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Control of Cell Identity in Pancreas Development and Regeneration

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Abstract

The endocrine and exocrine cells in the adult pancreas are not static, but can change differentiation state in response to injury or stress. This concept of cells in flux means that there may be ways to generate certain types of cells (such as insulin-producing β-cells) and prevent formation of others (such as transformed, neoplastic cells). We review different aspects of cell identity in the pancreas, discussing how cells achieve their identity during embryonic development and maturation, and how this identity remains plastic, even in the adult pancreas.

Keywords

Pancreas; Development; Regeneration; Cell Identity

Establishing Identity: Evolution of the Pancreas

In mammals, the pancreas regulates the response to feeding via an exocrine compartment, which produces and releases enzymes that digest proteins and lipids, and an endocrine compartment, which controls blood glucose levels by producing hormones such as insulin and glucagon. Even before food enters the mouth, its smell induces secretion of digestive enzymes[1] and insulin[2] during the so-called cephalic phase of digestion—a signaling pathway that begins in the brain and is transmitted via the vagus nerve. As food enters the digestive tract, digestive enzymes are secreted into the intestinal lumen and glucose-regulating hormones are released into the blood, resulting in a coordinated metabolic response.

This response evolved in multicellular organisms, and precursors of the mammalian pancreas can be traced far back in the phylogenetic tree. Worms and protochordates have gut cells that produce insulin-like peptides, whereas *Drosophila* have similar peptide-producing cells in the *pars intracerebralis* of the brain[3]. Among vertebrates, the hagfish, which has insulin-producing cells near the bile duct, is the most primitive to have a pancreas-like

structure. Ancient sharks have a tissue that more closely resembles the mammalian pancreas, containing a mixture of endocrine and exocrine cells, and more-advanced fish can have 2 distinct types of pancreata. Teleosts (such as zebrafish) have primary islets (also called Brockman's bodies), which are primarily composed of endocrine tissue, and also have secondary islets, which are embedded within a diffuse exocrine network.

During the evolution of amphibians and mammals, exocrine tissue came to occupy an increasingly large fraction of the pancreatic mass, whereas endocrine cells began to form well-defined, encapsulated islets (Fig. 1A). The fact that isolated insulin-producing cells appeared before exocrine tissue led to the proposal that β-cells are phylogenetic precursors of the mammalian pancreas[4]. The exocrine pancreas might have evolved in higher organisms via activity of the pancreas-specific transcription factor (Ptf)1, which regulates expression of exocrine-specific genes, in endocrine tissues. In support of this model, downregulation of Ptf1a in adult zebrafish exocrine cells results in their conversion to endocrine-like cells[5].

The developmental origin of β-cells is another interesting feature of pancreatic phylogeny. Remarkably, in vertebrates, insulin-producing cells develop from endoderm, whereas in flies they develop from ectoderm. As there is significant overlap among β-cell and neuronal signaling pathways[6], it is possible that during vertebrate evolution, central nervous system signaling pathways were also used to generate β-cells in the digestive tract[7]. In other words, a discrete regulatory module may govern endocrine identity. Based on the evolution of the pancreas, it is possible that this plasticity is related to an ancient and portable endocrine program, a module that may also underlie the high degree of cellular plasticity that is seen in the adult pancreas.

Formation of the Pancreatic Lineages

Specification

The endoderm gives rise to the tissues that line the gastrointestinal tract; specification of naïve cells requires precise integration of signals from several pathways, to ensure proper alignment of organ rudiments along the anterior–posterior axis. There is evidence that production of fibroblast growth factor (Fgf)4 by mesodermal cells posteriorizes endoderm in a concentration-dependent manner [8]. Similarly, retinoic acid (RA) signaling has been shown in several species, including mice and zebrafish, to control anterior–posterior patterning of gut organs and promote pancreatic identity[9, 10].

The pancreas is unique among gastrointestinal organs in that it derives from the dorsal and ventral portions of the endoderm[11]. The ventral part of the pancreas arises from anterior endoderm close to the liver anlage, whereas the dorsal pancreas forms from posterior endoderm cells; each part interacts with different surrounding tissues during development.

The first sign of the dorsal pancreas in mice is an epithelial thickening of the dorsal endodermal sheet at around embryonic day 9. Before that stage, the uncommitted endoderm cells of the forming gut tube receive signals from the notochord, an embryonic mesoderm signaling center that provides informational cues to the overlying neural tube and underlying endoderm[12]. Over time, the notochord is displaced by the dorsal aorta, which separates the endoderm from the notochord. Signals from the notochord such as activin and Fgf block expression of sonic hedgehog (Shh), a member of the Hh signaling family that regulates stomach and duodenal organ formation[13, 14]. Shh production in pancreas epithelium generates a molecular boundary and controls organ specification at the foregut midgut border. Signals generated by the aortic endothelium promote dorsal bud outgrowth and are

eventually replaced by the coalescence of mesenchymal cells around the evaginating epithelium of the pancreas[15–17].

