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Molecular Biology of Pancreatic Ductal Adenocarcinoma Progression: Aberrant Activation of Developmental Pathways

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Abstract

Embryonic development marks a period of peak tissue growth and morphogenesis in the mammalian lifecycle. Many of the pathways that underlie cell proliferation and movement are relatively quiescent in adult animals but become reactivated during carcinogenesis. This phenomenon has been particularly well documented in pancreatic cancer, where detailed genetic studies and a robust mouse model have permitted investigators to test the role of various developmental signals in cancer progression. In this chapter, we review current knowledge regarding the signaling pathways that act during pancreatic development and the evidence that the reactivation of developmentally important signals is critical for the pathogenesis of this treatment-refractory malignancy.

I. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the 10th most prevalent cancer in the United States, with an estimated incidence of 42,000 people in 2009,¹ and it is the fourth most common cause of cancer-related death. The prognosis of PDAC is poor, with only approximately 5% of patients surviving 5 years after diagnosis. The vast majority of PDAC patients die from complications of meta-static disease. Even patients who undergo pancreas resection for limited disease with no clinical evidence of metastasis have poor prognosis, as approximately 80% of these patients will also succumb to metastatic disease.² Thus, while cancers of all origins share many of the same hallmarks,³ PDAC is unique in its ability to form large tumors and metastasize, ultimately killing the host and circumventing treatments that are efficacious in other malignancies. In order to intervene in this particularly lethal cancer, it will be essential to understand the unique features of pancreatic biology and the accumulation of molecular events that are seen exclusively in pancreatic cancer. We will begin with a brief review of the major themes of pancreas development and adult exocrine pancreas biology. Then, we will review the molecular underpinnings of PDAC, highlighting the reemergence of developmental pathways during cancer progression (Table I).

II. Anatomy of the Exocrine Pancreas

The pancreas is an endoderm-derived organ located in the upper abdomen of vertebrates, adjacent to the stomach, duodenum, and spleen. It maintains an independent blood supply from other abdominal organs. It also features a connection to the intestines via the main pancreatic duct, which serves to empty secretions from the pancreas into the intestinal lumen.

The pancreas comprises two compartments with distinct functions. The endocrine pancreas functions as a central regulator of glucose and metabolic homeostasis. Cells that compose the endocrine pancreas are organized within structures called the Islets of Langerhans (Fig. 1). Islets of Langerhans are nestled within the pancreatic parenchyma, often intimately associated with blood vessels. While islets are scattered throughout the pancreas, they are densely concentrated in the tail. Within these islets, α -, β -, δ -, γ -, and PP-cells secrete the hormones glucagon, insulin, somatostatin, ghrelin, and pancreatic polypeptide, respectively, in response to metabolic and chemical cues from the local blood supply. While carcinomas of the endocrine pancreas occur, these are relatively rare, and are beyond the scope of this chapter.

The exocrine pancreas, from which PDAC arises, aids in the digestion of carbohydrates, fats, and proteins. Composing more than 90% of the organ, the exocrine compartment contains acinar and centroacinar cells interconnected by an elaborate epithelial-lined ductal network (Fig. 1). Acinar cells form discrete units, appropriately called acini, which, in cross-section, represent a circular cluster of polarized cells organized around a small concentric lumen. The apical portions of acinar cells face the middle of these acinar units, adjacent to the centrally positioned centroacinar cells. In response to the ingestion of food or drink, the upper intestine releases secretin and cholecystokinin, leading acinar cells to secrete inactive forms of digestive enzymes called zymogens. Zymogens undergo activation in an acidic environment; hence, concomitant release of bicarbonate by ductal cells maintains an alkaline microenvironment and prevents activation of digestive enzymes prior to exiting the pancreas. The lumens of the acinar units drain into a complex, interconnected network of epithelium-lined ducts which then anastomose to the main pancreatic duct, which in turn traverses the length of the pancreas. In most vertebrates, the main pancreatic duct represents the major conduit of drainage of pancreatic juice. The pancreatic duct then connects with the common bile duct, and pancreatic juice enters the intestine through the ampulla of Vater, located in the second portion of the duodenum.

