 2016;30:355–385. doi: 10.1101/gad.275776.115.
53. Aguirre AJ, Bardeesy N, Sinha M, Lopez L, Tuveson DA, Horner J, *et al*. Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. Genes Dev 2003;17:3112–3126. doi: 10.1101/gad.1158703.
54. Filippini D, Agosto S, Delfino P, Simbolo M, Piro G, Rusev B, *et al*. Immunoevolution of mouse pancreatic organoid isografts from preinvasive to metastatic disease. Sci Rep 2019;9:12286. doi: 10.1038/s41598-019-48663-7.
55. Kondratyeva LG, Didych DA, Chernov IP, Kopantzev EP, Stukacheva EA, Vinogradova TV, *et al*. Dependence of expression of regulatory master genes of embryonic development in pancreatic cancer cells on the intracellular concentration of the master regulator PDX1. Dokl Biochem Biophys 2017;475:259–263. doi: 10.1134/S1607672917040056.
56. Kopp JL, von Figura G, Mayes E, Liu FF, Dubois CL, Morris JPt, *et al*. Identification of Sox9-dependent acinar-to-ductal reprogramming as the principal mechanism for initiation of pancreatic ductal adenocarcinoma. Cancer Cell 2012;22:737–750. doi: 10.1016/j.ccr.2012.10.025.
57. Song SY, Gannon M, Washington MK, Scoggins CR, Meszoely IM, Goldenring JR, *et al*. Expansion of Pdx1-expressing pancreatic epithelium and islet neogenesis in transgenic mice overexpressing transforming growth factor alpha. Gastroenterology 1999;117:1416–1426. doi: 10.1016/s0016-5085(99)70292-1.
58. Fendrich V, Esni F, Garay MV, Feldmann G, Habbe N, Jensen JN, *et al*. Hedgehog signaling is required for effective regeneration of exocrine pancreas. Gastroenterology 2008;135:621–631. doi: 10.1053/j.gastro.2008.04.011.
59. Roy N, Takeuchi KK, Ruggeri JM, Bailey P, Chang D, Li J, *et al*. PDX1 dynamically regulates pancreatic ductal adenocarcinoma initiation and maintenance. Genes Dev 2016;30:2669–2683. doi: 10.1101/gad.291021.116.
60. Heller RS, Stoffers DA, Bock T, Svenstrup K, Jensen J, Horn T, *et al*. Improved glucose tolerance and acinar dysmorphogenesis by targeted expression of transcription factor PDX-1 to the exocrine pancreas. Diabetes 2001;50:1553–1561. doi: 10.2337/diabetes.50.7.1553.
61. Yu J, Liu SH, Sanchez R, Nemunaitis J, Rozengurt E, Brunicardi FC. PDX1 associated therapy in translational medicine. Ann Transl Med 2016;4:214. doi: 10.21037/atm.2016.03.51.
62. von Figura G, Fukuda A, Roy N, Liku ME, Morris Iv JP, Kim GE, *et al*. The chromatin regulator Brg1 suppresses formation of intraductal papillary mucinous neoplasm and pancreatic ductal adenocarcinoma. Nat Cell Biol 2014;16:255–267. doi: 10.1038/ncb2916.
63. Bailey P, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, *et al*. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature 2016;531:47–52. doi: 10.1038/nature16965.
64. Collisson EA, Sadanandam A, Olson P, Gibb WJ, Truitt M, Gu S, *et al*. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. Nat Med 2011;17:500–503. doi: 10.1038/nm.2344.
65. Kondratyeva LG, Safina DR, Chernov IP, Kopantzev EP, Kostrov SV, Sverdlov ED. PDX1, a key factor in pancreatic embryogenesis, can exhibit antimetastatic activity in pancreatic ductal adenocarcinoma. Cancer Manag Res 2019;11:7077–7087. doi: 10.2147/CMAR.S209940.
66. Sharma A, Zangen DH, Reitz P, Taneja M, Lissauer ME, Miller CP, *et al*. The homeodomain protein IDX-1 increases after an early burst of proliferation during pancreatic regeneration. Diabetes 1999;48:507–513. doi: 10.2337/diabetes.48.3.507.

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