The ventral pancreas gives rise to 2 distinct buds in mammals. One of these buds regresses soon after evagination, whereas the remaining bud branches into the surrounding mesenchyme. The endoderm tissues that give rise to the ventral pancreas buds are in direct contact with the lateral plate mesoderm; this tissue provides signals that initiate pancreas organogenesis at a specific point along the anterior–posterior axis. Signaling by activins, RA, and bone morphogenetic protein (BMP)7 induces the expression of pancreatic markers in ventral endoderm, similar to the roles of activin and RA in the dorsal bud[18]. Signals from the cardiac mesenchyme and septum transversum further distinguish the liver and pancreas anlagen in the ventral endoderm sheet. Increased Fgf signaling from the cardiac mesenchyme promotes differentiation of liver, instead of pancreas, which is reinforced by a delicate balance of transforming growth factor- β, BMP, and FGF signals from the septum transversum[19]. Therefore, interactions among different signaling pathways from the tissues that surround the ventral pancreas regulate its formation.

Mophogenesis and Lineage Segregation of Multi-Potent Progenitors

After organ specification, the pancreas epithelium thickens and eventually protrudes into the surrounding mesenchyme. Although researchers used to believe that the pancreas developed from a dense, cohesive epithelial bud that extended and branched, formation of a microlumen has been included in the process [20]. Detailed analyses of morphogenesis revealed that the organ forms from a single-layer epithelium that undergoes stratification; the inner cells develop without direct contact with either the primary central lumen or the basement membrane. Multiple micro-lumens form within the epithelium, eventually fusing to generate a single lumen with ductal tubes that line the internal space. There is increasing evidence that the surrounding mesenchyme promotes morphogenesis of the epithelial structures. Signaling by ephrin B2, expressed in pancreatic mesenchyme, through its receptor, EphB, on epithelial cells is required for structural changes, including the development of the microlumens[20]. The fusion of microlumens is followed by the segregation of the epithelial structures into distal tip and proximal trunk regions—morphologic changes that precede the differentiation of specific cell lineages within the pancreatic epithelium. Carefully timed morphogenetic movements of the pancreas epithelium therefore precede cell lineage specification during the early stages of pancreas formation.

The diverse endodermal cell types of the adult pancreas include digestive enzyme-producing acinar cells, duct cells that form the lumen through which acinar enzymes are transported towards the duodenum, centro-acinar cells that connect duct and acini, and hormone-producing endocrine cells located in islets of Langerhans. All these cell types derive from multi-potent progenitor cells present within the pancreas epithelium at early stages of development. Much of what we know about the lineage relationships among the different cell types has been determined using transgenic mice that allow for labeling of specific cell subsets during development. These studies have provided important information about the stepwise progression of endodermal cells within the endodermal sheet to lineage-restricted, multi-potent progenitors to fully differentiated cells with specialized functions in the adult organ.

Acinar Cells

Acinar cells are highly specialized and organized like bunches of grapes at the end of small ducts; they make up the bulk of the mature pancreas. They produce more than 20 different enzymes, including DNAses, proteinases, and lipases, which are required for digestion in the

duodenum. The first molecular feature of future acinar cells is expression of the digestive enzyme carboxypeptidase A1 (Cpa1) in cells at the distal tips of the branching epithelium. Interestingly, when Cpa1 expression is first detected, these distal cells still have the potential to differentiate into all pancreatic lineages, including acinar, duct, and endocrine cells[21]. Subsequently, when the epithelial tree expands, the distal tip cells commit towards the acinar lineage through activity of a trimeric transcription complex comprising Ptf1a, recombination signal binding protein for immunoglobulin-κ J region-like (Rbpj-l), and a common E-protein[22]. This complex regulates the expression of genes that encode digestive enzymes and genes required for acinar cell function[23].

Acinar cell development and expansion is guided by the temporal activities of embryonic signaling pathways, including Notch and Wnt signaling. Inhibition of the Notch signaling mediator Rbpj-k in pancreas epithelium reduces, but does not completely eliminate, acinar cell development[24]—probably because of its negative effects on the expansion of multi-potent pancreas progenitors. Sustained Notch signaling compromises differentiation of acinar cells, at least in part by inhibiting Ptf1a-mediated activation of acinar genes[25]. Increased Hh signaling in embryonic pancreas also compromises expansion of the epithelial cells that form the endocrine and exocrine compartments, including acinar cells[26]. Canonical Wnt signaling, mediated by β-catenin, is essential for the development of pancreatic acinar cells; sustained activation of the pathway promotes acinar cell proliferation[27–29]. More recent studies indicate that β-catenin does not control survival or functions of mature acinar cells, but is required for proliferation and regeneration of acinar cells upon injury [30, 31].

Endocrine and Duct Cells

Pancreatic endocrine and duct cells also derive from the multipotent progenitor cells (MPCs) located within the budding epithelium. In contrast to acinar cells, which remain at the distal tips, endocrine and duct progenitors segregate from their acinar counterparts to localize to epithelial cords. The mechanisms by which the duct and endocrine progenitors are generated and separate from acinar progenitors are being investigated. Early models proposed that duct and endocrine progenitors separated from the distal tip cells to form the stalk or trunk of the epithelial tree[21]. This model was based on conventional budding, extension, and branching patterns observed in other epithelial organs, and supported by real-time imaging of morphogenetic events in cultured pancreas rudiments[32]. As described above, more recent studies indicate the formation of an epithelial plexus, in which transient epithelial stratification is followed by de novo tubulogenesis, mediated through microlumen condensation[20]. This model introduces a more complex process in which profound changes in cell shape and organization promote the development of the branching epithelium. Studies are needed to determine the dynamics by which distal acinar-committed cells would become distinct from endocrine and duct progenitors.

