Transcriptional regulation of pancreas development and β-cell function

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Abstract: A small number of cells in the adult pancreas are endocrine cells. They are arranged in clusters called islets of Langerhans. The islets make insulin, glucagon, and other endocrine hormones, and release them into the blood circulation. These hormones help control the level of blood glucose. Therefore, a dysfunction of endocrine cells in the pancreas results in impaired glucose homeostasis, or diabetes mellitus. The pancreas is an organ that originates from the evaginations of pancreatic progenitor cells in the epithelium of the foregut endoderm. Pancreas organogenesis and maturation of the islets of Langerhans occurs via a coordinated and complex interplay of transcriptional networks and signaling molecules, which guide a stepwise and repetitive process of the propagation of progenitor cells and their maturation, eventually resulting in a fully functional organ. Increasing our understanding of the extrinsic, as well as intrinsic mechanisms that control these processes should facilitate the efforts to generate surrogate β cells from ES or iPS cells, or to reactivate the function of important cell types within pancreatic islets that are lost in diabetes.

Key words: Pancreas development, Transcription factor, Pdx1, Ptf1a, Diabetes

Introduction

In the past two decades, there has been increased interest in the molecular and genetic events regulating pancreas development. Numerous transcription factors and extrinsic signals that dictate the proper differentiation of cells into the endocrine and exocrine pancreas have been identified. Understanding the molecular mechanisms of mouse pancreas development has greatly facilitated our knowledge on how defects in the genes involved in pancreas organogenesis may cause human diabetes. It also opens the door towards establishing new therapeutic strategies against diabetes, such as by stimulating the differentiation of surrogate β cells from somatic cells or iPS cells. In this manuscript, I will give a brief outline of mouse pancreas development and discuss its physiological relevance to human diabetes and therapeutics.

Overview of mouse pancreas development

The vertebrate pancreas develops from one dorsal and two ventral evaginations in the endodermal epithelium [1, 2]. Early Pdx1 expression (E8.5-9.0) marks the pre-pancreatic endoderm prior to the onset of pancreatic bud formation [3]. In Pdx1-homozygous null mice, the pancreas is almost entirely missing, and pancreatic buds are arrested in early development. The pancreatic rudiment of Pdx1-null mouse embryos does however contain transient, first wave insulin-positive cells [4], and longer-lived glucagon-positive cells [3]. These observations indicate that Pdx1 is required for pancreas organ formation but not for the generation of first wave endocrine cells. Pdx1 is also expressed in the endodermal region, which develops into the caudal stomach, rostral duodenum, common bile duct, and gall bladder. In agreement with the expression profile of Pdx1, Pdx1-homozygous null mice showed shorter duodenal villi than wild-type mice at postnatal stages, and the loss of major duodenal papilla, which results in duodenal-biliary reflux and bile infection, finally resulting in the formation of brown pigment biliary stones [5]. Another key transcriptional activator required for pancreas
organogenesis is Ptf1a. Since Ptf1a was shown to trans-activate genes expressed specifically in the exocrine pancreas, such as amylase, Ptf1a was expected to be specifically required for exocrine cell function [6]. A subsequent study demonstrated that Ptf1a is required for the determination of the fates of all pancreatic cells. A genetic lineage tracing experiment using Ptf1a-Cre knock-in mice conducted by Kawaguchi et al. (Fig. 1) indicated that Ptf1a expression is restricted to the two pancreatic buds at E10.5 and that Ptf1a-expressing cells contribute to all pancreatic cell lineages, including pancreatic acinar, endocrine, and duct cells [7]. Importantly, the inactivation of Ptf1a alters the character of pancreatic progenitors such that their progeny proliferate and adopt normal fates of the duodenal epithelium, including the crypt, which is the stem-cell compartment of the duodenum. These experiments provide evidence that Ptf1a expression is specifically required for the acquisition of a pancreatic fate by undifferentiated Pdx1-expressing progenitors (Fig. 2).

Fig. 1  Genetic lineage tracing by Cre-mediated reporter-gene activation

Ptf1a Cre knock-in mice are crossed with ROSA26R mice, which carry the lacZ gene driven by the cell-type-independent ROSA26 promoter. In offspring obtained from this cross, Ptf1a-driven expression of LacZ is maintained in Ptf1a-expressing cells and their progeny.