Intercalated within the pancreatic parenchyma are mesenchyme-derived stromal cells, composed mostly of endothelial cells and quiescent fibroblasts. In non-diseased states, the stroma represents less than 1% of the pancreas. These cells are thought to primarily support the normal functioning of the epithelium by maintaining the intercellular structure of the organ. However, accumulating evidence has implicated pancreatic fibroblasts—especially the stellate cell, a specialized fibroblast—in additional tasks important for the normal functioning of the pancreas, including storage of Vitamin A, immune system regulation and surveillance, and maintenance of endothelial cell turnover.^{4,5} A complex, bidirectional interplay of signals between the stromal and epithelial compartments coordinates homeostasis within the organ. As will be discussed later, upon injury or carcinogenesis, signaling pathways active during pancreas development are reactivated, leading to the activation, accumulation, and diversification of the stromal compartment.

III. Development of the Exocrine Pancreas

Many cancers share common characteristics, as outlined previously³; however, each type of cancer is unique in its tissue of origin and developmental history. It is widely believed that the molecular events leading to carcinoma differ by tissue type and even by the specific cell of origin within a single organ. Consistent with these observations is the concept that key events leading to carcinogenesis involve the abnormal activation of developmental pathways specific to the ontogeny of the source or index cell.⁶ Indeed, recent work has supported the notion that this may be the case for PDAC. Thus, much insight into how PDAC forms has been attained by the assiduous study of normal exocrine pancreas development and mechanisms. Our understanding of early pancreas development comes from detailed studies

using the mouse as a model system. By employing a variety of techniques, most importantly lineage labeling technology, investigators have been able to describe many of the morphogenic and genetic events involved in pancreas formation.

A. Early Steps Preceding Pancreatic Budding

Regional specification of the pancreas anlagen occurs as early as gastrulation. In studies of chicken development, fibroblast growth factor 4 (FGF4) released by the mesectoderm enables a region of the mesoderm to become responsive to propancreatic signals secreted by the mesoderm-derived notochord. Later in gastrulation, retinoic acid (RA) and bone morphogenic proteins (BMPs) are released, similarly allowing for the pancreatic anlagen to respond to pancreas-forming cues later in development.^{7,8} The pancreas derives from two primordia located in the dorsal and ventral aspects of the endoderm-derived primitive gut tube, forming distinct pancreatic buds that will eventually fuse. Upon completion of gastrulation, a patch of dorsal midgut endoderm finds itself in contact with the notochord through embryonic day 8 in the mouse (E8.0), eventually giving rise to the dorsal pancreas.

Precursors for the ventral pancreas are positioned adjacent to the lateral plate mesoderm. This geography leads to intimate interactions between the mesoderm and endoderm that are required for pancreas organogenesis.

B. Signals from the Notochord

The mesoderm-derived notochord is required for the eventual development of the dorsal pancreas.^{9,10} Excision of this structure at the 8–9-somite stage leads to abolition of subsequent expression of pancreatic marker genes, including pancreas and duodenal homeobox-1 (Pdx-1; Ref. 11). Several soluble notochord-derived factors have been implicated in pancreas genesis. The best characterized of these factors, activin- β B, a member of the transforming growth factor β (TGF- β) family, and FGF2, underscore the complexity of mesenchymal–endodermal interactions.^{12–14} Culture of the prepancreatic endoderm at the 9-somite stage with these factors was shown to be sufficient to induce the expression of pancreas-specific markers in the absence of notochord.¹⁵ In these experiments, physiologic levels of activin- β B and FGF2 both led to suppression of endodermal Sonic Hedgehog (Shh) ligand, a key event in pancreatic specification (see below). Indeed, in embryos at this stage cultured *in vitro*, dorsal Shh expression was repressed when the notochord remained intact, but this repression was lost upon excision of the notochord, resulting in dorsal pancreas agenesis. These observations suggest that patterning at the gastrulation stage leads to the geographic specification of the future dorsal pancreas via the silencing of Shh expression.

Activin and FGF signaling lead to a cascade of events in the pancreatic endoderm. Intracellular signaling through these pathways serves to inhibit Shh expression by these cells, thereby inhibiting Hedgehog signaling through the Gli family of transcription factors.^{16,17} These events are integral for the early expression of Pdx-1 at E8.5 (all developmental stages refer to the situation in mice unless otherwise noted). Pdx-1 is the first pancreatic transcription factor to be expressed, and it marks the dorsal and ventral pancreatic buds.^{18,19} Indeed, ectopic expression of Shh leads to loss of Pdx-1 expression in the pancreatic anlage. In a correlative experiment, Patched-null mice, in which Hedgehog signaling is permanently activated, die at E9.5 with no expression of Pdx-1 in the pancreatic anlage.^{15,17} Expression of Pdx-1 is critical for early events in pancreas formation, as Pdx-1 null mice have arrested pancreas development at the bud stage.^{18–20} FGF-receptor signaling in the endoderm also activates Notch signaling in the pancreatic endoderm at around E8–8.5^{21,22} which may also play a role in the induction of Pdx-1 expression.