Most current models support the concept of a temporal restriction in cell differentiation potential, mirrored and promoted by differential expression of differentiation markers in various multi-potent and committed cell populations. For example, whereas the distal multi-potent cells are $Pdx1^+$, $Ptf1a^+$, $Cpa1^+$, and $cMyc^{high}$ [14], the emerging duct and endocrine restricted cells are $Sox9^+$, $Hnf1\beta^+$, $Foxa2^+$, and $Nkx6.1^+$ [33, 34]. Between embryonic days 13.5 and 15.5 (a period called the secondary transition), endocrine progenitors within the trunk epithelium form by reducing expression of Sox9 and Hnf1b. Concomitantly, neurogenin-3 (Ngn3), a transcription factor that is required for endocrine cell development, is transiently expressed. Its upregulation results in separation of endocrine cells from the duct lineage. Endocrine cells become separated from the trunk epithelium and cluster to form aggregates, eventually maturing into islets of Langerhans. Duct progenitors within the trunk retain Hnf1b and Sox9 expression and form a mature system of tubes that connects enzyme-producing acinar cells with the duodenum.

Development of the Human Pancreas

If basic science discoveries are to be developed into therapeutics, the experimental models we use must have relevance to human biology. It is therefore appropriate to ask whether the lineage relationships and morphologic and transcriptional changes observed during pancreatic development in rodents are similar to those that occur during human development. Surprisingly, there appears to be a great deal of overlap.

One of the most obvious differences pertains to overall organ morphology. Like its rodent counterpart, the human pancreas emerges from ventral and dorsal endoderm buds that arise as early as 5 weeks of gestation. In humans these 2 buds merge into a single organ a week later, ultimately coming to reside in the retroperitoneal space. By contrast, the mouse pancreas is more diffusely spread along the intestinal mesentery within the abdominal cavity. The species also differ in islet organization and composition. In murine islets, β-cells are found in the center of the islet, where they comprise 77% of the cells. In human islets, β-cells are interspersed with other endocrine cells and comprise 55% of the cells in the islet[35].

The transcription factors that regulate pancreas development seem to be conserved between rodents and humans. Mutations in PDX1 and PTF1A lead to pancreas agenesis in humans, as they do in mice[36, 37], and haplo-insufficiency for SOX9 results in Campomelic dysplasia, associated with abnormal pancreatic morphology and perturbed islet composition[38]. The finding that individuals with mutations in Ngn3 lack intestinal endocrine cells but do not develop diabetes led to the proposal that humans have a different or possibly redundant factor that controls pancreatic endocrine development[39]. However, subsequent analysis showed that the Ngn3 mutations identified in human patients are hypomorphic and permit sufficient islet development to maintain euglycemia[40]. Humans and mice therefore seem to have similar molecular pathways that control cell identity in the pancreas.

Maintenance of Cell Identity in the Adult Pancreas

More than 50 years ago, Conrad Waddington coined the term epigenetics to describe a process whereby stable (and heritable) changes in gene expression affect steps in cellular differentiation[41]. According to this view, differentiation represents a stable state. It might seem that substantial energy would be required to move a cell from 1 differentiated state to another. However, there is increasing evidence that the boundaries that maintain cellular identity can be easily overcome, which has important implications for developmental biology. Much of this emerging evidence comes from studies in the pancreas.

One of the first pieces of evidence that maintenance of cell identity requires active regulation of gene expression was the finding that deletion of Pdx1 from post-natal islets resulted in loss of the β-cells phenotype[42]. That study reported an increase in α-cells in the islets, although it was unclear whether Pdx1 resulted in conversion of β-cell progenitors to α-cells or there was a compensatory increase in α-cell numbers. More recently, Yang et al.[43] showed that ectopic expression of Pdx1 in endocrine progenitor cells causes them to adopt a β-cell fate, indicating that Pdx1 is a β-cell specification or maintenance factor.

DNA methylation status also seems to maintain the identity of β-cells in the adult pancreas. In adult β-cells, the promoter of the *Aristaless homeobox gene* (*Arx*) is methylated, resulting in its transcriptional inactivation, which also requires NK2 homeobox 2 [44]. Deletion of DNA (cytosine-5-)-methyltransferase 1, which maintains previously established methylation patterns on DNA, causes loss of β-cells in mice[45]. The mechanism for this loss of β-cells appears to involve reactivation of Arx; transgenic expression of Arx in β-cells causes their conversion to α-cells[46]. Furthermore, post-natal antagonism of Ptf1a leads to the conversion of exocrine cells into endocrine-like cells [5].

Taken together, these studies indicate that pancreatic cell identity must be actively maintained, either through transcription factor expression, chromatin modification, or a combination of these. These findings provide important information for exploring changes in cell identity during injury or following the expression of various reprogramming factors.

Plasticity in the Exocrine Pancreas

Types of Exocrine Injury and Animal Models

As the body's reservoir for proteases and lipases, the pancreas is prone to injury from the inadvertent release and activation of these enzymes. Under physiological conditions, enzyme activation is prevented by complex mechanisms in which enzymes are produced as inactive zymogens that only become activated upon entry into the intestinal lumen. However, bypass of these protective mechanisms can lead to a vicious cycle of enzyme auto-activation and acute pancreatitis.

Pancreatic injury can be caused by obstruction of the pancreatic ducts (by gallstones or tumors), toxins, drugs, and less-common causes. Injury is associated with death of acinar cells and neighboring islets, and can result in transient hyperglycemia. Remarkably, if the cycle of injury and auto-activation is disrupted, the pancreas can fully recover its normal histology and function.