Fig. 2  Roles for Pdx1 and Ptf1a in the differentiation of progenitors in the foregut endoderm

A) Pdx1-Ptf1a double-positive progenitor cells in the foregut endoderm can acquire a pancreatic cell fate. B) Signals from endothelial cells in the blood vessels stimulate Ptf1a expression in the dorsal pancreatic primordia. FoxA1 and FoxA2 induce Pdx1 activation in the dorsal pancreas. Pdx1 and Ptf1a cooperatively activate the proliferation and maturation of pancreatic progenitors.
Transcriptional regulation of Pdx1 in organogenesis

Regulatory mechanisms driving Pdx1 gene expression have been extensively studied among the genes involved in pancreas development. Alignment of the mouse, rat, and human Pdx1 gene sequences revealed three evolutionarily conserved regions, called ‘Area I-II-III’ (Fig. 3). The Area I-II-III region harbors binding sites for the maturity-onset diabetes of the young (MODY) transcription factors, such as HNF-1α and Pdx1 itself [8,9], as well as other islet-enriched transcription factors, including Foxa2/HNF3β, Pax6, MafA, and HNF-6 [10-14]. Sufficiency tests in transgenic mice and cultured cell lines identified islet-specific (PstI-BstEII fragment containing Area I-II) and β-cell-specific (XhoI-BglII fragment containing Area III and an adjacent 3’ region) cis-elements that overlap with Area I-II-III, suggesting that Area I-II-III may function specifically in the differentiation and maintenance of pancreatic islets [9, 10, 15, 16]. The crucial in vivo role of the Area I-II-III enhancer region was demonstrated by the observation that pancreata of mutant mice with a homozygous deletion of the enhancer were severely hypoplastic [17]. The ventral lobe of the mutant pancreas was completely missing owing to inactivation of Ptf1a in the ventral pancreas anlagen. Proximal to the duodenum, the dorsal pancreatic remnant contained a relatively large mass of acinar cells. Pancreatic endocrine cell differentiation in this zone was highly defective, with no well-differentiated islets observed. The portion of the dorsal pancreatic remnant distal to the duodenum developed as a cyst-like structure with no expression of pancreatic endocrine or acinar markers [17]. Thus, relatively high levels of Pdx1 are required for proper pancreas development.
specification and outgrowth/differentiation as well as gut/stomach enteroendocrine cell specification from the foregut endoderm. Low Pdx1 levels can drive the normal development of many aspects of gut epithelium differentiation, such as structures of the gastro-duodenal junction and Brunner’s glands. A major finding from these studies is that the various organs of the posterior foregut are sensitive to spatiotemporal and expression level differences of Pdx1, which are largely regulated by transcriptional networks that converge in Area I-II-III. Moreover, Pdx1 plays a role in specifying differentiation into the dorsal or ventral pancreatic bud, and the results from these studies suggest that the dorsal bud contains different classes of progenitors with respect to their ability to move through the pancreatic differentiation program [17].

In contrast to the principal control region of Area I-II-III, located between −2,800 and −1,600 base pairs (bp), the contribution of a highly conserved distal enhancer element between −6,530 and −6,045 bp, termed “Area IV”, remained less clear (Fig. 3) [18]. This distal enhancer is capable of directing pancreatic β-cell-selective reporter gene expression and potentiating proximal enhancer activity, independently of Area I-II-III [18]. It has been reported that both Foxa1 and Foxa2 predominantly occupy the distal Area IV enhancer during pancreas development [19]. Interestingly, they demonstrated that deletion of both Foxa factors from the pancreatic primordia causes almost complete pancreatic agenesis and loss of Pdx1 expression. These data suggest that Foxa1 and Foxa2 regulate Pdx1 expression via Area IV, working in addition to the formerly reported Foxa2 site in Area II.

