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Development of the Pancreas and Related Structures

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Anatomy of the Pancreas

The pancreas is a unique exocrine and endocrine organ located in the retroperitoneal region of the upper abdominal cavity. In humans, when fully formed, the organ has a distinct head, body, and tail, with the head of the pancreas contacting the duodenal region of the intestines (the main pancreatic duct drains into the duodenum) and the tail of the pancreas abutting the spleen. The greatest mass of the organ is present in the head, which is composed of tissue derived from two independent anlagen (see later). In other mammals, such as dogs and mice, the organ has a far less distinct structure and is identified as an amorphous pink tissue adjacent to the mesentery that runs along the upper intestinal wall.

The cells of the pancreas are arranged into distinct lobules composed primarily of the digestive enzyme-producing cells of the exocrine pancreas, which are arranged into acini (so-called acinar cells), the ductal structures that conduct these digestive enzymes to the intestines, and distinct clusters of endocrine cells, the islets of Langerhans, that secrete hormones and function to regulate glucose uptake and release and serum glucose levels. There are five recognized cell types within the islets, the α, β, δ, ε, and PP cells, which produce the hormones glucagon, insulin, somatostatin, ghrelin, and pancreatic polypeptide, respectively. The majority of the pancreatic tissue mass (more than 90–95%) is present within the exocrine compartment of the organ, with the islets of Langerhans, scattered throughout the tissue. The pancreas also has connective tissue, derived from the embryonic mesenchyme, which forms the septa that separate the many lobules of the organ. Mesenchyme-derived stromal cells are also present in the interlobular regions surrounding the pancreatic ducts, blood vessels, and nerves. In the following sections, we explore how these disparate cell types come together to form the pancreas.

Organogenesis in the Region of the Pancreas

Around day 14, the embryonic bilaminar germ disk is composed of a layer of epiblast and a layer of hypoblast. At this time, a faint groove appears along the longitudinal midline of the germ disk that develops into a structure called the primitive streak [1]. Around day 15, epiblast cells near the primitive streak undergo a morphologic change and migrate through the primitive streak into the space between the epiblast and hypoblast in a process known as gastrulation (Fig. 1.1). Some of the ingressing epiblast cells invade the hypoblast, which is eventually replaced by a new layer of epiblast-derived cells known as the definitive endoderm. Additional migrating epiblast cells occupy the space between the epiblast and the definitive endoderm to form a third layer of cells called the intraembryonic mesoderm (Fig. 1.1). As cells of the germinal disk migrate anteriorly to form a head process and lateral regions roll underneath to form an approximately cylindrical body shape, the endoderm is rolled into a tube that projects into the developing head region of the embryo surrounded by the mesoderm layer. This is the primitive digestive tube. The pancreas is specified by two separate outgrowths that arise on the dorsal and ventral surfaces of the primitive digestive tube. The epithelial cells of the pancreas originate from the interior lining of the primitive gut tube, which consists of a single layer of endoderm. A layer of mesenchyme, from which the muscle and

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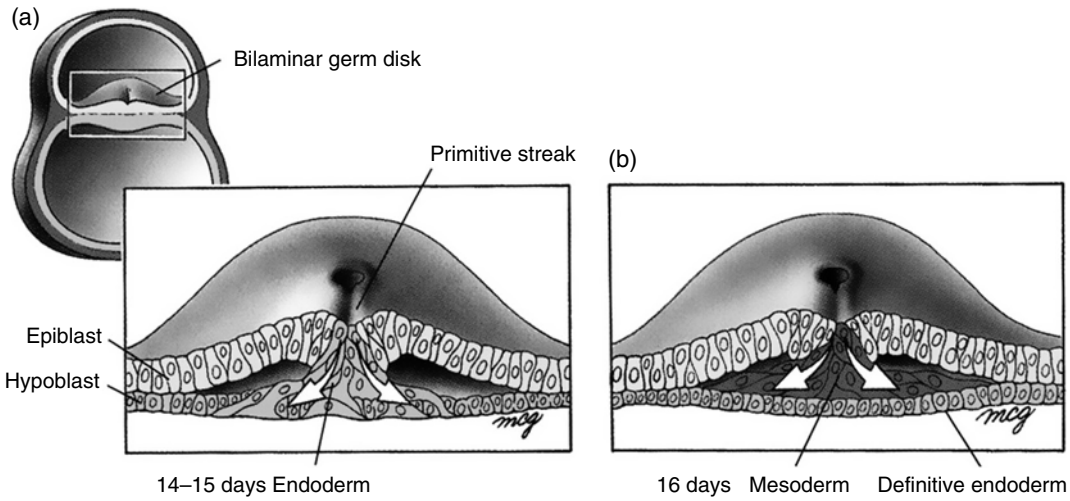

Figure 1.1 Germ disks sectioned through the region of the primitive streak, showing gastrulation. (a) On days 14 and 15, the ingressing epiblast cells replace the hypoblast to form the definitive endoderm. (b) The epiblast that ingresses on day 16 migrates between the endoderm and epiblast layers to form the intraembryonic mesoderm. *Source:* Larsen 2001 [1]. Reproduced with permission of Elsevier.

connective tissue of the gastrointestinal organs are derived, surrounds the endoderm.