A number of animal models have been developed to study the various forms of pancreatitis[47]. Mice injected with the cholecystokinin analog caerulein, which promotes secretion of digestive enzymes, are widely used (partly for convenience) to study pancreatic regeneration. These mice develop pancreatitis that resembles many aspects of human acute pancreatitis, including ultrastructural changes to acinar cells and pancreatic edema[48]. Like patients with acute pancreatitis, these mice recover and typically have normal pancreatic histology within a week after injury. Another model of pancreatic injury involves pancreatic duct ligation (PDL) in mice. In this model of acute obstruction of the pancreatic duct, a surgical suture is placed along the length of the main pancreatic duct, resulting in damage to distal acinar cells. Finally, partial pancreatectomy, in which 50%–90% of the pancreas mass is removed, has been widely used to study pancreatic injury. Following partial pancreatectomy, there is a partial recovery of islet mass. However, there is minimal recovery of exocrine pancreas mass despite cell cycle re-entry of some acinar cells, particularly at the wound site.

Cellular Responses to Injury

There is accumulating evidence that acinar cells activate regenerative mechanisms in response to injury and stress. Although the response to different stimuli varies, it generally includes transient changes in cell differentiation state or identity. Intriguingly, embryonic signaling pathways, named for their activity during specific stages of fetal development and pancreas organogenesis, become reactivated during regeneration[49]. Several studies have demonstrated roles for Notch, Wnt, and Hh signaling pathways in response to acinar injury in mice, including caerulein-induced injury.

In rodents, caerulein-induced pancreatitis causes transient de-differentiation of acinar cells towards a progenitor-like state, identified by upregulation of marker proteins normally excluded from adult acinar cells[31, 50]. However, these de-differentiated cells are not necessarily identical to cells that existed during pancreas embryogenesis, but instead express markers found in other mature pancreas cell types. For example, this process leads to the transient expression of proteins normally observed in duct cells, including Sox9 and cytokeratin 19, indicating that acinar cells temporally lose their identity.

Different results were obtained from mice that express a transgenic diphtheria toxin receptor under control of the Pdx1 promoter[51]. When transgenic mice were treated with diphtheria toxin, acinar and endocrine cells died, whereas duct cells did not; the mechanisms that protect duct cells are not known. The duct cells instead proliferate, and duct-derived cells acquire acinar and endocrine properties. Similar to the transient de-differentiation of acinar cells observed following caerulein administration, duct-derived acinar cells reactivate parts of the pancreatic developmental program. Therefore, depending on the type and severity of injury, distinct cell types and mechanisms regulate acinar cell regeneration.

Analyses of transgenic animals have revealed essential roles for Notch signaling in caerulein-induced pancreatitis. The Notch pathway becomes reactivated upon caerulein administration, and reduction of Notch activity via genetic elimination of its receptors or pharmacologic inhibitors impairs acinar cell regeneration[52]. In the pancreas of adult mice, Notch signaling is only active in duct and centro-acinar cells, supporting the concept that upregulation of Notch signaling in injured acinar cells promotes transient trans-differentiation towards the duct cell lineage[53]. The important role of Notch signaling in maintaining cellular identity in the exocrine pancreas was illustrated by the observation that elimination of Rbpj-k, a co-factor for Notch-mediated gene transcription, promoted transdifferentiation of duct and centro-acinar cells into acinar cells[53].

Similarly, the Wnt and Hh signaling pathways have been implicated in acinar regeneration. Caerulein administration transiently activates Hh signaling mediators and targets; inhibition of this pathway impairs acinar regeneration[54]. Canonical Wnt signaling, defined by the nuclear activity of β-catenin, is also transiently increased in injured acinar cells[31]. Notably, canonical Wnt signaling is absent from mature acinar cells, and inappropriate stabilization of β-catenin stimulates acinar cell proliferation[27]. Therefore, de-regulation of signaling events normally absent from acinar cells promotes the transient acquisition of a progenitor-like state. Removing the injury stimulus results in rapid reactivation of the mature acinar identity, marked by loss of the inappropriate embryonic signaling events. Tight control of activation and de-activation of embryonic signaling pathways is therefore an important feature of acinar cell regeneration.

Changes During Regeneration

There are many questions about the identity of de-differentiated acinar cells. Although many studies have reported the loss of mature acinar cells markers and inappropriate reactivation of factors expressed by pancreas progenitor cells during organogenesis, it is not clear whether the process is a true reversal to a cellular state present in the embryonic pancreas. In other words, we do not know whether de-differentiated acinar cells return to a state they progressed through during ontogeny. Alternatively, the reactivation of signaling pathways might provide protective mechanisms by which the acinar cells can undergo regeneration and repair. Acinar cells are complex machines that produce enormous amounts of digestive enzymes. Reduced protein synthesis (indicated by reduced production of transcription factors required to maintain acinar function as well as expression of enzyme mRNAs) might provide cells with the opportunity to repair injury-related damage. However, it is important to note that de-differentiation generates a vulnerable state, because oncogenic stimuli, such as oncogenic Kras, can divert the regenerative process to initiate neoplastic transformation[55].

How Does Pancreatitis Lead to Cancer?

