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Determinants of Pancreatic Islet Development in Mice and Men: A Focus on the Role of Transcription Factors

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Endocrine differentiation · Pancreas development · Transcription factors · Beta cells

Abstract
The development of the endocrine pancreas is regulated by several cell-matrix interactions that generate a diverse array of intracellular signals determining the progression of a multipotent progenitor to a mature endocrine cell. This process involves interactions between the epithelium, mesenchyma, and endothelial cells. Later in development, coordinated signaling contributes to the maintenance of the differentiated endocrine cell phenotype. It has been demonstrated that key factors as well as the sequence of events involved in mouse pancreatic development is conserved in humans. This review will discuss our current knowledge in mouse as well as human pancreatic development and highlights some important transcription factors associated with human disease.

Introduction

The mammalian pancreas is a mixed exocrine and endocrine gland that produces digestive enzymes and hormones. The enzymes are produced by cells of the exocrine portion, while the hormones are synthesized by cells clustered in the islets of Langerhans. The islet secretion profile is comprised of insulin (beta cells), glucagon (alpha cells), somatostatin (delta cells), ghrelin (epsilon cells) and pancreatic polypeptide (PP cells). These endocrine cells play a central role in glucose homeostasis. Insulin released from beta cells after a meal promotes the uptake of glucose into target organs, such as skeletal muscle. The action of insulin is counterbalanced by glucagon, a hormone produced by alpha cells that acts on the liver to stimulate glycogenolysis and gluconeogenesis [1]. Diabetes mellitus results from a dysfunction in insulin and glucagon signaling. In type 1 diabetes, beta cells are progressively destroyed by autoimmunity, whereas in type 2 diabetes, the pancreatic beta cell is unable to compensate for the metabolic demand of the peripheral tissues. For millions of people suffering from type 1 diabetes, beta-cell replacement therapy offers a possible treatment. Unfortunately, this approach has been hampered by a scarcity of cells for transplantation and the preexisting autoimmunity which destroys the transplants. Today, a promising solution to this problem is directing the differentiation of stem cells into beta cells. However, the pluripotency of stem cells, while potentially advantageous, poses a significant challenge. In order to overcome this challenge, it is necessary to understand how a beta cell matures under normal circumstances. For example, progenitor cells follow an endocrine fate under the influence of a complex network of genes and hormones that operate before and after birth

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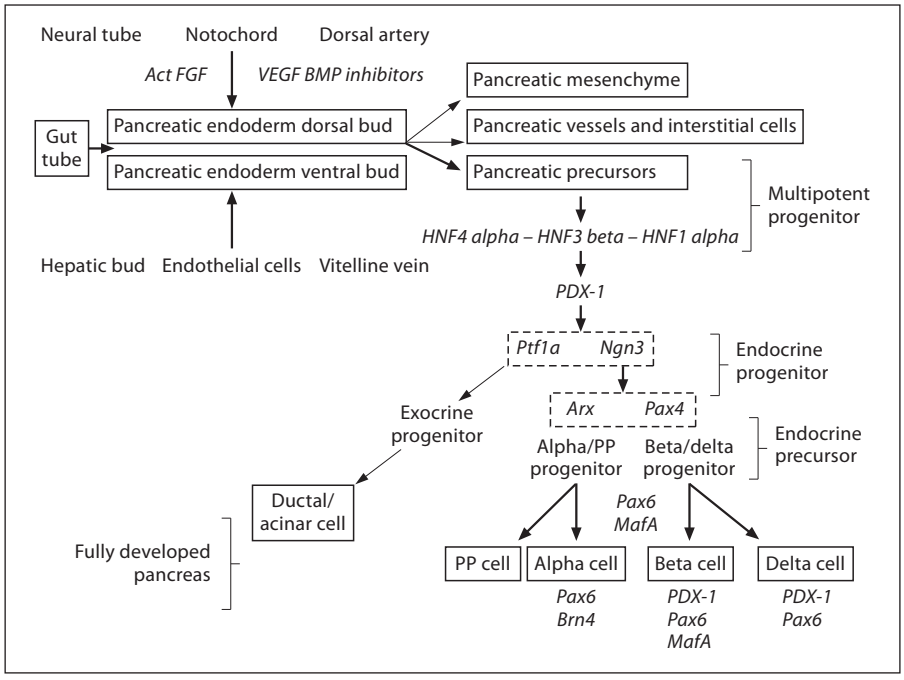

Fig. 1. Schematic model representing the transcription factors implicated in the specification of the endocrine pancreas based on the temporal expression and the phenotypic consequences of specific gene deletions.