The anterior regions of the endoderm form the foregut; regions posterior to the foregut form the midgut and hindgut. The most anterior regions of the foregut give rise to the esophagus and stomach. Just posterior to the foregut, the endoderm is continuous with the yolk sac, which extends outside the embryo, in a region known as the anterior intestinal portal. Endodermally derived cells close to the anterior intestinal portal specify the pancreas. The duodenum and liver are also specified by foregut endoderm in this region.

Thus, many gastrointestinal tissues are specified at the same time from a fairly restricted region of the gut endoderm. How are each of these organs specified in the appropriate anatomic location, and how do they differentiate properly into mature functional organs? The epithelial organs of the developing embryo originate as buds from the endoderm as the appropriate temporal and spatial cues are received. Thus, proper initiation and location of endodermally derived organs are regulated by the activation status of important signal transduction pathways involved in animal development, including the hedgehog, Notch, and fibroblast growth factor (FGF) signaling pathways.

Early Pancreatic Development

During the fourth week of gestation, two buds appear on the dorsal and ventral sides of the foregut near the anterior intestinal portal. These epithelial buds indicate the specification of the pancreas. These buds initially grow and differentiate independently, but later fuse to form a single organ. The anlage on the dorsal side, the dorsal pancreatic bud, appears first and gives rise to the dorsal pancreas. The cells of the dorsal pancreas will give rise to the head, body, and tail of the mature pancreas. The second pancreatic anlage appears shortly after the appearance of the dorsal pancreatic bud. This bud, which appears on the ventral side of the gut tube, is appropriately called the ventral pancreatic bud and develops into the ventral pancreas, which forms part of the head of the pancreas. Both pancreatic buds develop simultaneously, and the proliferating epithelial cells grow as projections into the surrounding mesenchymal tissue. During this time, the development of the intestines, and importantly the duodenum, continues. Rotation and asymmetric growth of the duodenum move the originally ventral part to a dorsal location, carrying with it the ventral pancreas and the primordial common bile duct. As the duodenum begins to rotate into its appropriate anatomic location, the ventral pancreas also rotates around the gut tube such that the ventral and dorsal pancreata lie adjacent to each other. These pancreatic rudiments then fuse to form a single organ. While both developing pancreatic buds independently form pancreatic ducts, the lumens of which are continuous with the lumen of the primitive gut, after they fuse their primary ducts anastomose to form the main pancreatic duct (Fig. 1.2). The region of the primary duct of the ventral pancreas proximal to the duodenum fuses with the primary duct of the dorsal pancreas and becomes the primary drainage into the duodenum, entering the duodenum immediately adjacent to the common bile duct. The proximal region of the primary duct of the dorsal pancreas sometimes

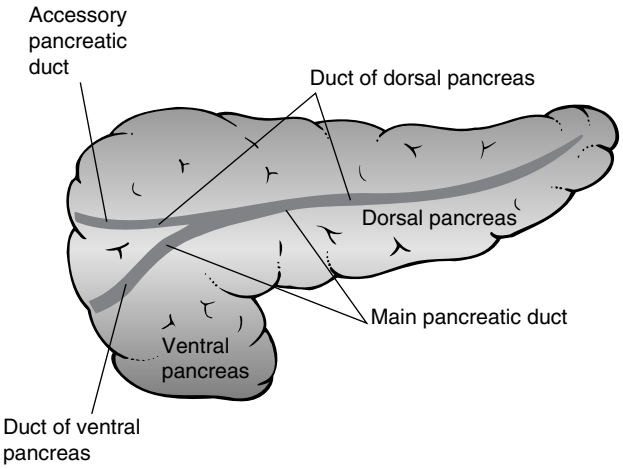

Figure 1.2 Contributions of the dorsal and ventral pancreas to the definitive organ. The ventral pancreas becomes most of the head. The dorsal pancreas becomes the remainder of the head, plus the body and tail. The duct of the dorsal pancreas contributes a large part of the main pancreatic duct plus the accessory duct. The duct of the ventral pancreas becomes the part of the main duct nearest the duodenum.

remains as an accessory drainage but often regresses. The ducts sometimes fail to fuse, in which event two independent duct systems drain into the duodenum.