Pancreatitis is associated with the development of pancreatic ductal adenocarcinoma (PDA). Individuals with a history of pancreatitis have a more than 2-fold increase in risk for PDA, whereas those with hereditary pancreatitis (due to mutations in the gene encoding trypsin)

have a more than a 50-fold increase in risk[56]. This association also in exists in mice, in that pancreatitis stimulates progression of premalignant pancreatic intraepithelial neoplasm (PanIN) lesions[57].

It is not known precisely how inflammation increases the risk of cancer progression. One possibility is that the cellular turnover prompted by acinar cell death and replenishment simply causes an increase in DNA replication—which is normally quite low in the pancreas—providing an opportunity for replication infidelity and disease-causing mutations. Alternatively, inflammation could lead to the accumulation of reactive oxygen species (ROS), which can also serve as a mutagen. One additional possibility is that inflammatory cells themselves provide signals that promote proliferation of pancreatic epithelial cells that already contain oncogenic mutations. This hypothesis is supported by the finding that inhibiting inflammation with the corticosteroid dexamethasone leads to a reduction in PanIN lesions in animals with cancer-causing mutations[58]. It is important to increase our understanding the role of pancreatic inflammation in initiation and progression of PDA, which could provide an opportunity for immunomodulatory intervention, as recently demonstrated[59].

A new discovery about the cellular source of pancreas cancer could link inflammation, cell plasticity, and carcinogenesis. Over the past several years, studies that used compartment-specific transgenic mice to determine the so-called cell-of-origin of pancreatic cancer have indicated that pancreatic acinar cells can give rise to PDAs [60, 61]—a somewhat surprising result, given the ductal nature of this malignancy. Recently, Kopp et al. [62] showed that not only are acinar competent to give rise to PDA, but ductal and centro-acinar cells are refractory to oncogenic transformation, indicating that carcinogenesis requires the conversion of acinar cells into a duct-like state. This finding associates changes in cellular identity with oncogenic potential, consistent with the recent finding that cholangiocarcinoma (a form of hepatic ductal cancer) can arise from hepatocytes [63]. As described above, induction of experimental pancreatitis (such as with caerulein) leads to a de-differentiation of acinar cells, which is a strong stimulus for pancreatic cancer progression. Inflammation might therefore accelerate tumorigenesis by causing cells to change their identity, making them more competent to undergo malignant transformation.

Plasticity in the Endocrine Pancreas

In the human pancreas, loss of endocrine cells (principally β-cells) occurs via autoimmune destruction (resulting in type 1 diabetes; T1DM) or β-cell failure, as a result of increased stress (resulting in type 2 diabetes mellitus; T2DM). Although NOD mice, which are a models of autoimmune β-cell destruction in humans, have been widely used to study T1DM, most studies of β-cell regeneration have relied on toxin-based or genetic models.

Streptozotocin (STZ) is induces rapid death of β-cells. The limited recovery of β-cells following exposure to STZ was initially interpreted as evidence that the toxin kills putative stem cells in addition to differentiated β-cells[11]. Despite a longstanding search for pancreatic stem cells with the capacity to give rise to β-cells, lineage-tracing studies indicate that most post-natal β-cells are derived from pre-existing β-cells[64]. Moreover, genetic ablation experiments using ectopic expression of diphtheria toxin or c-myc have shown that mature β-cells retain a robust regenerative capacity—even after more than half of the cells in the islet are killed, β-cells replicate and β-cell mass returns to near normal levels[65, 66].

Although replication appears to be the dominant method by which the endocrine compartment of the pancreas maintains or recovers its mass, under extreme circumstances the pancreas can exploit cell plasticity as a means of recovery. For example, Thorel et al. [67] used a diphtheria toxin-based model to achieve a near-complete genetic ablation of β-cells,

similar to that observed in patients with T1DM. Under these circumstances, the authors showed that newly generated β-cells came from α-cells—evidence for a previously underappreciated degree of endocrine cell plasticity under physiological circumstances. It is not known whether similar cell fate conversions occur following STZ-induced injury.

Findings from Talchai et al.[68] indicate that alterations in cell identity could underlie β-cell failure in patients with T2DM. In that study, mice lacking Foxo1 in β-cell gradually lost β-cell mass due to loss of β-cell identity, rather than cell death (with β-cells losing differentiation markers and a subset becoming α-like). Importantly, the same phenomenon of β-cell de-differentiation (or more appropriately un-differentiation) was observed in 2 mouse models of T2DM. Therefore, although changes in cell identity, such as conversion of β-cells to α-cells, do not provide a robust means for β-cell recovery following injury, loss of β-cell identity can be involved in pathogenesis of diabetes.

Exocrine–Endocrine Interconversion

In addition to the findings that β-cells can arise from agr;-cells, there has been longstanding interest in whether β-cells can arise from non-endocrine cells. In particular, many investigators have focused on the possibility that β-cells arise from rare stem cells or differentiated cells within the exocrine compartment. Although new findings are continuously reported, there is growing consensus that expansion of β-cells in young animals, including humans, is mainly achieved via proliferation of existing insulin-producing cells. In older mammals, β-cell replication is reduced significantly, to the point that very few proliferating cells are detected[69–71]. Inhibition of β-cell apoptosis or induction of neogenesis from non-endocrine cells might replenish the β-cell mass. Evidence to support this hypothesis comes from transgenic mice in which exocrine injury was established via PDL.