(fig. 1; table 1). Advances have also been made in defining the role of environmental factors in endocrine pancreas development [2]. Nutrients such as glucose and oxygen are necessary for endocrine cell differentiation [3]. As a result, the identification of factors that influence the growth and differentiation of endocrine precursor cells has important implications in the treatment of diabetes. Pancreatic development seems to be conserved among species (rodent, sheep, and human), thus the rodent model (mouse) has been a powerful tool for understanding pancreatic development. This has allowed for the elucidation of signals and the development of potential tools to improve the in vitro generation of functional beta cells from stem and/or progenitor cells. The following review will discuss our current knowledge of the stages of pancreatic development including differentiation, proliferation, and maturation and compares this process between mouse and human with particular attention to the role of transcription factors in endocrine cell development.

Pancreatic Morphogenesis

The pancreas derives from two independent thickening or 'anlage' dorsally and ventrally of the endodermal germ layer. However, the development of each anlage is not identical and requires different signaling from the surrounding tissues. The dorsal bud develops in the proximity of the notochord and the splanchnic mesenchyme, which forms the dorsal aorta. Some of the signals involved are activin, fibroblast growth factor (FGF), transforming growth factor-beta (TGFβ), retinoid acid, vascular endothelial growth factor, bone morphogenetic protein inhibitors, and hedgehog-type ligands. The ventral bud, initially developing as two independent endodermal regions, grows in close contact with the liver and bile duct epithelium, and is brought together at the time of gut tube closing (fig. 1). The epithelium then invades the surrounding mesenchyme, which will signal proliferation in the adjacent epithelial buds of the pancreatic primodium. Subsequently, the invasion is followed by branching, differentiation, and maturation of the pancreatic primordium. Consequently, the microlumens coalesce to form a continuous lumen around which the ductal network will develop, leading to the formation of the ductal tree [4, 5]. Intercellular signaling through TGFβ, Notch, FGF, Wnt, and Hedgehog signaling are then crucial for proper endocrine and exocrine pancreatic development [6, 7]. Similar to humans, alpha cells are the first cells to appear in fetal rodent pancreas. Alpha cells are found at embryonic day (e) 9.5, followed by beta cells at e13.5, delta cells at e15, and PP cells at e18 (fig. 2; table 1) [8].

Table 1. Critical period of pancreatic development in mouse

Embryonic day	Occurrence	Morphological changes	Gene expression
e8–8.5	Primary transition	No visible pancreatic epithelium	Isl1 in dorsal mesenchyme; Cdk4 expansion of pancreatic tissue
e8.5–9.5	Embryo rotation	Closure of the gut tube	PDX-1 activation
e9.5–10	Budding	Ventral bud appears; pancreatic epithelium evagination; presence of glucagon cells	Ngn3, Nkx6.1, Pax6, Isl1, Hes1, HNF6, NeuroD
e10.5–11		Epithelial evagination branching and innervation; presence of co-expressing glucagon insulin cells	Ptf1
e12	Branching		
e13.5	Secondary transition	Differentiation of beta cells (few scattered mature beta cells) and exocrine compartment	PDX-1, Nkx6.1, and Nkx2.2 expression limited to beta cells
e15	Aggregation of islets; tertiary transition	Presence of delta cells; acinary structure achieved	MafA and MafB
e18		PP cells appear	
Postnatal	Growth	Endocrine and exocrine growth; formation of acini	

