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Authors

Nair, Gopika
Hebrok, Matthias

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Islet formation in mice and men: Lessons for the generation of functional insulin-producing β cells from human pluripotent stem cells

Gopika Nair and **Matthias Hebrok**

Diabetes Center, Department of Medicine, University of California, San Francisco, CA 94143, USA.

Abstract

The Islets of Langerhans are crucial ‘micro-organs’ embedded in the glandular exocrine pancreas that regulate nutrient metabolism. They not only synthesize, but also secrete endocrine hormones in a modulated fashion in response to physiologic metabolic demand. These highly sophisticated structures with intricate organization of multiple cell types, namely endocrine, vascular, neuronal and mesenchymal cells, have evolved to perform this task to perfection over time. Not surprisingly, islet architecture and function are dissimilar between humans and typically studied model organisms, such as rodents and zebrafish. Further, recent findings also suggest noteworthy differences in human islet development from that in mouse, including delayed appearance and gradual resolution of key differentiation markers, a single-phase of endocrine differentiation, and prenatal association of developing islets with neurovascular milieu. In light of these findings, it is imperative that a systematic study is undertaken to compare islet development between human and mouse. Illuminating inter-species differences in islet development will likely be critical in furthering our pursuit to generate an unlimited supply of truly functional and fully mature β -cells from human pluripotent stem cell (hPSC) sources for therapeutic purposes.

Section I: Introduction

Islets emerge via the aggregation of five discrete endocrine cell types (each producing insulin, glucagon, somatostatin, pancreatic polypeptide or ghrelin in the adult organism) that are intimately associated with endothelial cells and neuronal processes to function together as a single unit. Dysregulation of islet function perturbs glucose homeostasis and eventually leads to diabetes. Efforts are underway to generate insulin-producing β -cells from hPSCs in the hope of treating diabetes. Unfortunately, current differentiation protocols produce β -like cells that possess limited glucose responsiveness, only in static insulin secretion assays, and

Correspondence Matthias Hebrok University of California, San Francisco Diabetes Center, Department of Medicine 513 Parnassus Avenue, HSW 1116, Box 0540 San Francisco, CA 94143-0573 Tel: 415-514-0820, Fax: 415-564-5813 mhebrok@diabetes.ucsf.edu.

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hence are not fully mature[1]. In particular, these hPSC differentiation protocols have relied heavily on information gleaned from pancreas development in animal models, specially rodents[2]. However, critical differences have been well-established between human and mouse adult β -cells, including the regulation of the insulin promoter and thus insulin gene expression[3], expression of glucose transporters[4, 5], responsiveness to neuropeptides [6, 7], and the repertoire of cell-cycle regulators[8]. Besides these molecular dissimilarities, gross islet cytoarchitecture is also markedly different between the two species [9]. This implies disparities should also exist during development. Consequently, implementing developmental mechanisms elucidated exclusively in animal models in hPSC differentiation may not be sufficient to successfully generate pristine mature human β -cells in vitro. In support of this notion, new insights into human pancreas organogenesis do indeed point to deviation from rodent development. Although limited by histological analysis of cadaveric fetal tissue of different gestational ages or ex vivo organogenesis, an overview of human pancreas development is materializing. In this review, we summarize the emerging differences between human and mouse islet development and morphogenesis, and comment on the implications of such differences on our attempts to generate human β -cells in a dish.

Section II: Early pancreas development: From foregut to endocrine specification

Extensive knowledge of molecular and morphological events that regulate mouse pancreas development has been acquired over the last twenty years through pioneering lineage tracing techniques using sophisticated transgenic mouse models[10]. The pancreas arises from two diametrically juxtaposed anlagen located on the dorsal and ventral portions of the developing foregut endoderm. In mouse and chick, notochord-derived signals promote the exclusion of Sonic Hedgehog (Shh), a member of the Hedgehog family of secreted signaling molecules, in the presumptive pancreatic endoderm prior to dorsal bud formation. The absence of Shh in this area permits expression of Pancreatic and duodenal homeobox factor 1 (Pdx1), a transcription factor essential for pancreas development[11], as early as embryonic day 8.75 (e8.75) in mouse when the notochord is still in contact with the endodermal sheet. While SHH expression is also excluded from the human dorsal foregut epithelium slated to develop into pancreas, PDX1 expression is delayed, and detected only after gut closure and separation of the dorsal aorta and notochord by mesenchyme (29-31 days post conception(dpc)) [12](Fig. 1; Table 1). Other transcription factors, including Ptf1a, Gata4, and Gata6 also mark pancreas specification, and their importance in human pancreas development is evidenced by several reports of pancreatic agenesis and permanent neonatal diabetes mellitus (PNDM) caused by mutations in these genes[13-16]. Unlike the situation in rodents, the expression of GATA4 is delayed during human development, appearing at the same time as PDX1. Also, SOX17, a definitive endoderm marker whose expression is lost in rodent pancreas epithelium, persists in the presumptive human pancreatic endoderm[12]. After specification, pancreatic buds rapidly grow into the surrounding mesenchyme, which produces proliferative signals such as FGF10 and FGF7[17], resulting in the formation of a multipotent pancreatic epithelium (30-33 dpc in humans). This immature epithelium is characterized by the expression of Pdx1, Ptf1a, Gata4, Sox9, Nkx2.2, Hnf1b, Foxa2 and Nkx6.1[18] in mouse. Many of these factors were