Using a PDL model, Xu et al. demonstrated the appearance of $Ngn3^+$ cells within the duct epithelium[72]. The multi-potency of these newly formed endocrine progenitors was confirmed through transplantation into Ngn3-mutant pancreatic buds cultured in vitro. However, it is not clear whether $Ngn3^+$ cells derived from duct cells or other cells associated with the ducts. Recent findings indicate that the duct cells are not the progenitors of endocrine or insulin-producing cells in adult tissues. Specifically, Solar et al. performed lineage-tracing experiments using $Hnf1bCre^{ER}$ transgenic mice to demonstrate that tamoxifen-induced Cre-recombination within the pancreas was confined to duct and centro-acinar cells, excluding acinar and endocrine cells[34]. Labeled cells failed to give rise to endocrine cells under either control conditions or after PDL, indicating that mature duct cells do not serve as endocrine progenitors. Further evidence refuting a ductal origin for new endocrine came from studies of $Sox9Cre^{ERT2}$ mice, in which Cre-activity was also confined to duct and centro-acinar, but not endocrine cells, in the adult pancreas. Lineage tracing experiments under control and injury conditions revealed that $Sox9^+$ cells do not give rise to mature insulin-producing cells[33]. Although PDL induces the expression of Ngn3, these cells do not complete the endocrine differentiation process, due to the absence of transcription factors such as Nkx6.1 and Pax6. Although these data do not support the concept that adult duct cells can give rise to mature endocrine cells, current lineage-labeling reagents do not permit all duct cells to be tagged. Further studies are needed to determine whether subpopulations of duct cells exist that might still possess duct-endocrine differentiation capacities.

Roles for Cellular Plasticity in Therapy

Studies of pancreatic cell identity and differentiation might lead to methods to engineer β-cells from other somatic cell types. Given the close developmental relationship between the

pancreas and liver, and the discovery of ectopic hepatic foci in the pancreas under pathologic and experimental conditions, researchers have sought to interconvert pancreatic and hepatic cell types. These experiments have involved in vitro studies of pancreatic cell lines[73] and in vivo analyses of the effects of overexpressing pro-pancreatic or pro-endocrine transcription factors, such as Pdx1 and Ngn3 (reviewed in [49]). Mis-expression of Ngn3 was reported to induce hepatic oval cells (a putative progenitor cell in the liver) to adopt an islet-cell fate[74].

Other attempts to apply cell plasticity to therapeutic development have been based on the discovery that a small number of transcription factors can reprogram terminally differentiated cells to a pluripotent state[75]. Inspired by this finding, Melton and colleagues performed a screen for combinations of transcription factors that induced acinar cells to adopt a β-cell identity. Remarkably, pancreatic acinar cells that expressed the endocrine transcription factors Pdx1, MafA, and Ngn3 from a viral vector were able to produce, process, and secrete insulin. These cells were protected mice from the effects of STZ[76], although the reprogramming effect appeared to be incomplete[77].

In addition to the liver and the exocrine pancreas, other closely related endoderm derivatives, such as the intestine, could be substrates for new β-cells. More than 10 years ago, human intestinal K-cells (a type of enteroendocrine cell) were shown to correctly process and secrete insulin[78]. The potential for intestinal cells to serve as a source of insulin-producing cells has more recently been demonstrated through studies in which deletion of the Foxo1 transcription factor in the adult intestine causes enteroendocrine precursor cells to adopt a β-like phenotype[79]. Enteroendocrine progenitor cells normally express Ngn3, just as pancreatic endocrine progenitors do, making the intestine a plausible reservoir for ectopic β-cell production.

Conclusion

All cells in the body arise from embryonic precursors through the coordinated activity of *trans*-acting transcriptional regulators and *cis*-acting modifications in DNA. Work from many laboratories over the past 15–20 years has identified a regulatory code that determines cell identity in the pancreas, and evidence is beginning to accrue that cell identity can be manipulated in vivo, via careful alterations to this code. These advances are directly relevant to tissue engineers who seek to generate functional cells and tissues for therapy, starting with material taken directly from patients. Moreover, such studies are germane to those of the normal response to injury and the molecular and cellular events that mediate (or impede) recovery. In this sense, the pancreas, which has long been of interest to developmental biologists, provides a good model for scientists working in the emerging field of regenerative medicine.

