Published in final edited form as:

Curr Opin Endocrinol Diabetes Obes. 2014 April ; 21(2): 77–82. doi:10.1097/MED.0000000000000047.

Pancreas development in humans

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Abstract

Purpose of review—We highlight some of the major recent advances in characterizing human pancreas development and endocrine cell differentiation.

Recent findings—Extensive research efforts have helped to define crucial events in the mouse pancreas organogenesis. Information gained from these studies was used to develop human embryonic stem cell (hESC) differentiation protocols with the goal of generating functional glucose-responsive, insulin-producing human β -cells. In spite of remarkable progress in hESC differentiation, current protocols based on mouse developmental biology can produce human β -cells only *in vivo*. New differentiation markers and recently generated reagents may provide an unprecedented opportunity to develop a high-density expression map of human fetal pancreas and pancreatic islets that could serve as a reference point for *in vitro* hESC differentiation.

Summary—Integrating an increased knowledge of human pancreas development into hESC differentiation protocols has the potential to greatly advance our ability to generate functional insulin-producing cells for β -cell replacement therapy.

Keywords

endocrine cell birth; endocrine cell proliferation; fetal human pancreas transplantation; islet formation; pancreas development

INTRODUCTION

Although extensive research over the past decade greatly advanced our understanding of critical steps in mouse pancreas development (reviewed in [1]), information about embryonic development of the human pancreas remains limited. These deficits in our knowledge are rooted in the extremely limited access to relevant early (<8 weeks gestation) and later gestational-aged human pancreatic tissue (>22 weeks gestation). Consequently, most human embryonic stem cell (hESC) [2,3,4*] or human induced pluripotent stem cell-

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Conflicts of interest

There are no conflicts of interest.

based differentiation protocols [5–7] focused on the generation of mature, single hormone-expressing, glucose-responsive human β -cells use information gained from studies of mouse rather than human islet development. In large part, this dependence on mouse developmental biology is due to the paucity of information on the markers, transcription factors, and mechanisms underlying human endocrine progenitor cell differentiation at very early stages, and the maturation of human β -cells with normal glucose-stimulated insulin secretion during later development. In this review, we summarize current information about human pancreas development (Table 1) and relate these findings to key morphogenetic events of mouse pancreas organogenesis highlighting areas that will require further investigation.

PANCREAS BUDDING

Human pancreas formation is first evident at gestational day 26 (G26d). It begins with dorsal bud formation that is followed by the appearance of two ventral buds at approximately G30d [8,9[¶]]. The left ventral bud gradually regresses, whereas the right ventral bud starts to migrate posteriorly at approximately G35d and fuses with the dorsal pancreatic bud upon gut rotation at 6–7 weeks of gestation (G6–7w) [8,10,11]. According to several reports, failure of the left ventral bud to regress could lead to the pathological condition known as annular pancreas (reviewed in [10]). In humans, the dorsal pancreas gives rise to the majority of the pancreas, including the upper part of the head (caput pancreatis), the isthmus (isthmus pancreatis), the body (corpus pancreatis) and the tail of the pancreas (cauda pancreatis). The right ventral bud gives rise to the inferior part of the head [10,11].

KEY POINTS

- Molecular markers of early pancreatic differentiation are highly similar in mouse and human.
- The first endocrine cells that appear in the human pancreas are insulin-expressing cells (G7.5w) and they remain the most prevalent endocrine cell type during the first trimester.
- Majority of α , β , and δ -cells in the fetal human pancreas express a single hormone and reach approximately a 1 : 1:1 ratio by the last trimester, which is maintained through birth.
- Acinar cell-specific genes show a distinct transition at around G11–15w, which may represent the beginning of secondary transition in the human pancreas differentiation program.

Our knowledge of early events in human pancreas organogenesis, such as the foregut endoderm patterning and pancreatic bud formation, is exceptionally limited due to the scarcity of tissue specimens and ethical concerns. However, Jennings *et al.* [9[¶]] reported recently that prior to the appearance of a discernible dorsal bud (G25–27d); the dorsal prepancreatic endoderm is in contact with the notochord, leading to exclusion of sonic hedgehog (SHH) expression from this endodermal region. Exclusion of the SHH expression by the notochord provides permissive signals for pancreas development in both chick and mouse embryos, suggesting that this mechanism is highly conserved and may be responsible

for providing permissive signals in human pancreas development as well. The transient notochord–endoderm contact is disrupted by the paired dorsal aortae that fuse in the midline with surrounding mesoderm at G29–31d. Whereas pancreas and duodenum homeobox 1 (PDX1) expression in the mouse can be detected as early as embryonic day (E8.5) when the endoderm is still in contact with the notochord, PDX1 expression in humans is undetectable in the dorsal pancreatic endoderm region until the paired dorsal aortae fuse at G29–31d [9^{••}, 12]. The fused dorsal aorta is then separated from the dorsal bud at G35–37d by the splanchnic mesoderm. During subsequent development, both pancreatic buds extend into the surrounding mesenchyme, which provides fibroblast growth factor 7 and fibroblast growth factor 10 signaling for the growth and proliferation of pancreatic epithelial cells [13].