also expressed in the human counterpart; nevertheless, a striking difference is the absence of NKX2.2 expression, which does not appear until after cells have committed towards the endocrine lineage[12] (Fig. 1).

The next major event in pancreas development is the segregation of multipotent progenitor cells (MPCs) into spatially distinct ‘tip’ and ‘trunk’ domains. The tip progenitors initially possess the ability to develop into all pancreatic epithelial cell lineages, including acinar, duct, and endocrine cell types. Subsequently, cells that remain at the distal tip of the epithelial structure undergo acinar differentiation, while cells proximal to the tips become bipotent (endocrine/duct) trunk progenitors. The separation of pro-acinar tip cells and trunk cells is thought to be established by an antagonistic relationship between the transcription factors Nkx6.1 and Ptf1a[19]. Ptf1a is progressively restricted to the tip cells, whereas Nkx6.1, Sox9, and Hnf1b are localized exclusively to the trunk domain, also known as the epithelial cords. Although Sox9 is co-expressed with Nkx6.1 in the epithelial cords that give rise to both endocrine and duct cells, it is later confined to the duct lineage in adult. The resolution of the tip and trunk domains is complete by ~e14 in mouse when Nkx6.1 is excluded from the tips. In human, however, the process is more gradual with the extended presence of NKX6.1 in the tips until 49-52 dpc (approximately corresponding to e15.5 in mouse). In addition, SOX9 expression is found in human acinar cells until 10-14 weeks post conception(wpc) [12] (Fig. 1). The allocation of progenitors to the two domains is also influenced by tubulogenesis, a morphological event by which the extensive duct network is generated[20]. Tubulogenesis in the pancreas does not follow a stereotypical branching pattern that involves iterative tube extension; instead it occurs through epithelial stratification, acquisition of cell polarity, microlumen formation, microlumen fusion to form a plexus, and finally remodeling into a complex 3D network of tubules[21]. While microlumens are also observed in human pancreatic buds[12], suggestive of similar tissue reorganization, additional work is needed to fully explore the exact nature of this process.

The Sox9⁺ bipotent trunk domain is poised to generate either endocrine or duct cells contingent on the activation of Neurogenin3 (Ngn3), a pro-endocrine transcription factor. Ngn3 is necessary for commitment to the endocrine fate. As such, Ngn3-deficient mice fail to develop all endocrine cells, and die from diabetes 1-3 days after birth[22]. Bi-allelic mutations in NGN3 were reported to cause PNDM in humans[23], implying a similar role in human islet development. In mouse, a biphasic transient wave of Ngn3 expression [24] is observed, wherein the second wave corresponds to the secondary transition, an extensive terminal differentiation event in the developing pancreas that peaks at e15.5. In stark contrast, only a single-phase of NGN3 expression is observed in human that starts at 8wpc (corresponding to e16 in the mouse) and peaks in the second trimester between 10-14wpc[25] (Fig. 1). During the peak period, only 3-4.5% of SOX9⁺ trunk cells express NGN3, indicating a rather low efficiency of endocrine cell induction. This small number of endocrine precursors suggests that a prolonged period of endocrine cell specification is required to generate sufficient numbers of islet cells in humans. However, several critical questions still remain. Why do only certain Sox9⁺ cells activate Ngn3 expression? How does the niche control this differential cell fate? These mysteries are only beginning to be solved. For example, recent studies demonstrated that graded levels of Notch regulate the ductal vs. endocrine lineage choices as well as the tip vs. trunk fates of MPCs. MPCs with active

Notch signaling acquire a trunk fate [26], and further within the trunk domain, high Notch signaling activates the expression of a downstream transcription factor Hes1 that promotes duct fate over endocrine commitment [27]. Whether these mechanisms are active in human fetal pancreas is unknown. A recent study examined HES1 expression in 16wpc and 19wpc old human pancreata, stages at which ducts should express Hes1 by extrapolation of mouse findings, but only found significant Hes1 expression in acinar tissue [28]. Investigation of Notch targets in earlier stages of human development are needed to delineate the role of Notch signaling in fate decisions.