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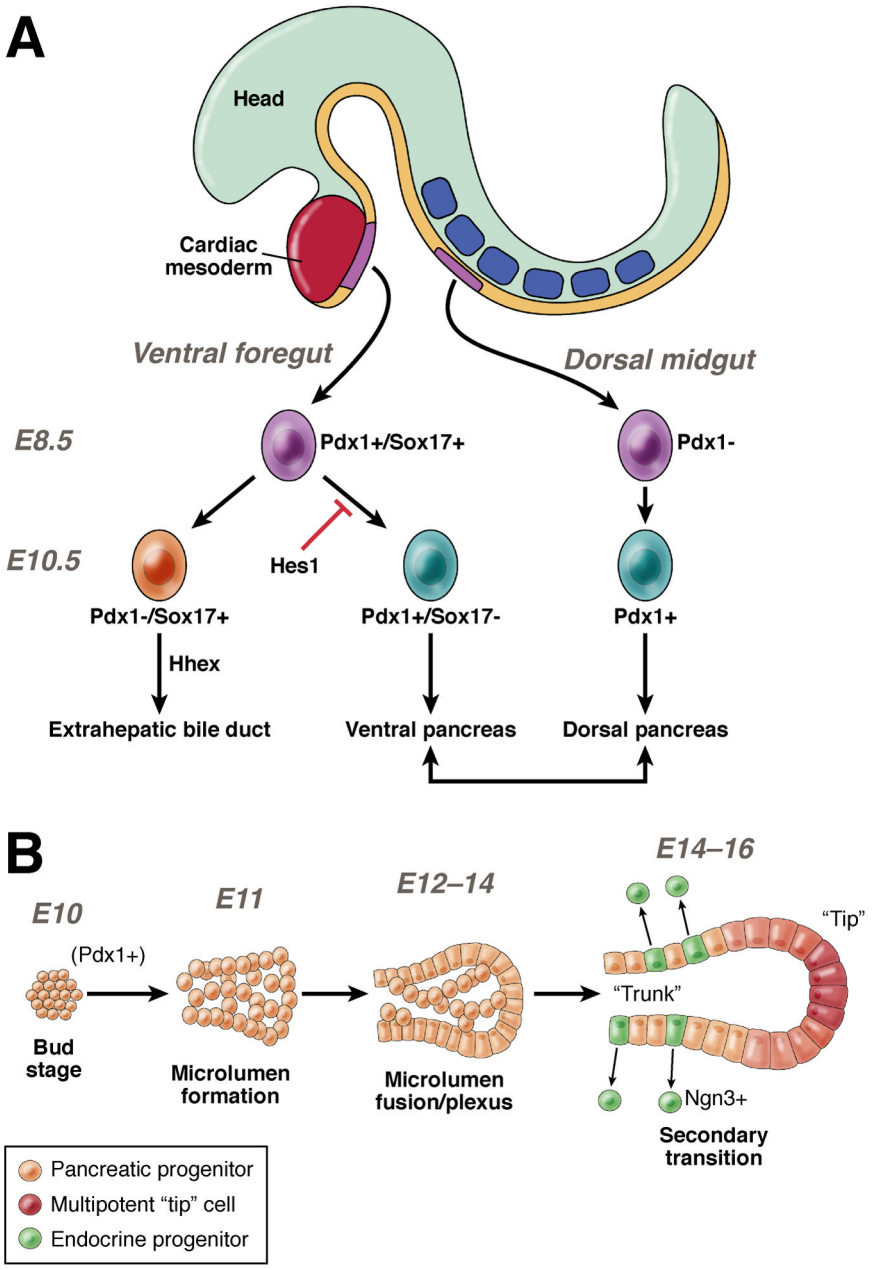

Figure 1. Developmental of the Mammalian Pancreas

(A) Molecular regulation of pancreas development in the mouse. Following gastrulation, 2 patches of endoderm (yellow)—1 in the ventral foregut and 1 in the dorsal midgut—receive signals from adjacent structures resulting in pancreatic specification (purple). Cells in both regions express the Pdx1 transcription factor whereas cells located in the ventral foregut patch also express the Sox17 transcription factor. Over time, $Pdx1^{+}/Sox17^{+}$ cells resolve into a Sox17 single-positive population (which gives rise to the extrahepatic biliary tree) and a Pdx1 single-positive population (which gives rise to the ventral pancreas). Subsequently, the ventral and dorsal pancreatic buds merge during rotation of the gut. *(B)* Cells become polarized within the growing pancreatic buds, forming microlumens which fuse to form a tubular plexus. During the secondary transition, a period marked by a large increase in

endocrine and exocrine differentiation, the tubular plexus resolves into better defined ductal structures. The tips of these primitive ducts initially remain multipotent, having the capacity to give rise to all pancreatic cell types including acinar cells, while the trunks are committed to ductal and endocrine fates. Endocrine progenitor cells, detectable by the expression of Ngn3, delaminate from the trunks, eventually aggregating as Islets of Langerhans.