EPITHELIAL TUBE/MICROLUMEN FORMATION

Between G30d and G33d the early human pancreatic buds are composed of stratified epithelium containing cells that express several transcription factors such as PDX1, SRY (sex-determining region Y)-box 9 (SOX9), Nirenberg and Kim homeobox 6.1 (NKX6.1), GATA transcription factor 4 (GATA4) and forkhead box A2, but not Nirenberg and Kim homeobox 2.2 (NKX2.2) [9^{••}, 14]. Although expression of pancreas transcription factor 1A (PTF1A) in human embryonic pancreas has not been reported so far, this panel of transcription factors closely resembles the signature of multipotent pancreatic progenitors cells (MPCs) reported in the mouse, suggesting that the molecular markers of early pancreatic differentiation are highly similar in mouse and human. At G30–33d, the stratified epithelium of the dorsal bud has a primary central lumen and microlumens [9^{••}].

Starting from G45–47d, the pancreatic epithelium undergoes active growth and branching morphogenesis. During this process, pancreatic epithelium is embedded in the loose mesenchyme and is surrounded by a dense peripancreatic mesenchyme. At approximately G7–8w, the epithelium begins to ramify and starts to form a lobular pattern [9^{••}, 11]. Whether human fetal pancreatic epithelium undergoes similar morphogenetic processes as reported in the mouse, such as plexus formation and remodeling, awaits future detailed investigation.

TIP–TRUNK SEGREGATION

In humans, tip–trunk compartmentalization of the pancreatic epithelium is initiated as early as G7w, with GATA4 expression (a proacinar and tip cell marker) detected in the periphery of the pancreatic epithelium. At this stage, the GATA4⁺ tip cells also co-express MPC markers SOX9 and NKX6.1, indicating that MPCs are being relocated to the tip domain. At G14w, tip–trunk segregation is completed when GATA4⁺ tip cells cease to express SOX9 and NKX6.1 [9^{••}]. It is still unclear if all MPCs relocated to the tip domain are GATA4⁺ or if the GATA4⁺ population is only a subset of proacinar cells. Future studies including an additional marker for tip MPCs and proacinar cells, such as PTF1A, would advance our understanding of the tip–trunk segregation process. The analyses performed thus far have been primarily based on image snapshots from a limited region in the pancreatic section and may not represent the overall tissue morphology. This makes it rather difficult to determine

whether the tip–trunk compartmentalization process is synchronized across different pancreatic regions or if it is more prominent in the periphery of the pancreatic epithelium.

ENDOCRINE CELL BIRTH

In contrast to the presence of glucagon-expressing cells in the early mouse pancreatic bud, hormone-expressing endocrine cells are not detected in the early human embryonic pancreas until G7.5–8w, 3 weeks after the initial outgrowth of the dorsal bud [8,9[¶],14,15[¶]].

Therefore, it seems unlikely that there is a human equivalent of the mouse primary transition. The first endocrine cells that appear in the human pancreas are insulin-expressing cells. They can be detected as early as G7.5w and remain the most prevalent endocrine cell type during the first trimester [8,9[¶],14,15[¶]]. This is followed by the appearance of glucagon and somatostatin-expressing cells at G8w, and pancreatic polypeptide and ghrelin-expressing cells at G9w [8,15[¶]]. Although the timeline of endocrine cell emergence is well established, it is currently unknown whether the birth of human endocrine cells takes place only in the trunk region of the pancreatic epithelium as described in the mouse.

Although only limited studies have been performed, the expression of several transcriptional regulators specifically marking endocrine cell progenitors and precursors has been determined at mRNA and protein levels at G9–21w developmental stages. These include neurogenin3 (NGN3), paired box 6, NKX2.2, NKX6.1, ISLET1, neurogenic differentiation 1, and Paired box 4 [9[¶],14,16]. The spatio-temporal expression pattern of these transcription factors in human fetal pancreas suggests that similar transcriptional regulatory mechanisms operate in both humans and mice. NGN3 protein, the endocrine progenitor marker, is detected in the pancreatic epithelium as early as G8w concomitant with the appearance of hormone-expressing endocrine cells [9[¶]]. The number of NGN3⁺ cells gradually increases during subsequent development and these cells become scattered throughout the epithelial clusters (G8.5–10w). The majority of NGN3^{HI} cells do not co-express the trunk marker, SOX9 [9[¶]], but the presence of NGN3⁺PDX1⁺, NGN3⁺INSULIN⁺, and NGN3⁺GLUCAGON⁺ co-expressing cells in the early human fetal pancreas (G8–11w) has been reported [14]. Endocrine cells fail to develop in the mouse pancreas in the absence of NGN3 [17]; however, a few β -cells are still able to form in humans with an NGN3 homozygous mutation [18], suggesting that there might be an NGN3-independent pathway for endocrine cell development in the human pancreas. It will be interesting to determine if neurogenin1 and neurogenin2 are also expressed in the human fetal pancreas.