Section III: Determination of endocrine cell types: Are Ngn3⁺ cells pre-committed?

After Ngn3 expression, pro-endocrine cells exit the cell cycle, delaminate into the mesenchyme and trigger downstream endocrine genes in rodents. Concerted activity of several transcription factors (Pdx1, Nkx6.1, Sox9, Nkx2.2, Neurod1, Ngn3, Pax4, Arx, Rfx6, Pax6, and others) orchestrate the formation of the five individual endocrine cell types (α , β , δ , ϵ and PP) present in adult islets. For instance, opposing functions of Arx and Pax4 determine α vs. β/δ fate[29]. However, we do not yet fully understand how a given Ngn3⁺ endocrine-committed cell makes the decision to differentiate into a specific endocrine subtype. The concept that not all Ngn3⁺ cells are alike in their developmental potential and that their fate is spatio-temporally restricted is gaining consensus [30-32]. Johansson and co-authors proved the existence of competence windows for the generation of different endocrine subpopulations in mouse; temporally controlled activation of Ngn3 in Pdx1⁺ pancreatic epithelium showed that Ngn3⁺ cells predominantly formed α -cells [30, 33] at e8.5, β - and PP-cells between e10.5 and e.12.5, and δ -cells from e14.5 [30]. On the contrary, the first endocrine cell type to be detected in human is the β -cell (6 wpc)[34, 35], followed by α -cells(8-9wpc), δ -cells(10wpc), and PP-cells (17wpc), in that order[35]. These findings raise a number of logical questions: does islet cell fate allocation occur prior to or during Ngn3 expression? If Ngn3⁺ cells are pre-biased, what cell-autonomous factors govern the acquisition of one of the various islet cell fates? How would differential expression of such factors be determined? Could it be through the location of cells along the epithelial cords and/or via extrinsic age-dependent signaling from the mesenchyme? These questions are only beginning to be addressed, but there is already some evidence that Nkx6.1 expression is mandatory before Ngn3 activation for commitment to the β -cell lineage [36]. A recent report indicated that repression of NeuroD1 by Nkx2.2 earlier in a Pdx1⁺ progenitor population, but not in Ngn3⁺ endocrine precursors, results in acquisition of α -cell fate[32]. In human PDX1⁺ progenitors, however, only NKX6.1, not NKX2.2, is present prior to NGN3 expression. Thus, the regulatory interactions between NKX2.2 and NEUROD1 are probably not present in human cells, and this may explain why the first endocrine cells to arise in human development are β -cells, and not α -cells. Summarily, these observations insinuate that events preceding Ngn3 expression regulate endocrine cell fate choices. Whether there is any functional significance for the appearance of insulin-producing cells before other cell types in human remains to be investigated. Possibly, these early β -cells participate in paracrine insulin signaling that may be required to maintain the Sox9⁺-progenitor pool as demonstrated in other organ systems [37-39].

While the majority of endocrine cells in the human fetal pancreas only express a single hormone, polyhormonal cells have been observed by several groups, especially early in development [35, 40-43]. Although the numbers of insulin and glucagon co-expressing cells varied between the different studies, the general trend was progressively decreasing percentages of double-positive cells through development with the highest numbers in the first trimester (20-30% of total insulin⁺/or glucagon⁺ cells). These hormone co-expressing cells were always scattered as single cells or found in small aggregates near the epithelial cords, supporting the notion that they likely represent newly generated endocrine cells [35, 41, 42]. Previously, Cre recombinase-mediated lineage tracing in mouse had revealed that cells that activate the insulin promoter at some point in their life do not develop into an adult α -cell and vice versa[44], signifying that insulin-glucagon double-positive cells do not contribute to adult islets. Nonetheless, this finding does not rule out extremely low promoter activity not sufficient to activate Cre recombinase expression. In human, however, the presence of ARX, but not of PDX1, NKX6.1 or MAFA, was demonstrated recently in insulin-glucagon double-positive cells in the developing human pancreas, leading the authors to conclude that the double hormone positive state is a transitional phase in α -cell development[41]. Additional studies are required to resolve the relevance of polyhormonal expression in developing endocrine cells.