**Endothelial signals and dorsal pancreas bud formation**

The notochord and dorsal aorta have been associated with the induction of dorsal pancreas bud formation in the endoderm. Initially, the dorsal midline endoderm lies adjacent to the notochord. It has been reported that the notochord sends out a signal that induces dorsal pancreatic formation, and initiates and maintains the expression of genes required for pancreas development, such as Pdx1 and Isl1, by excluding sonic hedgehog expression from the endoderm [20, 21]. Slightly later at E8.75–E9.0, when robust Pdx1 expression can be detected in the dorsal pancreatic region, the notochord spatially separates from the endoderm. This dorsal displacement of the notochord away from the dorsal pancreatic endoderm coincides with the fusion of the aortas at the midline, and eventually the endodermal cells of the dorsal aorta become located immediately adjacent to the most dorsal pancreatic endoderm [1]. At this stage, endothelial cells in blood vessels appear to provide signals that induce pancreas development. Removal of the dorsal aorta in Xenopus embryos results in a deficiency of insulin expression in vivo. Transgenic ectopic expression of VEGF induces ectopic vascularization in the posterior foregut, thereby leading to ectopic insulin expression and islet hyperplasia [2]. Furthermore, it has been reported that mice deficient for flk-1, which lack endothelial cells show normal initial Pdx1 expression in the dorsal pancreatic bud, suggesting that the signals provided by the endothelium are dispensable for the induction of Pdx1, whereas Ptf1a is selectively lost in the dorsal pancreas [22]. In the ventral pancreas, both factors are independent of endothelial signals. Thus, the initiation of dorsal and ventral pancreas development occurs via different mechanisms due to the different tissue contexts of the two pancreatic primordia. On the other hand, mice deficient for Hlxb9/Mnx lack a dorsal pancreatic bud owing to the absence of Pdx1 activation, suggesting that Hlxb9/Mnx1 may be located upstream of Pdx1 in the dorsal bud specification program (Fig. 2) [23, 24]. However, the role of Hlxb9/Mnx1 in Pdx1 activation may be indirect. As Hlxb9/Mnx1 is also expressed in the notochord, which is a source of signaling for dorsal pancreatic development, it may have non-cell autonomous functions on dorsal pancreas specification via notochord-derived signaling molecules. As described in the previous paragraph, the deletion of both FoxA1 and FoxA2 in pancreatic progenitor cells results in the loss of Pdx1 activation, thereby leading to almost complete pancreas agenesis [19]. This observation raises the possibility that FoxA1 and FoxA2 directly drive Pdx1 activation in the dorsal pancreas through the Area IV enhancer (Fig. 2).

**Collaboration between Pdx1 and Ptf1a in specifying pancreatic progenitors**

Accumulating lines of evidence support the Ptf1a-Pdx1 feed-forward loop in driving pancreatic bud outgrowth (Fig. 2). Mice with a homozygous deletion of Area I-II-III, which contains a Ptf1a-binding site, show a significant reduction in Pdx1 expression, both in the
dorsal and ventral pancreatic buds. At the same time, expression of Ptf1a in the dorsal bud is significantly reduced and that in the ventral bud is completely absent in mutant mice [17]. During pancreatic bud outgrowth (E9.5-11.0), Ptf1a appears to play a major role in mediating early Pdx1 expression throughout the pancreas, by binding to Area III in the Pdx1 promoter. Two teams independently reported that Area III in the Pdx1 gene contains a functional Ptf1a-binding site and that Ptf1a transactivates Pdx1 in an Area III-dependent manner [25, 26]. In agreement with this, a LacZ reporter driven by Area I-II (Area I-II-LacZ) does not recapitulate the endogenous expression profile of Pdx1 during pancreatic bud outgrowth in vivo, whereas Area I-II-III-LacZ can drive LacZ expression throughout the pancreas, further supporting the role of Ptf1a in driving Pdx1 expression in early pancreatic progenitors.

These observations suggest that the cooperative actions of Pdx1 and Ptf1a are crucial for the undifferentiated foregut endoderm to acquire a pancreatic cell fate and the subsequent propagation of early pancreas progenitors. This idea was supported by an experiment using Xenopus, in which the ectopic expression of Ptf1a within the Pdx1-expressing undifferentiated gut endoderm switches the progenitors towards a pancreatic fate, resulting in the formation of a giant pancreas [27]. More recently, a unique characteristic of the cooperative actions of Pdx1 and Ptf1a in pancreas specification was reported. The transient ectopic expression of Ptf1a at E9.0 in the Sox17-positive undifferentiated endoderm outside the Pdx1-expressing domain can reprogram the progenitors of the antral stomach, rostral duodenum, and extra-hepatic bile duct to become mature pancreatic tissues, including the exocrine and endocrine compartments by inducing Pdx1 [28]. In contrast, the ectopic expression of Ptf1a at E12.5 was able to induce only exocrine tissue. These results further suggest that Ptf1a is the primary determinant of pancreatic fate specification and that the competence of each progenitor to differentiate in response to Ptf1a is spatiotemporally regulated. Therefore, the timely coactivation of Ptf1a and Pdx1 in progenitor populations with competency may be conceptually applicable for the efficient induction of functional β cells from iPS or ES cells in vitro.