ISLET FORMATION AND DISTRIBUTION

Cole *et al.* [19] proposed that islet formation involves delamination of differentiated hormone-expressing cells that undergo epithelial–mesenchymal transition (EMT). For example, their study suggested that β -cells delaminating from the pancreatic epithelium transiently express the mesenchymal marker vimentin and EMT regulators Snail1 and Snail2. In contrast, the delamination of islet endocrine precursors in mouse pancreas precedes endocrine cell differentiation and oftentimes these precursor cells delaminate in clusters (termed ‘cluster delamination’; [1]). It is not known if cluster delamination occurs in humans, or whether a single cell delamination followed by endocrine cell clustering is a

predominant mechanism of human islet formation, since numerous single hormone-expressing cells are frequently found within the epithelium [11]. The latter concept is further supported by *in vivo* lineage-tracing analysis performed by Scharfmann *et al.* [20], who found that endocrine cells within a single human islet originate from more than one progenitor.

Information about the spatial distribution of developing endocrine clusters in the fetal human pancreas is very limited. The study by Jeon *et al.* [21] suggests that clustering of insulin and glucagon-producing cells begins in the central region of the pancreas and shows that these centrally located endocrine cell clusters tend to be much larger than those at the pancreas periphery. However, it is not known whether the organization of human endocrine cells resembles the ‘endocrine cords’ found in the mouse pancreas. Piper *et al.* [8] reported that endothelial cells begin to associate with small endocrine cell clusters at G10w and fetal islets contain vascular structures by G14w [22]. Further studies focused on characterizing architecture of the developing human pancreas are needed to define how islet formation is integrated with the development of vascular and neuronal components.

The relationship of endocrine cell populations within developing islet clusters has so far been mostly characterized for α and β -cells. It appears that islet clusters initially contain 3–4-fold fewer α -cells than β -cells (G9–13w), but by G14–16w α and β -cells reach approximately a 1 : 1 ratio [15[•],21], and this ratio is maintained through birth [23^{••}]. Surprisingly, during the last trimester, the proportion of β -cells is also equal to δ -cells as recently reported by Gregg *et al.* [23^{••}], which differs from the developing mouse pancreas where β -cells are the most abundant cell type during the secondary transition and at birth. Several groups examined proliferation of endocrine cells during G9–38w period, but proliferation indexes varied significantly depending on markers used for analyses [15[•], 16,21,22,24]. Notably, the clusters of β -cells contained fewer replicating cells than the groups of α or δ -cells [16,21], and β -cell proliferation in the third trimester was lower than in the neonatal period [23^{••}].

The vast majority of α , β , and δ -cells in the fetal human pancreas appear to express a single hormone [15[•]]. The considerable number of bihormonal cells co-expressing insulin and glucagon (20–40% of α and β -cells) was only noted between G9w and G16w, and significantly declined by G21w [15[•],21]. This is quite different from the current protocols for directed differentiation of hESCs, which often generate not only insulin-positive cells, but also a significant population of bihormonal (i.e. insulin/glucagon, insulin/somatostatin, glucagon/somatostatin) and polyhormonal cells (i.e. insulin/glucagon/somatostatin) both *in vitro* and *in vivo* [25[•],26[•]].

EXOCRINE TISSUE DEVELOPMENT

Compared to endocrine pancreas development, fewer studies have been devoted to exocrine pancreas development. Most of the information on human exocrine development is gained from morphological studies [10,27–29]. As early as G8w, several carboxypeptidase A1 (CPA1)-positive pyramidal cells can be found budding from the pancreatic epithelium, probably representing the early proacinar population [9^{••},30]. Apart from CPA1 expression

at this stage, other proacinar markers such as GATA4, MIST1 and pancreatic secretory trypsin inhibitor are also expressed in this population.

Acinar cell-specific genes show a distinct transition at around G11–15w, which could represent the beginning of human secondary transition based on the initiation of cell-specific differentiation program. Several digestive enzymes and acinar-specific markers such as carboxylester lipase, chymotrypsinogen, trypsinogen, elastase1, protease, serine, 1 (trypsin1) [PRSS1], regenerating islet-derived 1A, and regenerating islet-derived 3G show a dramatic increase in their expression starting at G11w and reaching a plateau at G15–19w, whereas amylase can only be detected from G23w onwards. The expression pattern of PTF1A, the major regulator of acinar cell development and acinar cell-specific gene expression in mice, has not been shown so far in the human pancreas.