C. Early Signals from the Aortic Endothelium

Midline fusion of the dorsal aorta occurs at E8.5, leading to the interposition of the aorta between the notochord and the endoderm. Work by Melton and colleagues showed that endothelial cells from the aorta are required for dorsal budding as well as later endocrine differentiation.²³ In fact, removal of the dorsal aortae in *Xenopus* embryos before fusion led to the lack of dorsal pancreas bud formation, presumably by preventing the induction of Pdx-1-expressing cells in the dorsal anlage.^{23,24} This observation led to the implication of vascular endothelial growth factor A (VEGF-A) as a factor involved in the specification of the developing pancreas. FGF10 is also released by the merged aortic endothelium and acts on the endoderm to promote the expression of Ptf1a, a key transcription factor necessary for normal pancreas development.^{25–27}

D. Interactions with the Pancreatic Mesenchyme

After fusing of the aorta, the pancreatic mesenchyme becomes the primary signaling partner of the developing pancreatic endoderm. The early embryonic mesenchyme is thought to elaborate numerous signals that lead to the careful orchestration of important molecular events in the developing pancreas. As a direct result of midline aorta fusion, contact between the mesoderm-derived notochord and the underlying endoderm is disrupted, followed by condensation and brisk expansion of the surrounding mesenchyme around what will become the dorsal pancreas at approximately E9.0–9.5.^{9,28,29} Analogous events occur in the area of the ventral pancreas a short time thereafter. Subsequently, evagination of the pancreatic buds takes place, followed by elongation. It is at this point in time that mesenchymal proliferation rates are at their highest.³⁰ The apical regions of the buds then undergo branching morphogenesis, forming outgrowths at acute angles, unique to pancreatic development. The sharp angles lead to the close approximation of the epithelium at branch points, with comparatively little mesenchyme between the basal ends of the branching epithelia, leading to the first signs of asymmetric distribution of mesenchyme within the developing pancreas. As will be discussed below, this redistribution of mesenchyme likely impacts the signals to the epithelia, affecting lineage decisions later in development. Fusion of the dorsal and ventral buds begins around E13 of mouse development, whereupon the cellular architecture of the pancreas starts to mature, and cells of the exocrine and endocrine lineage differentiate and organize accordingly. Throughout these stages of development, complex and diverse molecular interactions between the mesenchyme and the pancreatic epithelium take place. The careful geographic and time-dependent orchestration of epithelial–mesenchymal interactions has been well studied. However, given the complexity of these interactions, much is still unknown.

E. Key Signaling Pathways Involved in Exocrine Pancreas Development

Not surprisingly, a host of signal transduction pathways are utilized to form the pancreas. Because of space constraints, we will focus on pathways prominent in both exocrine pancreas development and in the progression of PDAC.

1. HEDGEHOG SIGNALING—Hedgehog signaling occurs when a soluble ligand is secreted by a neighboring cell (Fig. 2). Three ligands are known to exist in mammals: Shh, Indian Hedgehog, and Desert Hedgehog. While each features different expression patterns and chemical structures, all elicit similar downstream effects as they bind to the same receptors.³¹ In the absence of ligand, the Patched receptor (Ptch) inhibits the activity of the G-coupled receptor protein Smoothed (Smo). Upon ligand binding, Smo dissociates from Ptch. Liberated Smo then initiates a cascade of events leading to the translocation of Gli transcription factors into the nucleus.

As mentioned previously, repression of the Hedgehog signaling pathway is required for pancreas development. Forced expression of Shh in Pdx-1-expressing cells after E9.5 leads to loss of Pdx-1 expression in the dorsal pancreatic anlage and pancreatic agenesis, while ectopic expression of Shh later in pancreas development (E12.5) leads to the expected loss in pancreatic mass but also to expansion of the mesenchyme.³² In the intestine, Shh induces the adjacent mesenchyme to differentiate into smooth muscle¹⁶; hence, the absence of smooth muscle in the pancreas may be due to Shh repression at the earliest stages of development. The observation that epithelial-to-mesenchymal Hedgehog signaling, normally silenced during development, can induce stromal proliferation in the pancreas has provided clues about the mechanism of stromal expansion during pancreatic carcinogenesis. Interestingly, repression of Hedgehog signaling does not seem to be required for budding and development of the ventral pancreas.