Section IV: Islet cytoarchitecture and Acquisition of form

Despite decades of research, islet morphogenesis remains a poorly understood area as we lack critical information regarding factors that trigger clustering of newly forming endocrine cells into specialized islet structures. Moreover, large inter-species diversity exists in islet architecture and cell composition [9], yet the rodent islet structure with its core-mantle organization is often considered the canonical islet morphology. β -cells constitute the primary component of rodent islets (~80% of cells), whereas human and non-human primate islets have relatively fewer β -cells (~60%), and more α - (~30%) and δ -cells (~10%) [45, 46]. There are two opposing opinions regarding the arrangement of endocrine cells within the human islet; in the first view, human islets have a modified core-mantle structure where smaller groups of β -cells form anatomical subdivisions surrounded by non β -cells [47]. The alternative view dictates that endocrine cells are randomly distributed along islet microcapillaries with no apparent subdivisions[45]. More heterotypic β - α cell interactions were discerned than homotypic β - β or α - α cell interactions in investigations that supported the second premise[45, 46]. A combination of the two theories was described in a trilaminar model proposed by Bosco et al., where a β -cell layer was sandwiched between two non β -cell (mostly α -cell) layers. This trilaminar plate was lined on both sides with blood vessels, and most β -cells extended cytoplasmic extensions through the α -cell layers such that they were in direct contact with endothelial cells[48]. A recent semi-automated analysis of human islets also demonstrated a slight preferential homotypic attachment of β cells amidst increased intermingling with α -cells[49], indicating β - and α -cells are not randomly mixed. Despite the controversy concerning human islet organization, it is apparent that profound differences exist between rodent and human islet architecture. These differences have been implicated in distinct functions. In contrast to mouse islets, human islets do not exhibit synchronized Ca^{2+} oscillations in response to glucose, but do show a Ca^{2+} response to low

glucose (due to presence of more α -cells)[45]. Additionally, the increased number of heterotypic α - β contacts in human islets suggests a prominent role for α -cells in β -cell function, consistent with prior reports that showed juxtacrine glucagon signaling as well as α - β cellular contacts positively regulate glucose stimulated insulin secretion (GSIS)[50, 51]. Studies that correlate the relative arrangement of endocrine cells with their functional outcome are necessary to illuminate the influence of islet architecture on β -cell function.

Endocrine cells assemble to form islets close to the time of birth in the mouse, whereas in human, islet organization begins as early as 12 wpc. The first sign of islet morphogenesis in humans is preceded by scattered single endocrine cells that arise within the epithelial cords (6-9 wpc). At 10 wpc, α -or/and β -cells group into small clusters, and by 12 wpc they aggregate into a structure resembling the typical core-mantle mouse islet organization with β -cells in the center and α - and the other endocrine cells in the periphery. Soon after, the α - and β -cells expand and re-organize into juxtaposed homotypic clusters (also called bigeminal islets [40]) by 17 wpc. Finally, in the third trimester these homotypic aggregates undergo further remodeling to attain the adult human islet morphology that consists of intermingled endocrine cells [35, 40, 42, 52] (Fig. 1). The cell migration processes involved in the assembly of the mature islet structure are unknown, but one might speculate that signals from endothelial cells guide this event considering the alignment of endocrine cells along intra-islet vasculature in adult islets[45]. Finally at birth, human islets have equal numbers of α -, β - and δ -cells, and the adult composition is established by a neonatal burst in β -cell proliferation concomitant with a decline in δ -cell numbers [53].

Section V: Form follows function: Does islet morphogenesis induce maturation?

Association of diabetes with aberrant islet morphology in mice and human alludes to a correlation between the form and functional status of islets. However, it is not clear whether acquisition of islet structure causes functional maturation of β -cells or vice versa. Maturation of rodent β -cells into functional metabolic regulators has been associated with the appearance of a key transcription factor, MafA[54], and a secreted protein, Ucn3[55]. A switch from MafB (homolog of MafA expressed in immature α and β cells) to MafA occurs perinatally in mouse β -cells, and coincides with islet formation [54, 56]. In contrast, adult human β -cells maintain MAFB expression along with MAFA. MAFA mRNA is also detected in the developing human pancreas albeit at low levels[43], but convincing presence of nuclear MAFA protein has not been demonstrated until 21 wpc[35, 41]. Examination of third trimester fetuses and neonatal islets are required to determine the timing of MAFA appearance in humans. Notably, deletion of MafA in mice significantly disturbed islet morphology and impaired GSIS by affecting genes involved in glucose metabolism, insulin granule docking and insulin synthesis[56]. On a similar note, mutations in SUR1, a K^+ ATP channel subunit that confers β -cell function and is required for the induction of membrane depolarization, results in disrupted islet architecture[57] and causes NDM in humans[58]. Thus, it appears that factors involved in β -cell function also are necessary for islet structure.