**Exocrine versus endocrine cell specification in the developing pancreas**

During subsequent stages of development, the expression of Ptf1a and carboxypeptidase 1 becomes restricted to cells in the tips of the branching epithelium, whereas markers such as Nkx6.1, Sox9, and Hnf1β become exclusively localized to the trunk portion of the organ (Fig. 4) [29]. The tip region is formed by undifferentiated progenitor cells (multipotent...
pancreatic progenitor cells; MPC) at around E12.0. The number of MPC formed in the pancreatic bud by E12.5 determines the final size of the adult pancreas [30]. It is believed that the activation of notch signaling plays a pivotal role in the maintenance of the undifferentiated state of MPC [31, 32]. The multipotency of MPC in the tip region quickly disappears within the next day and cells residing in the tip region alter their characteristics to become progenitors of acinar cells (pro-acinar) until E14.5 (Fig. 4) [29]. On the other hand, epithelial cells positive for Nkx6.1, Sox9, and Hnf1β in the trunk region constitute bi-potent progenitors that can produce both endocrine and duct cells [33]. With time, a portion of cells in the trunk region activate high levels of Neurogenin3 (Ngn3) and they become restricted to the endocrine fate. During the secondary transition, these endocrine progenitors delaminate from the tubule structures of the trunk region by epithelial-mesenchymal transition, and then become clustered to form islet structures (Fig. 4) [34]. Lineage-tracing experiments have shown that all endocrine cells originate from Ngn3-expressing progenitors [35]. A null mutation in Ngn3 results in the complete loss of endocrine cells, which further supports the central role of Ngn3 in endocrine cell specification [36]. HNF1β-, Sox9-, and Nkx6.1-positive cells remaining in the trunk tube give rise to pancreatic duct cells. The formation of tip-trunk patterning is governed by the balance between Ptf1a and Nkx6.1 expression. Ptf1a is required for tip formation, whereas Nkx6.1 is required for trunk formation. The cross-antagonism between Nkx6.1 and Ptf1a in multipotent progenitors governs the equilibrium between endocrine and acinar cell differentiation required for normal pancreas development [37].

After delamination from the trunk epithelium, Ngn3-expressing cells give rise to all endocrine cell lineages of the adult pancreas, including α, β, δ, e, and PP cells. Endocrine cell specification and maturation is believed to be controlled by the complex interplay among numerous transcription factors and extrinsic cues (Fig. 5). But exactly how and when Ngn3-positive progenitors promote the development of one endocrine cell fate over another is not known.

**Fig. 5** Transcription factors involved in acinar, duct, and endocrine specification

Numerous transcription factors expressed at each stage of pancreas development. -Pax4 and -Nkx2.2 indicate that e-cells develop in the absence of Pax4 and Nkx2.2. [Revised from Pan FC et al. [58]]
Transcriptional regulation of endocrine cell differentiation

To date, numerous transcription factors have been reported to control endocrine cell differentiation (Fig 5). Mutations in some of these genes have been linked to human diabetes, including MODY (Pdx1 [MODY4] [38], Ptf1a [39], NeuroD1 [MODY6] [40], HNF1β [MODY5] [41], Rfx6 [42], Pax4 [43], and Pax6 [44]. Among them, Pax4 is a transcription factor that plays a crucial role in the differentiation of pancreatic progenitors into insulin-producing β cells. Mice with a Pax4 gene disruption show an absence of mature β cells and δ cells, and the number of α cells is increased [45]. Although Pax4 gene mutations have not been found in MODY families, a rare missense mutation in Pax4 (Arg121Trp) has been described in the Japanese type 2 diabetic population [43]. Japanese carriers of the Arg121Trp variant are characterized by either a transient insulin dependence at diabetes onset or a rapid progression toward insulin deficiency, suggesting that Pax4 mutations lead to severe β-cell dysfunction in humans. As Pax4 encodes a putative transcriptional repressor, the function of Pax4 is executed via the suppression of some positive regulators that specify an α-cell fate [46, 47]. Indeed, Pax4 directly inhibits Arx expression, which is a primary specification factor for α cells [48, 49]. The ectopic expression of Arx induces β-to-α cell conversion, whereas the misexpression of Pax4 induces α-to-β conversion when introduced in mature endocrine cells [50, 51]. Pax4 and Arx reciprocally antagonize each other, thus specifying β- and α-cell fate, respectively. It was shown that the misexpression of Pax4 in mouse adult α cells and embryonic α cells induces their neogenesis and subsequent conversion into β-like cells, suggesting its therapeutic potential against hyperglycemia [51]. A subsequent study demonstrated that the repression of Arx expression is essential for inducing α-to-β cell fate conversion [52]. Very importantly, long-term GABA exposure recapitulates Pax4-induced α-to-β cell conversion, in which duct-lining progenitor cells initially adopt an α cell fate, and then subsequently undergo α-to-β-like cell conversion [53]. These neo-generated β-like cells can repeatedly reverse chemically induced diabetes in vivo. These series of studies on Pax4 and Arx highlight the possibility that basic research in developmental biology of the pancreas may lead to the discovery of a groundbreaking strategy for a diabetes cure.