2. FGF SIGNALING—The fibroblast growth factors comprise a group of heparin-binding proteins that share similar structures.³³ These factors are involved in a variety of functions, including angiogenesis and mitogenesis. There are 14 FGFs known with varying expression domains and targets. FGFs mediate their actions after binding to one of four FGF receptors (FGFRs) on the target cell. FGFRs are subject extensive alternative splicing and glycosylation, resulting in a multitude of affinities and specificities for various FGF ligands. Binding of FGFR leads to the activation of a number of signal transduction pathways via tyrosine phosphorylation.³⁴ Multiple pathways are activated by FGFRs, including (1) the Ras-Raf1-MEK-MAPK pathway; (2) the phosphatidylinositol-3 kinase (PI3K) pathway; and (3) the phospholipase-C-gamma (PLC2 γ)-phosphatidylinositol 4, 5-bisphosphate (PIP2) axis. These signaling cascades ultimately result in proliferative changes and effects on cellular differentiation.

Endoderm-to-mesenchymal FGF signaling plays an important role in pancreatic development. FGF4 elaborated by the pancreatic anlage, probably in concert with mesenchymal-derived FGF, serves to delineate the developing pancreatic mesenchyme early in development.^{35,36} Without this key molecular signal to the regional mesenchyme, the proper formation of the endoderm-derived pancreas does not take place.³⁶ Later in development, once the pancreatic mesenchyme has undergone asymmetric distribution, FGF4 can cause the mesenchyme to persist, thereby enhancing exocrine differentiation of the adjacent epithelium.³⁷

Several studies, including pioneering work from the Scharfman laboratory, have shown that mesenchyme-derived FGFs play a vital role in pancreas development. FGF1, 4, 7, 10 are all expressed by the pancreatic mesenchyme during development.³⁸ These ligands bind to the dominant FGFR expressed in the pancreatic epithelium, FGFR2B.³⁹ The first sign of mesenchymal-to-epithelial FGF signaling occurs at approximately E9.0, upon fusion of the aortic rings. FGF10 produced by the newly fused aorta leads to transcriptional changes in the dorsal pancreatic bud, resulting in the expression of pancreas-specific markers. FGFs elaborated from the cardiogenic mesenchyme similarly induce the expression of pancreatic markers in the ventral pancreas.⁴⁰ FGF1, 7, and 10 signaling throughout the development of the pancreas promotes pancreatic epithelial proliferation, without eliciting cellular differentiation^{38,41–43} before E16. Later in development, these mesenchymal factors are thought to guide pancreatic cells toward the exocrine lineage and inhibit endocrine differentiation by constraining the proliferation of endocrine progenitors.^{37,44} Finally, it has been shown that FGF signaling can lead to Notch pathway activation in the pancreatic epithelium, which will be elaborated upon below.^{22,45} Later in development, signaling through FGFR3 inhibits endocrine differentiation and islet formation, possibly via the Notch pathway.⁴⁶

3. NOTCH SIGNALING—The Notch pathway has been implicated in many important basic events during mammalian organogenesis, including the delineation of tissue borders, the determination of cell fate, and differentiation within an organ.^{47–49} Signaling occurs when one of four receptors (Notch 1–4) binds to one of five known Notch ligands (Delta-like 1, 3, 4, and Jagged1, 2) expressed by an adjacent cell (Fig. 3). A series of proteolytic cleavages then occurs near the transmembrane domain of the Notch receptor, culminating in the release of Notch intracellular domain (NICD) by γ -secretase. NICD translocates to the nucleus and complexes with the transcription factor RBPJK, switching it from a transcriptional repressor to activator. This leads to the transcription of select basic helix-loop-helix proteins, including hairy/enhancer of split 1 (Hes1), which mediate the downstream effects of Notch.