Conversely, islet formation enables cell-cell coupling through Gap-junctions and Connexin-36 signaling, and contributes to functional maturation of β -cells[59]. Aside from

endocrine cell coupling, the neurovascular milieu, which forms a significant structural component of islets, has also been implicated in islet maturation. In mouse, islet vascularization begins during islet cell differentiation (e13.5 onwards), and by birth islets are fully vascularized. While the neuronal processes surround the developing endocrine clusters by e15.5, islet innervation, guided by vascular scaffolds, is completed later around the time of weaning [60]. On the other hand, vascularization and innervation of human fetal pancreas is completed long before birth. During human islet development, CD34⁺ endothelial cells are present in close proximity to small clusters of β/α -cells at 10 wpc. By 12.5 wpc, they penetrate newly forming islets, and by 20 wpc both blood and lymphatic machineries are in place[61]. The human neuronal component, however, differs between adult and fetal stages; adult islets have few nerve fibers, and these are intimately associated with islet microvasculature rather than endocrine cells[62], while fetal islets have a higher density of nerves, ganglions and neuroinsular complexes (NIC). Interestingly, the highest density of NIC was detected in the third trimester (27-38 wpc) when the typical adult mosaic islets form, suggesting a role for neuronal control in islet morphogenesis[63]. Remarkably, norepinephrine- β -adrenergic signaling from sympathetic axons was shown to establish islet cytoarchitecture and β -cell maturation in mice[64].

Maturation of human β -cells is a subject that had not received much attention up until now. Hrvatin and colleagues purified human fetal (14wpc) and adult β -cells, and characterized their transcriptome to unearth genes that are differentially expressed in mature β -cells. They found significant enrichment of genes involved in metabolic processes, such as generation of metabolites, oxidation-reduction, electron transport chain and monosaccharide utilization, and vesicle-mediated secretory processes in adult β -cells. Ucn3, a marker of mature mouse β -cells, however, was not significantly upregulated in the human context[65]. A similar analysis on >27wpc fetal β -cells, when the mosaic islets develop and have maximal neuronal associations, would be interesting, and will shed light on whether islet morphogenesis induces maturation in humans.

Section VII: Conclusions and application to hPSC differentiation

By comparing human and mouse islet organogenesis, we learn that key events are grossly conserved, though differences in timing and developmental factors are evident between species. For example, human pancreas and islet formation is marked by late detection of PDX1 only after separation of the foregut from the dorsal aorta, delayed resolution of tip and trunk progenitors, single-phased transient NGN3 induction, absence of NKX2.2 before endocrine commitment, presence of significant numbers of polyhormonal cells, appearance of β -cells before other cell types, innervation and formation of the unique mosaic islet structure before birth. It has become further apparent that polyhormonal cells represent either early endocrine or α -cell-fated cells during human pancreas development. Polyhormonal cells were the hallmark of β -cell differentiation from hPSCs until recently. The absolute requirement of NKX6.1 for commitment to the β -cell lineage has led to the development of two improved protocols that tweak the early pancreatic progenitor stage. These protocols produce monohormonal β -like cells that have suboptimal functional capacity, responding to glucose only in static GSIS assays [66, 67]. Apart from incorrect lineage specification, the difficulty in generating functional β -cells may be due to the lack of

pertinent maturation cues. Thyroid hormone was employed by these two studies to induce MAFA expression [66, 67]. However, using one marker, for instance MAFA, as readout for maturity in hPSC differentiation could be misleading, and a combination of several metabolic processes and transcription factors are necessary to mark mature human β -cells as elucidated in section V.