Signaling Governing Early Pancreatic Development

Early pancreatic development and establishing pancreatic identity are governed by the interplay between several critical transcription factors and intercellular signaling pathways. PDX1 and PTF1A are among the earliest transcription factors expressed in the pancreatic progenitor populations, and their functions are critical for pancreatic development [2–5]. In mice, PDX1 expression is first detected in the primitive gut tube at embryonic day 8.5 (E8.5), which corresponds to ~25–27 days in humans. PDX1 expression demarcates the prospective pancreatic domain, which is then followed by PTF1A expression in pancreatic endoderm at E9.5 [5–7]. Mice lacking either transcription factor display pancreatic agenesis [2,3,5,8].

In addition to the transcription factors, several key intercellular signaling pathways between gut endoderm and mesenchyme, including the hedgehog, FGF, Notch and Hippo pathways, play important roles in establishing the pancreatic identity and controlling the expression of these transcription factors. Research studies have shown that sonic hedgehog (SHH), the ligand of the hedgehog pathway, is excluded from the prospective pancreatic region, but is present in the region of foregut that becomes the duodenum, and ectopic expression of SHH in the pancreas induces an intestinal fate, suggesting that SHH signaling may specify a duodenal versus pancreatic fate in the posterior foregut [9,10]. Another well-understood pathway mediating the mesenchymal–epithelial interaction is the FGF signaling pathway, in particular the FGF10–FGFR2 ligand-receptor pair. During early pancreatic development, FGF10 is highly expressed in the primitive mesenchyme, whereas its receptor FGFR2 is present in the pancreatic epithelium [11]. Mouse genetic experiments demonstrated that FGF10 provides the pro-proliferative signal to promote the expansion of the progenitor pool in the pancreatic epithelium [11]. In addition, FGF10 signaling from the mesenchymal cells is critical for maintaining the epithelial expression of SOX9 [12]. SOX9 is another transcription factor critical for early pancreatic development, and it exerts its function in part by controlling the expression of the FGF10 receptor FGFR2 [12,13]. Together, the complex regulatory loop between these signaling pathways and transcription factors in the epithelium and mesenchyme coordinates early organ growth and the establishment and maintenance of pancreatic identity.

Differentiation of Pancreas Cell Types

The acinar, ductal, and endocrine cells of the pancreas are all produced through the proliferation and differentiation of the epithelial cells of both pancreas primordia. The cells appear homogeneous during the early stages of development as they proliferate and grow into the surrounding mesenchyme as finger-like projections. The epithelial cells form undifferentiated tubules that branch and anastomose as they penetrate into the mesenchyme to generate a tubular network, which resembles an immature (and nonfunctional) duct system. The acinar cells appear as clusters of cells at the ends of branches of this tubular network. The endocrine cells appear as cells that delaminate from the tubular epithelium and reaggregate in isolated clusters embedded within the developing parenchyma. The existing cells within these small isolated endocrine clusters proliferate, and these clusters therefore expand to form the islets.

Apparent differentiation of pancreas epithelial cells into endocrine cells can be identified beginning at 12 weeks of gestation with the detection of endocrine granules. Most of the endocrine differentiated cells identified at this time express glucagon and are therefore believed to be α cells. Importantly, lineage-tracing experiments performed in mice demonstrated that these early α cells do not act as endocrine progenitors, as β cells, the predominant cell type in the mature islet, are derived from glucagon-negative cells [14]. Differentiation of

acinar cells is detected at approximately 16 weeks, as identified by the appearance of zymogen granules. Interestingly, not all enzymes are elaborated at once—detection of trypsinogen does not occur until approximately 22 weeks. The digestive enzyme-positive cells arise as clusters from the undifferentiated tubules, the expansion of which is rapid such that the acinar cells become the dominant population within the organ. Although they are not yet mature acinar cells, the cells in the acinar clusters display some of their hallmark features, including basolaterally located nuclei. As differentiation continues, the cells become arranged in recognized acini and defined lobules surrounded by connective tissue. The ductal system arises after maturation of the immature tubular network. The specific morphologic changes that accompany this change are unclear, although some work suggests that Wnt signaling is involved in this transition [15].