Transcription Factors

Most of the understanding about endocrine pancreatic development has arisen from animal models in which several transcription factors have been genetically manipulated (table 2). The array of transcription factors important for pancreas development can be broken down and classified according to their expression pattern: (1) transcription factors found in early non-hormone progenitor cells [e.g. neurogenin 3 (Ngn3)]; (2) transcription factors found in cells that produce each of the endocrine hormones [e.g. NeuroD1, islet-1 (Isl1), and paired box 6 (Pax6)], and (3) transcription factors found in a specific hormone-producing cell type [e.g. Nkx6.1, Nkx2.2, Pax4, Arx, and brain 4 (Brn4)] [9].

Transcription Factors Found in Early Non-Hormone Progenitor Cells

The pancreatic buds contain undifferentiated precursor cells that are specified towards either the endocrine or exocrine lineage. All cells that derive from the endoderm – endocrine, exocrine, and ductal cells – have been shown to express duodenal homeobox factor-1 (PDX-1) (fig. 1, 2) [10]. The Notch signaling system is critical for the decision between endocrine and progenitor/exocrine fates in the developing pancreas [11]. Similarly, Sox9 in conjunction with the Notch pathway maintains the progenitor state [12]. Bone morphogenetic protein-4 also blocks the differentiation of progenitor cells, instead promoting their proliferation [13]. A pair of opposing transcription factors, Ptf1a and Ngn3, acts as the first fate-determining factors in the branching of pancreatic progenitors into the endocrine and exocrine pancreas. The pair of opposing transcription factors 'Ptf1a-Ngn3' directs this differentiation (fig. 1) [14]. Ptf1a gives rise to exocrine cells, while Ngn3 drives pancreatic precursors towards the endocrine cell fate [15–17]. During the differentiation of islet cells, Ngn3 regulates the cell cycle; its down-regulation allows the mature islet cell population to expand [18]. Cyclin-dependent kinase 4 and its downstream transcription factor E2f1 regulate the activation of Ngn3, increasing the pool of endocrine precursors [19]. Downstream of Ngn3 is Rfx6, which has been shown to play a crucial role in the development, maturation, and function of endocrine cell lines [20].

It has been proposed that endocrine cells develop directly from a dividing Ngn3+/PDX-1+/Nkx6.1+/Ki-67+

Table 2. Role of transcription factors associated with mouse and human pancreatic development