Other endocrine cell types, especially α -cells form a significant component of human islets and aid β -cell function as described in section IV. Therefore, generating pure β -cell populations in isolation with the intent of fully replicating all regulatory aspects of insulin secretion is counter-intuitive. We can also infer that important roles may be played by neuronal and endothelial cells due to their increased presence in the third trimester of human development during which islet morphogenesis and maturation occurs. Supplementing the hPSC cultures with these cell types or addition of factors derived from these cells, including CTGF and norepinephrine, at appropriate times may enhance the generation of mature β -cells (Fig. 2). Moreover, the focus should now be on inventing conditions to maintain terminally differentiated β -cells in their fully functional state. This may even necessitate the construction of micro-organoids with all the various islet cell types. As described above, immaturity has also been attributed to lack of proper coupling between oxidative phosphorylation and glycolysis, underscoring the magnitude of research that needs to be conducted in hPSC-derived β -cell metabolism. In conclusion, we anticipate that additional insights gained from human development will provide the ultimate blue print to produce bona fide human β -cells in vitro in the coming years.

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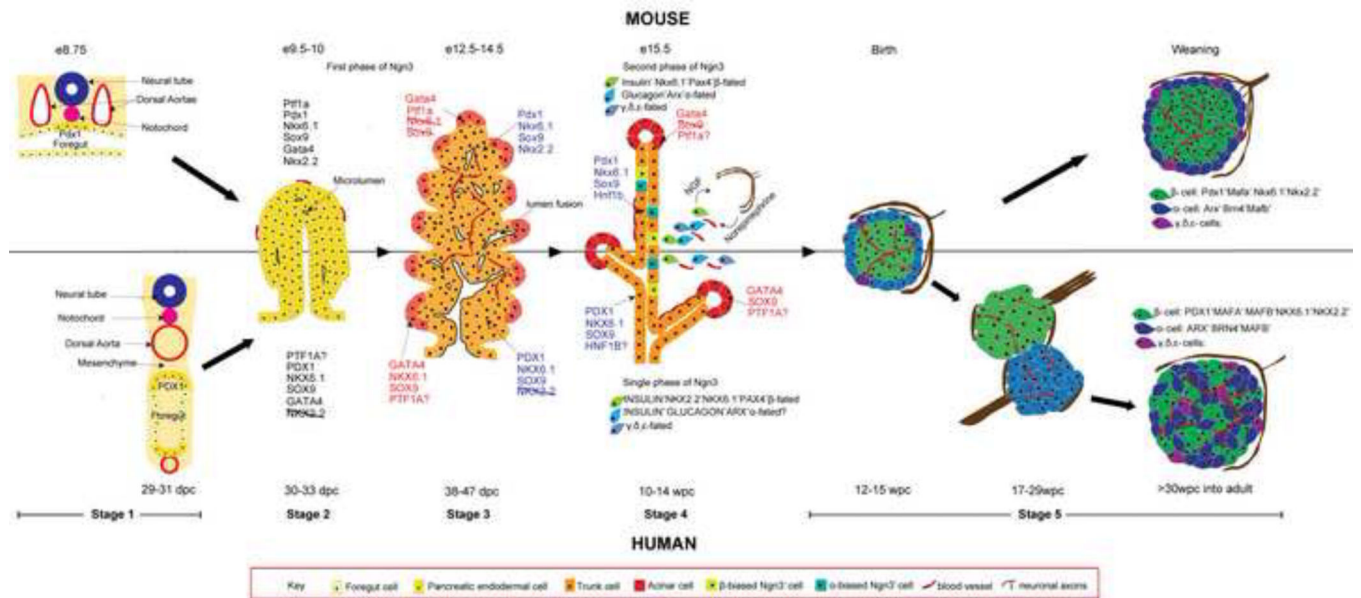


Fig. 1.