Transcriptional Mechanisms Underlying Pancreatic Cell Fate Decision

Much information about pancreatic cell fate determination and cell type differentiation has been obtained from studies in animal models. Elegant genetic and cell-based experiments in mice have identified a gene regulatory network controlled by many transcription factors to specify different cell lineages in the developing pancreas.

Development of the Endocrine Lineage

Endocrine cell specification begins with the expression of NGN3, a bHLH (basic helix–loop–helix) transcription factor, in a subset of progenitor cells within the trunk region of the pancreatic bud [16–18]. The NGN3-expressing cells eventually give rise to all endocrine cell types: insulin-producing β cells, glucagon-producing α cells, somatostatin-producing δ cells, ghrelin-producing ε cells, and pancreatic polypeptide-producing PP cells [16–18]. NGN3 initiates endocrine lineage specification by inducing the expression of downstream transcription factors, including NeuroD, NKX2.2, PAX4, and ARX. Among them, NKX2.2, NeuroD, and PAX4 play key roles in the specification of β cells [19–21]. Mutant mice lacking any of these transcription factors display a phenotype of dramatic or total loss of β cells [19–21]. Further studies revealed that the opposing actions of PAX4 and ARX determine the fate choice between α and β cells. During endocrine differentiation, loss of ARX leads to a complete loss of α cells, but a concomitant increase in β and δ cells [22], whereas loss of PAX4 results in an opposite phenotype with loss of β and δ cells and expansion of α cells [20,22]. It is believed that this effect on cell fate choice is mediated by the reciprocal transcriptional repression between these factors.

Differentiation of Acinar Cells

Pancreatic acinar cells are primarily derived from precursor cells in the tip region, and their differentiation is coordinated by the transcription factor PTF1A, a master regulator of pancreatic development. Prior to exocrine differentiation, PTF1A forms a complex with the bHLH transcription factor RBP-Jk, and is required for activation of RBP-Jl, an acinar-specific paralog of RBP-Jk [23,24]. The more active RBP-Jl then replaces RBP-Jk to form the complex with PTF1A, thereby directly inducing the expression of many acinar-specific genes, including secretory peptides and digestive enzymes [23,24]. Interestingly, PDX1, another factor important for early pancreatic morphogenesis, is also involved in acinar differentiation. Although not essential for initial acinar specification, it appears that PDX1 is required for terminal differentiation of acinar cells [25]. Other transcription factors, such as NR5A2 and MIST1, are also required for acinar differentiation and homeostasis, likely through the interaction with the PTF1A/RBP-Jk/l complex [26,27].

Ductal Cell Differentiation and Lineage Plasticity

During development, NGN3-positive cells in the trunk region of the pancreatic bud give rise to endocrine cells, whereas NGN3-negative trunk epithelial cells contribute to the ductal system [28,29]. A number of transcription factors, such as SOX9, PROX1, HES1, and HNF6, are expressed in the ductal lineage and play various roles in ductal differentiation, including primary cilia formation in the ductal epithelial cells [30–33]. The Notch signaling pathway is the main determinant for promoting and maintaining the ductal cell identity [31]. Although the three lineages (endocrine, exocrine, and ductal) are specified during early development, the adult pancreatic cells from different lineages show remarkable plasticity and trans-differentiation capacity in pancreatic injury, pancreatitis, and tumorigenesis, which may shed light on the mechanisms underlying these pancreatic pathologies.

Development and Disease

Molecules important in the development of the pancreas are also causally associated with pancreatic disorders. Several of the signaling pathways involved in normal

pancreas development, such as the Notch, hedgehog, Hippo/YAP and Wnt signaling pathways, are commonly dysregulated in pancreatic ductal adenocarcinomas [34–40]. Aberrant activation of Wnt signaling drives the development of other pancreatic tumor types such as acinar carcinomas, pancreatoblastoma, and mucinous cystic neoplasms [41–43].

In diabetes, mutation of the transcription factor PDX1, which is important for pancreas specification and for proper β-cell maturation and function, is a cause of maturity-onset diabetes of the young (MODY) [44]. Other transcription factors that are critical for β-cell development (as determined by genetic studies in the mouse), such as hepatocyte nuclear factor 1α (HNF1α), HNF1β, HNF4α, and NeuroD, are all also mutated in additional MODY complementation groups [44]. More recently, scientists have utilized our growing understanding of normal pancreas development to promote the differentiation of induced pluripotent stem cells into insulin-producing cells in a new potential therapeutic approach for diabetes [45–47].

Collectively, these findings illustrate the importance of key regulators of pancreas development and differentiation in pathologic disease states and how knowledge of normal pancreas development may drive new therapeutic strategies for pancreatic diseases.

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