Factor	Day of onset	Role	Mouse phenotype	Human phenotype	Ref.
Hlxb9	e8	(+) notochord, dorsal gut, and ventral endoderm; required for dorsal pancreatic development	–/–: failure to develop dorsal pancreatic lobe; small islet cells and decreased number of beta cells		[72]
PDX-1	e8.5	(+) in pancreatic buds and beta and delta cells; regulates beta-cell function	–/–: failure of pancreatic bud to expand, hyperglycemia and early death +/–: normal pancreatic development with diabetic phenotype	+/–: MODY4 and T2DM –/–: pancreatic agenesis	[34, 73, 74]
Isl1	e9	(+) dorsal pancreatic mesenchyme and exocrine pancreas; required for dorsal exocrine and all endocrine development	–/–: no dorsal pancreatic mesenchyme and exocrine cells; loss of differentiated islet cells	+/–: non-sense mutation (Q310X) found in 1 diabetic patient	[75, 76]
Sox9	e9	(+) in PDX-1+ progenitors; maintains Notch signaling	–/–: increase in apoptosis and decrease in proliferation of PDX-1 progenitors +/–: glucose intolerance		[12, 77]
Pax6	e9	(+) in endocrine cells of dorsal and ventral buds; functions in alpha- and beta-cell differentiation	–/–: absence of alpha-cell lineage, decrease in beta, delta, and PP cells; conditional inactivation: diabetic phenotype and early death		[78, 79]
Nkx2.2	e9.5	(+) in undifferentiated pancreatic epithelium and endocrine progenitors followed by (+) in alpha, beta, and PP cells; maintains normal beta-cell function	–/–: lack of beta cells, decreased number of alpha and PP cells; large population of ghrelin+ cells; severe hyperglycemia, early death		[23]
Pax4	e9.5	(+) in endocrine progenitors; essential for beta- and delta-cell maturation	–/–: reduction in beta- and delta-cell numbers, increase in alpha and ghrelin cells		[80, 81]
NeuroD1	e9.5	(+) in developing and mature beta cells	–/–: reduction in beta-cell number, failure to develop mature islets; hyperglycemia, and perinatal death	–/–: neonatal diabetes +/–: MODY6	[82, 83]
Ngn3	e9.5	(+) in all endocrine progenitor cells	–/–: devoid of pancreatic endocrine lineages; abnormal acinar morphogenesis		[15, 16]
Arx	e9.5	(+) in endocrine progenitors, dependent upon *Ngn3* expression; Opposes *PAX4* and promotes alpha/PP cells; represses beta/delta cells	–/–: lack of alpha-cell population; increase in beta and delta cells; early-onset hypoglycemia and early death after birth	Arx mutation: transient hyperglycemia	[84, 85]
Ptf1a	e10	(+) in pancreatic progenitors; regulates exocrine differentiation	–/–: p48 (*Ptf1a* subunit): lack of exocrine pancreas with relocation of endocrine pancreas to the spleen; Hypomorphic mutation: pancreatic hypoplasia, glucose intolerance, and mis-specification of progenitors; delay of branching morphogenesis and exocrine differentiation	Permanent neonatal diabetes associated with pancreatic agenesis	[86–88]
Nkx6.1	e10.5	(+) in *Ngn3*+ endocrine precursors, later restricted to beta cells; necessary for second wave of beta-cell differentiation	–/–: embryos with normal pancreas size, but reduced islet size with a reduction in beta-cell number		[26]
MafB	e12.5	(+) alpha and beta cells, becomes restricted to alpha cells; required for beta-cell terminal differentiation	–/–: reduced number of alpha and beta cells throughout development; production of beta cells delayed until e13.5 with the onset of *MafA*		[89]

T2DM = Type 2 diabetes mellitus; –/– = homozygous; +/– = heterozygous; (+) = expression; MODY = maturity-onset diabetes of the young.