Illustration of pancreas morphogenesis in mouse and human. The top half of the figure depicts mouse while the bottom half portrays human development. Stage 1: Pancreas specification: appearance of Pdx1 marks the presumptive pancreatic region in the foregut epithelium. In mouse Pdx1 appears before gut closure when the notochord is in contact with the gut, but in human, PDX1 is delayed and appears only after separation of notochord and aorta from the gut. Stage 2: Formation of pancreatic epithelium: Following Pdx1 expression and Ptf1a activation, pancreatic buds containing MPCs grow into the surrounding mesenchyme. MPCs exhibit similar transcript profile between mouse and human with the exception of NKX2.2. Microlumens are already visible at this stage. Stage 3: Segregation of the tip and trunk domain: MPCs differentiate into pro-acinar tip cells (indicated by light red) and bipotential epithelial cords (light orange) due to opposing functions of Ptf1a and Nkx6.1. This process is dynamic with rampant tubulogenesis. In human, the separation occurs gradually with the tip cells still expressing NKX6.1 and SOX9 at the corresponding age in mice. Stage 4: Endocrine differentiation (also secondary transition in mouse). Terminal differentiation of tips to acinar fate and trunk cells to endocrine/duct fate occur during this period. The second wave of Ngn3 expression coincides with this period in the mouse, whereas in human, this period occurs after embryogenesis and a single phase of Ngn3 expression is observed. The time point indicated denote the peak period of endocrine differentiation in both species. Stage 5: Islet morphogenesis. In mouse, sympathetic axons regulate islet cell clustering by β -adrenergic signaling while a parallel is unknown in human. Moreover, islet formation along with vascularization is completed only at birth in the mouse, whereas in human, islet morphogenesis extends through the second and third trimester but is completed before birth. Initially fetal human islets resemble mouse islets in its architecture. Then, they open up to give rise to juxtaposed homotypic α/β clusters, and finally acquire the intermingled architecture of adult islets in the last trimester. Innervation of mouse islets is completed at weaning (orange arrow), whereas human islets lose their dense pre-natal neuronal associations. In addition, axons contact vasculature in human adult islets (orange

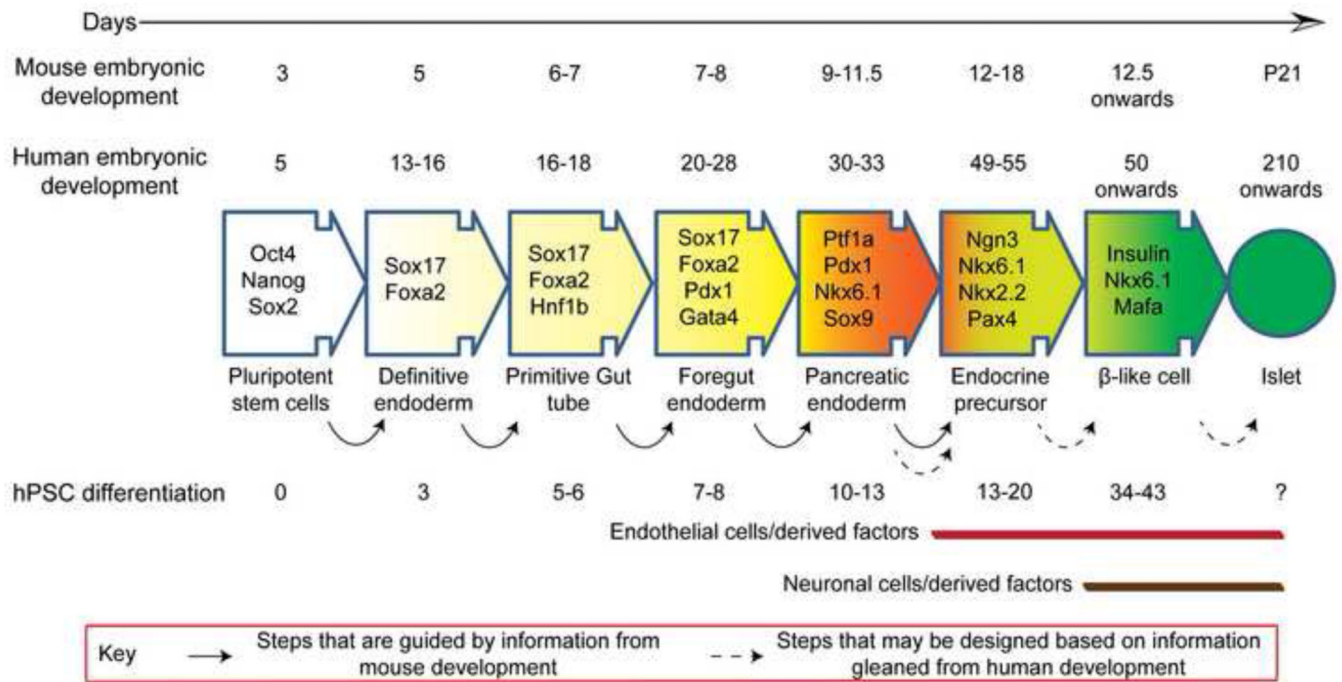
arrowhead). The approximate embryonic/fetal age at which the various stages occur are designated respectively for the two species. Progressively darker shades of the colors representing epithelial and endocrine cells indicate differentiation to more committed fates over time. e-embryonic day; dpc-days post conception; wpc-weeks post conception