Maintenance of β cell identity and dedifferentiation

Dedifferentiation of β cells has been described in some genetically engineered mouse models of diabetes. Mice with β-cell specific FOXO1 inactivation that are subjected to the metabolic stress of pregnancy or aging develop late onset diabetes associated with reduced β cell number and a reciprocal increase in α cell mass [54]. Lineage tracing experiments demonstrated that β cells do not undergo cell death but dedifferentiate into Ngn3-positive cells that do not produce insulin. These hormone-negative cells derived from β cells may represent endocrine/multipotent progenitors, as these cells express early markers, such as L-Myc, Oct-4, and Nanog, in addition to Ngn3. Lineage tracing experiments also provided evidence that these immature cells appear to occasionally redifferentiate to become non-β hormone-producing cells (mainly α cells). Wang et al. reported that mice overexpressing a mutant KATP channel that is defective in ATP binding develop hyperglycemia, in which β cells are dramatically decreased and many remaining insulin-negative cells express high levels of Ngn3 [55]. Intriguingly, amelioration of hyperglycemia by insulin treatment drives these Ngn3-positive cells to redifferentiate into insulin-producing β cells. These observations indicated that the dedifferentiation of β cells may provide an alternative mechanism as to how β-cell mass and function is affected under diabetic conditions and how persistent hyperglycemia is crucially involved in regulating this process.

Pdx1 appears to play a dominant role in maintaining β cell identity. MIP-CreER-mediated inactivation of Pdx1 in mature β cells results in severe hyperglycemia with a prompt loss of the transcriptional profile, ultrastructure, and physiological signature of β cells and the adoption of those of α cells. One of the key findings of this study was that Pdx1 can actively repress α cell fate [56]. On the other hand, the forced expression of Pdx1 in mature α cells did not convert these cells into β cells, but rather converted these cells into a stable population of endocrine cells lacking hormone expression [57]. Interestingly, the authors reported in the same study that the forced expression of Pdx1 in α cells at more immature stages represses α cell fate and concurrently induces switching to a β cell fate. These observations suggest the time- and context-dependent role of transcription factors in regulating cellular identity.
Conclusions

As discussed in this review, ideas and methodologies, such as genetic lineage tracing, that have been developed for elucidation of the developmental biology of the mouse pancreas, have also greatly contributed to our understanding of the molecular mechanisms involved in adult pancreatic function and its disruption in human disease. Hopefully, future studies on pancreas development will not only deepen our basic understanding of organogenesis, but should also contribute to translational efforts towards the cure of diabetes. For instance, a promising way to induce pancreas generation from iPS cells would be to recapitulate pancreas development in culture dishes. To this end, we need to investigate how the detailed mechanisms of pancreas development can be recapitulated in culture dishes, and once we are able to create a mature pancreas, we must determine how to maintain its function in the recipients.

Acknowledgements

I thank Ms. Suda for her secretarial assistance. I also thank Drs Hirotaka Watada, Chris Wright, Yoshiya Kawaguchi, Takaaki Matsuoka, Hideaki Kaneto, and Takeshi Miyatsuka for their advice and collaboration. I also acknowledge the support from JSPS KAKENHI (16K09765) and AMED-CREST program.

Conflicts of interest

The author declares that there are no conflicts of interest associated with this manuscript.

References