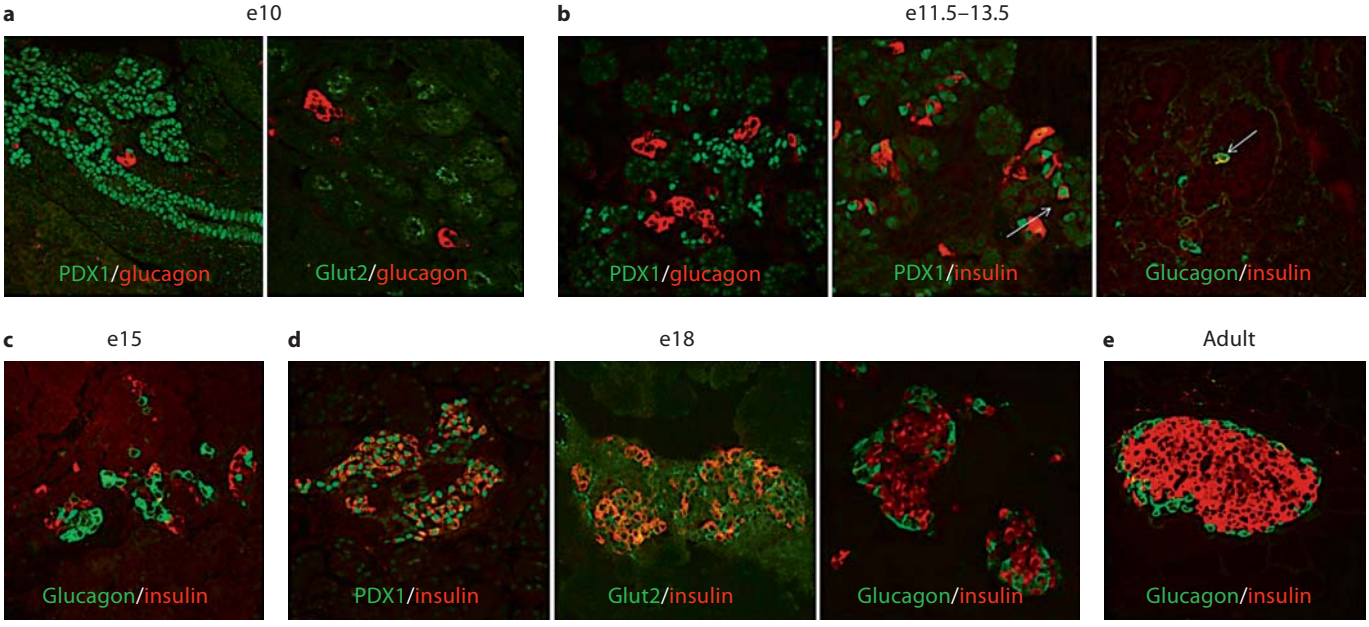

Fig. 2. Temporal endocrine differentiation. Photomicrographs illustrate confocal microscopic images. **a** The pancreas of an e10 embryo immunostained for PDX-1 (green), glucagon (red), and glucose transporter 2 (Glut2) (green) shows expression of glucagon as the first hormone to appear in the primitive endoderm. **b** The pancreas of an e11.5–13.5 embryo immunostained with PDX-1 (green), glucagon (red), and insulin (red) shows the presence of co-expressing glucagon insulin cells found during the protodifferentiated state (arrows) followed by the appearance of mature insulin cells at e13.5. **c** Photomicrograph of sections of pancreas of e15 mice illustrates the presence of insulin or beta cells surrounded by glucagon or alpha cells at these stages of development. **a**, **b**, **d** PDX-1 immunostaining (green) at various stages shows that at e10.5–e15.5, PDX-1 is expressed throughout the pancreatic epithelium and is subsequently down-regulated in acini and ducts while being maintained in beta cells. **a**, **d** Glut2 immunostaining (green) at various stages shows that at e10, Glut2 is expressed in cells that do not express any other hormone and is subsequently found in the cytoplasm of insulin or beta cells. **d**, **e** Beyond e17.5, endocrine cells aggregate into recognizable islets, with insulin or beta cells at their cores and glucagon or alpha cells distributed peripherally in a similar pattern found in adult rodent pancreas (**e**).

progenitor cell population into a mature non-dividing NeuroD+/Isl1+/Pax6+/Ki-67– cell [16]. Following the initiation of the endocrine program, a set of transcription factors is necessary to then convert Ngn3-labelled cells into alpha, beta, delta, and PP cells.

Transcription Factors Found in Cells That Produce Each of the Endocrine Hormones

The second level of branching is directed by another pair of opposing transcription factors: Pax4-Arx [14]. Pax4, a paired box gene, also plays a central role in the differential specification of endocrine precursor cells. It is expressed around e9.5 in both of the pancreatic buds and becomes progressively restricted to beta cells until e15 [21, 22]. Arx, a member of the aristaless-related paired-class homeobox gene family, is restricted to alpha- and beta-cell precursors and delta cells [9]. Arx appears to specify the alpha-cell fate, whereas Pax4 first allows the commitment towards a beta-/delta-cell fate by repressing Arx and subsequently inducing precursor cells towards a beta-cell fate through the inhibition of the delta-cell destiny [9, 22]. It has been shown that both Pax4 and Arx require the activity of Nkx2.2. Nkx2.2 belongs to the NK class of homeodomain-encoding genes, and its expression is initiated at e9.5 in the dorsal epithelium, becoming progressively restricted to alpha-, beta-, and PP-cell subtypes [23]. In addition, Nkx2.2 and Pax4 control Arx gene activity in committed beta-cell precursors [24]. Nkx6.1, an additional member of the NK class, is also detectable at e9.5 in both pancreatic buds and becomes specifically restricted to beta cells [25, 26].