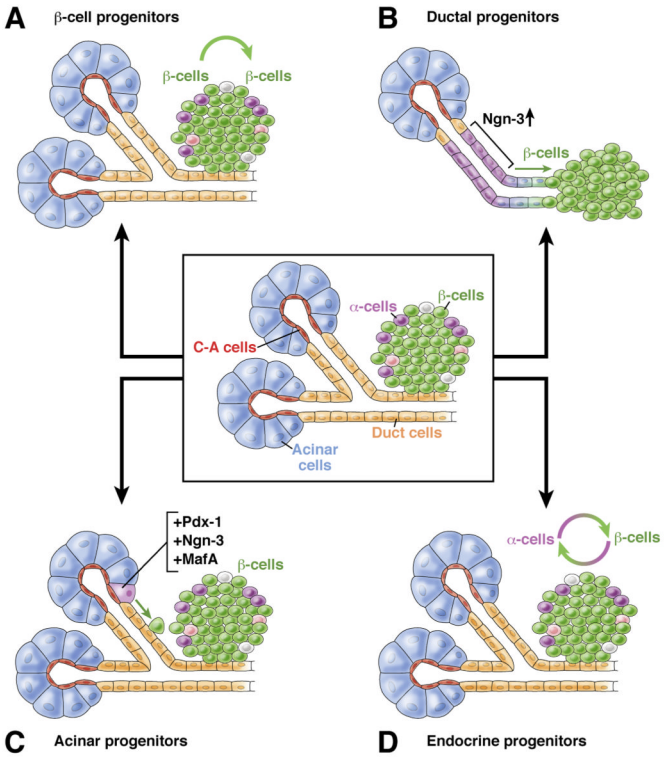

Figure 2. Cellular Plasticity in the Adult Pancreas

Depiction of the structural elements of the exocrine pancreas, including acinar, centro-acinar (C–A), and duct cells, and the endocrine islet, including α- and β-cells in the center of the schematic. *(A)* β-cells serve as progenitors for β-cells and expand via proliferation. *(B)* Upon injury, duct or duct-associated cells begin to express Ngn3, which could result in the formation of endocrine cells, including β-cells. *(C)* Viral expression of *Pdx-1*, *Ngn-3* and *MafA* in adult acinar cells promotes reprogramming towards β-cells. *(D)* α-cells can transdifferentiate into β-cells following their depletion, whereas β-cells can become α-like cells or undifferentiated cells during stress or following deletion of Foxo1. (Adapted from Puri & Hebrok, 2010.)

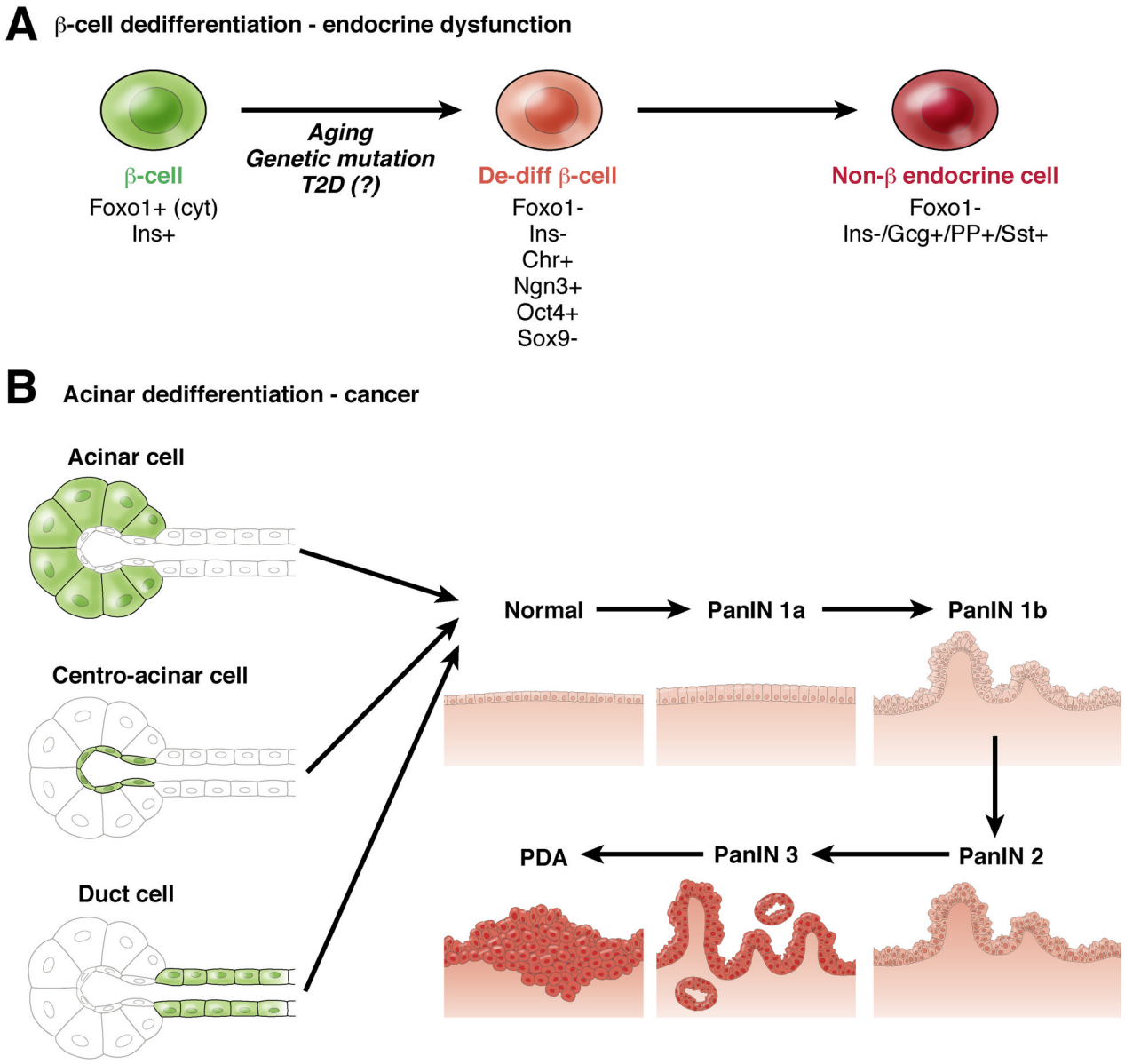

Figure 3. Loss of Cell Identity in the Adult Pancreas

(A) β-cells undergo a process of "de-differentiation" or "un-differentiation," either via genetic mutation (e.g. Foxo1 elimination) or physiological changes, (e.g. aging, T2D). These empty β-cells have reduced expression of markers of mature β-cells and increased expression of markers found in progenitors, including Ngn3, and Oct4. Further progression results in non-β-cell endocrine cells that express other endocrine hormones. (Adapted from Puri & Hebrok, 2012). *(B)* Exocrine cells, including acinar, centro-acinar, and duct cells, are considered to be progenitors to PanINs and PDA. (Adapted from Morris et al, 2010).