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**Fig. 2.**

Timeline of pancreas development, in vivo, in mouse and human compared with in vitro differentiation of β -cells from hPSCs. hPSC differentiation protocols have been developed largely based on information gained from pancreas development in mice. Current protocols generate sub-par β -like cells that have modest glucose responsiveness but do not resemble mature β -cells of an islet in their metabolic and secretory properties. New data from human pancreas development, such as involvement of neuronal and endothelial cells in later stages of organogenesis when β -cell maturation predominantly occurs, may be used to instruct better differentiation protocols that result in bona fide β -cells. The timeline of hPSC differentiation is based on two recent studies; Pagliuca et al. [66] and Rezania et al. [67]

Table.1
Comparison of events and their time of appearance in mouse and human pancreas development.

Developmental Stage	Mouse		Human	
	Events/Processes	Embryonic age	Events/Processes	Fetal age
Pancreas specification	Shh exclusion from prospective pancreatic region in the dorsal endoderm (11)	e8.75	Shh exclusion from prospective pancreatic region in the dorsal endoderm (12)	25-29 dpc
	Pdx1 appears in dorsal endoderm before gut closure when the notochord is still in contact with foregut(10) Gata4 present in ventral endoderm and required for gut closure(68) Sox17 absent in dorsal foregut endoderm(69)	e8.75 e8 e8.5	Pdx1 appears only after gut closure and separation of foregut from notochord and dorsal aorta by the mesenchyme(12) Gata4 present in foregut endoderm only after gut closure (12) Sox17 present in dorsal foregut endoderm(12)	29-31 dpc 29-31 dpc 25-31 dpc
Pancreatic buds/epithelium	MPCs express Pdx1, Ptf1a, Sox9, Gata4, Nkx6.1 and Nkx2.2(10,18) Microlumens found(20,21)	e9-e11.5 e10.5	MPCs express Pdx1, Sox9, Gata4, Nkx6.1 but not Nkx2.2 (12) Microlumens detected (12)	30-35 dpc 30-33 dpc
	Tip and Trunk domain formation	e14.5 e14.5 e14.5	Nkx6.1 is still present in tip cells whereas Gata4 is restricted in tip cells (12) Nkx6.1 exclusion from tips complete (12) Mutually exclusive expression of Sox9 and Gata4 expression (12) Trunk domain has Sox9, Hnf1b, Nkx6.1 but not Nkx2.2 (12)	49-52 dpc 10 wpc 14 wpc 49dpc - 14wpc
Endocrine differentiation	Biphasic Ngn3 expression, second wave peaks at e15.5(24) α -cells appear first (30,33) presence of few polyhormonal cells(44)	e8.5-e11 and e12-e18 e8.5	Single wave of Ngn3 expression peaking 10-14wpc (25,12) β -cells appear first (34,35) 20-30% early endocrine cells are polyhormonal (35,40-43) Nkx2.2 appears after endocrine commitment (12)	8-18 wpc 6 wpc <10 wpc
	Islet morphogenesis	Islets have core-mantle structure with β -cells in the center and α , δ , ϵ and PP cells in the periphery (9,46,49) Islets formed close to birth(10) β -cells outnumber other cell types at birth and form majority of islets cells(24,46)	>e18	Islets have mosaic structure with intermingled β , α , δ , ϵ and PP cells (9,45-49) Islets formation completed in 3rd trimester (42) Undergoes a transient mouse-islet like morphology in 2nd trimester(35,40,42,52) β -cells equal in number with α , δ -cells at birth and undergo neonatal proliferation to reach baseline mass (35,41,53)

Developmental Stage	Mouse		Human	
	Events/Processes	Embryonic age	Events/Processes	Fetal age
Islet Vasculatization	Begins during islet cell differentiation(60) Completed at birth(60)	e13.5 e20	Begins during islet cell differentiation(61,34) Completed in 2nd trimester(61)	10 wpc 20 wpc
Islet Innervation	Adult Islets are highly innervated with direct contact between axons and endocrine cells(62) Begins during islet cell clustering (60,64) Completed at weaning(60)	e15.5 P21	Adult Islets are poorly innervated with contact mostly between axons and smooth muscle cells of the endothelium(62) Begins during islet cell differentiation(63) Highest density of neuroinsular complexes in 3rd trimester and diminishes greatly in adult(63)	12 wpc 27-38 wpc

e-embryonic day; dpc-days post conception; wpc-weeks post conception