Transcription Factors Found in a Specific Hormone-Producing Cell Type: Maintenance of the Islet-Cell Subtype

Beta-Cell Lineages

It has been proposed, on the basis of the glucose transporter 2 (Glut2) gene fetal expression pattern [27], that there may be two separate beta-cell lineages: the '*first wave or protodifferentiated'* beta cell, also called insulin cell co-expressing glucagon that appears at e10.5, and the '*mature or second transition*' beta/insulin cell that appears at e13.5 and persists during adult life (fig. 2).

First Wave. The earliest endocrine cells to appear are multihormonal and co-express glucagon [25, 28]. Although early studies contested the existence of these cells [29], this point has now been confirmed repeatedly by us [30] and numerous groups [25, 28, 31–33]. Double- and triple-staining studies of early pancreatic buds have shown that the vast majority of the insulin-glucagon co-expressing cells are negative for PDX-1, Nkx6.1 [25], or Pax4 [22], suggesting that these cells derive from a different progenitor cell. They usually appear in clusters surrounded by glucagon cells and express activin [33]. Furthermore, there is some evidence that insulin-glucagon cells proliferate [28], suggesting a contribution to the pool of either glucagon or insulin cells.

Second Transition. During the second transition, mature beta cells arise directly from non-hormonal protodifferentiated epithelial cells that express Glut2, nerve growth factor receptor TrkA, PDX-1, and Nkx6.1 [27, 34–36], as well as amyloid polypeptide, PC3/1, PC2, NKx2.2, glucokinase, and MafA [37, 38]. In mature organized insulin cells, PDX-1, which is stabilized by the retinoblastoma protein [39], transactivates the insulin gene and other genes involved in glucose sensing and metabolism such as Glut2 and glucokinase [27]. Glucose sensing is the initial event in the pathway for glucose-stimulated insulin secretion from the pancreatic insulin cell [40]. PERK (EIF2AK3) expression during fetal life is also required for the differentiation of beta cells and the development of normal islet architecture [41]. Other important transcription factors involved in beta-cell identity are NeuroD and MafA which both activate insulin transcription. MafA is a beta cell-specific transcription factor that binds to the insulin promoter, playing a crucial role in beta-cell maintenance [42]. In addition, MafA interacts with PDX-1 and NeuroD to activate insulin transcription [43]. NeuroD, while not required for endocrine cell differentiation, may be involved in promoting cell cycle exit and has been associated with maturity-onset diabetes of the youth (MODY) in humans (table 2) [5].

Alpha-Cell Lineages

Despite the significant differences between alpha and beta cells, it has been shown that both cells share major similarities [44]. Recent evidence suggests that alpha cells can transdifferentiate into beta cells [45–47], thus it is important to fully understand how alpha cells develop. After the endocrine progenitor develops, another set of opposing transcription factors will direct the development of alpha cells. Arx and forkhead box A2 (FOXA2) are implicated in the initial or terminal differentiation of alpha cells. In addition, FOXA1, Pax6, Brn4, and Isl1 are involved in the preproglucagon transcription and maintaining of alpha-cell function [48, 49].

MicroRNAs and Epigenetic Modifications

During development, the pancreas expresses many small non-coding RNAs called microRNAs (approx. 20 nt). microRNAs are post-transcriptional regulators that are integrated into an RNA-induced silencing complex to repress translation, resulting in gene silencing [50]. Evidence suggests at least 125 microRNAs are involved in pancreatic development. It has been found that microRNAs are important in regulating ductal, exocrine, and endocrine development, particularly beta-cell neogenesis [51].

Recently, epigenetic modification has also been shown to alter the expression of genes involved in beta-cell differentiation. Specifically, histone deacetylases class II (HDAC), which are expressed in the endocrine pancreas, are key regulators in controlling the pancreatic cell lineage by inhibiting beta-cell (HDAC 4, 5, and 9) and delta-cell development (HDAC 4 and 5) [52].