At the ultrastructural level, zymogen granules are seen beginning around G14w and they accumulate between the Golgi apparatus and the apical pole [10,30]. By G20w, zymogen granules increase in number and display proteolytic activity [10]. The lobular organization of acini appears at G16–20w. It becomes more accentuated and dense at the tissue periphery and then throughout the gland between G25w and G41w [10].

Although mRNA expression of several differentiated ductal cell markers such as cytokeratin 19, carbonic anhydrase 1, mucin 1, cystic fibrosis transmembrane conductance regulator and aquaporins has been detected from G11w onwards, ductal cell differentiation has not been clearly defined. Adda *et al.* [10] reported the appearance of definitive ductal structures such as intercalated, intralobular and interlobular ducts at G24–32w, but the differentiation status and heterogeneity of ducts in different regions of the epithelium is still unknown. In particular, it remains unclear when these ducts undergo terminal differentiation and lose their competency to produce endocrine cells, and it will require further detailed analysis in the future to make this determination.

STUDIES OF HUMAN PANCREAS DEVELOPMENT EX VIVO

As we reviewed above, the vast majority of data on human pancreas development comes from static histological studies of several developmental ‘snapshots’ (G8–21w). Furthermore, the scarcity of histological specimens during later development (>G22w) limits studies of human β -cell maturation [31,32[¶]]. To overcome these limitations, Scharfmann and colleagues developed a model for longitudinal and more mechanistic studies of human pancreas development and endocrine cell function [33,34^{¶¶}]. The model is based on transplantation of entire fetal human pancreas at G8–9w either into the renal subcapsular space or under the muscle epimysium of immunodeficient mice [33,34^{¶¶}]. The transplanted human fetal pancreas differentiated into functional insulin-producing cells and these cells were able to maintain normoglycemia in mice made diabetic by alloxan injection 3 months after pancreas transplantation [33]. Using BrdU pulse/chase experiments, Castaing *et al.* [35] produced evidence of clonal acinar cell development through proliferation of differentiated acinar cells. In contrast, they concluded that endocrine cell development was regulated by the proliferation of pancreatic progenitor cells (PDX1⁺), followed by β -cell differentiation [35]. This model may allow longitudinal and mechanistic assessment of

human pancreas organogenesis. It will be important to directly compare and align the processes occurring during *ex vivo* and *in vivo* human pancreas development.

CONCLUSION

Emerging information about early events in human pancreas development, expression of pancreas/endocrine cell transcriptional regulators, endocrine cell clustering and islet formation have provided initial insight into the complex mechanisms involved in pancreatic endocrine cell differentiation. Detailed and systematic mapping of human pancreas organogenesis may lead to generation of new therapies for type 1 and 2 diabetes.

Acknowledgments

We thank Kristie Aamodt and Eric Bankaitis for their critical reading of the manuscript.

This work was supported by NIH (DK72473, DK89572, DK89538), and the Vanderbilt Diabetes Research and Training Center (DK20593).

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Table 1**Key events in human pancreas development**

Gestational day (d)/week (w)	Carnegie stage	Events/processes
G25–27d	9–10	Transient notochord–dorsal prepancreatic endoderm contact
G26d	9	Dorsal bud formation
G29–31d	11–12	Dorsal aortae fusion and contact with dorsal prepancreatic endoderm
		PDX1 expression detected in pancreatic buds
G30d	12	Formation of two ventral buds
G35–37d	15	Separation of fused dorsal aorta from endoderm by splanchnic mesoderm
		Dorsal bud outgrowth
G6–7w	18–20	Fusion of dorsal and ventral buds
		Active outgrowth and branching morphogenesis of pancreatic epithelium
G7w	20	Initiation of tip–trunk compartmentalization process
G7.5w	21	Appearance of first endocrine cells (insulin ⁺ cells)
G8w	23	NGN3 expression and NGN3 ⁺ endocrine progenitors found
		Appearance of glucagon ⁺ and somatostatin ⁺ cells
		Few CPA1 ⁺ cells detected in the peripheral epithelium
G9w	N/A	Appearance of pancreatic polypeptide ⁺ cells
G11w	N/A	Initiation of acinar differentiation
G11–14w	N/A	Initiation of islet vascularization
G12w	N/A	Initiation of active endocrine cell birth, delamination and islet clustering
G14w	N/A	Tip–trunk compartmentalization process completed
G24–32w	N/A	Definitive ductal structures (intercalated, intralobular, interlobular ducts) become apparent

CPA1, carboxypeptidase A1; N/A, not available; NGN3, neurogenin3; PDX1, pancreas and duodenum homeobox 1.