Human Pancreatic Development

In humans, the pancreas develops as a ventral and dorsal outgrowth, first visible at days 25–26 of gestation [53]. The human pancreatic bud elongates into a loose mesenchymal bed [54]. By 35 days of gestation, the ventral pancreatic bud begins to rotate and eventually comes into contact and fuses with the dorsal bud during the 6th week of development or gestational age (wGA) [55–61]. Similar to rodents, the mesenchymal tissue plays an important role in cell fate differentiation [62]. From 8 to 10 wGA, pancreatic ducts are surrounded by the mesenchyme and eventually branch and form lobular structures, becoming clearly defined by 11 wGA. Endocrine differentiation has

been shown to follow a spatial and temporal pattern where the endocrine mass increases from the center of the organ, in central epithelial ducts, toward the periphery in a centrifugal manner [63]. By 9–11 wGA, the mesenchymal tissue contains scattered hormone-negative Ngn3-positive endocrine cells [62], and, similar to rodents, the first endocrine cells have been found to be associated with the ductal epithelium [64]. These endocrine cells are found at a distance away from the mesenchymal tissue, suggesting that the mesenchyme could have a repressive effect on the development of endocrine tissues [63]. The critical window of differentiation of endocrine cells in humans is from 9 to 23 wGA [62]. Assan et al. [65] demonstrated that glucagon cells are the first cells that appear in the fetal pancreas. Glucagon cells are found at 7 wGA [65], followed by insulin, somatostatin, and PP cells at 8–10 wGA [58]. It has also been shown that a subpopulation of primitive endocrine cells that co-express insulin, glucagon, and somatostatin appears at 8 wGA. This subset of primitive cells has a low proliferation rate, suggesting that the 'mature endocrine cells' arise from progenitors by differentiation instead of proliferation [63]. Despite the similarity to rodents, human islets and mouse islets differ considerably in architecture and composition in that alpha, beta, and delta cells are dispersed throughout the islet [66].

Single-cell transcriptional analysis from adult islets has identified two populations of cells: larger cells with a dense cytoplasm that constitute the majority of the islet population and distinctly smaller cells. The large mature beta cells express insulin, PDX-1, Glut2, Nkx2.2, Ngn3, Nkx6.1, Pax6, Isl1, and NeuroD [67]. Islets are seen as early as at 11 wGA, and vascularized structures appear by 20–23 wGA [62, 68]. The peak proliferation of glucagon cells occurs at 20 wGA, and at 23 wGA for insulin and somatostatin cells. [62]. In contrast to rodents, human fetuses are able to develop a robust insulin response to secretagogues [69].

As studies of the human pancreas are limited, we rely on animal studies to elucidate the progression of pancreatic development. It has been shown that certain mutations seen in animal models can also affect human pancreatic endocrine development, such as mutations in PDX-1 expression, which is associated with pancreatic agenesis and MODY4 (table 2). Similarly, changes in glucokinase activity have also been associated with large islets, highlighting the importance of transcription factors as well as enzymes in human endocrine pancreas differentiation and function [70].

Similar to rodents, studies have shown microRNAs to play a role in targeting genes and transcription factors that are essential to pancreatic development. A total of 212 microRNAs have been found to be expressed in 10–22-week-old fetuses. Four microRNAs have been shown to increase their expression, 35 microRNAs have had decreased expression, and 173 microRNAs remained unchanged [71].

Conclusion

The development of the endocrine pancreas is regulated at several levels by cell-cell and cell-matrix interactions and locally produced and circulating peptides as well as nutrients that generate diverse intracellular signals. The analysis of mouse loss-of-function phenotypes has represented a powerful tool for the attribution of specific gene functions and has demonstrated the importance of multiple systems in determining pancreatic cell fate determination. However, regardless of all the knowledge gained over the last decade, it has not been possible to successfully develop functioning beta cells from non-pancreatic cells or endocrine precursors. With collective research efforts yielding new information in this field, we hope to be brought closer to generating a cure for diabetes.

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