In specific reference to signals arising from mesenchymal–epithelial interactions, the Notch pathway is indirectly activated by paracrine FGF signaling. Mesenchymal FGF ligands bind to FGFR2B on the pancreatic epithelium during development. Through elegant transgenic experiments, Notch signaling was shown to be activated by FGFR2B signaling, culminating in an increase in the expression of the Notch effector molecule Hes1.^{22,36} Other downstream effects of Notch signaling include the downregulation of p57, a cyclin-dependent kinase inhibitor, and effects on the Ptf1a transcriptional complex.^{25,50–52} Later in development, mesenchymal-to-epithelial FGF signaling tilts the scales in favor of exocrine differentiation. After the appearance of mature pancreatic buds, there is a decline of FGF and Notch signaling in the developing exocrine pancreas,⁵³ concurrent with the initiation of acinar differentiation.

It is thought that the bulk of Notch signaling in the pancreas occurs between epithelial cells, in a process commonly known as lateral inhibition. Several studies in mice have demonstrated that epithelial Notch signaling is critical for cell fate determination within the pancreas. But, as with most signaling pathways during development, the dynamics of lineage specification through Notch signaling is complex. Early in development, Hes1 expression serves to maintain cells in a progenitor state by inhibiting the expression of neurogenin3 (ngn3; Refs. 54,55;). Later in development, however, a shift in the glycosylation of the Notch receptor in a subpopulation of pancreatic progenitor cells leads to the induction of ngn3 expression, resulting in endocrine differentiation.⁵⁶ The timing of Ngn3 induction (secondary to repression of Notch signaling) may be the critical factor that determines endocrine cell fate.⁵⁷ In addition, it was shown by conditional gene ablation that Jagged1, the dominant Notch ligand in the pancreas, can act as a competitive inhibitor of Notch signaling in the embryonic pancreas, while postnatally, it has its expected role as pathway activator.⁵⁸ Notch signaling is thought to be persistently active in adult centroacinar cells, the terminal ductal epithelial cells found abutting the acinus.⁵⁹ However, the precise lineage of these cells is unknown. Because Notch signaling is apparently quiescent in mature exocrine cells, the centroacinar cell has been theorized to be a rare progenitor cell present in the adult pancreas⁶⁰ and a candidate cell of origin for PDAC.⁶¹

4. TGF- β SIGNALING—The TGF- β signaling family represents a group of diverse and versatile pathway constituents that play important roles in a number of biological processes, including embryonic development, extracellular matrix production, and apoptosis. For a more in-depth review of this pathway in pancreas development, the reader is referred to two comprehensive reviews in the subject.^{62,63} Signaling is first initiated when a ligand from the TGF- β superfamily binds to a specific receptor. There are six types of ligands which, upon receptor engagement, result in unique downstream effects. Four of these play active roles in pancreas development and have been implicated in PDAC progression: (i) TGF- β family ligands, (ii) activins, (iii) BMPs, and (iv) growth differentiation factors (GDFs). Upon binding of ligand to a serine–threonine kinase receptor, the kinase domains undergo

conformational change allowing for phosphorylation of receptor-regulated SMADs, intracellular proteins that shuttle from the plasma membrane to the nucleus.

The TGF- β family of ligands plays a key role during pancreas development, as well as mediating terminal differentiation events. All three TGF- β isoforms (TGF- β 1, 2, and 3) are expressed in the embryonic pancreatic epithelium as early as E12.5.^{64,65} The mesenchyme does not express this ligand, and, during early exocrine differentiation, the major type II receptor for this ligand, TGFBR2, is exclusively located in the epithelium.⁶⁴ As acinar cells are first specified, TGF- β ligand expression becomes sequestered in this compartment.^{64,66} By E18.5, TGF- β 2 expression is found only in the pancreatic ducts. These data suggest that in later development, acinar-to-ductal TGF- β signaling coordinates the precise formation of the ductal network. This notion was confirmed when mice expressing a dominant-negative form of TGF- β 2 displayed aberrant redundant pancreatic ducts.⁶⁷

The two activin ligands (Activin A and B) play important roles during pancreas development. One role of Activin B is to promote the specification of the dorsal pancreatic anlage by inhibition of Shh secretion (See Signals from the Notochord). Later in development, however, the expression of these ligands is restricted to cells of the endocrine lineage,^{68,69} and inhibition of Activin signaling is thought to be critical for the formation of the exocrine compartment through the secretion of follistatin, an inhibitor of Activin signaling.⁷⁰ Continued Activin signaling prevents branching morphogenesis.⁷¹ Thus, Activin signaling later in pancreas development provides a general proendocrine and antiexocrine cue. Indeed, exogenous activin supplementation has been used to promote beta cell differentiation in embryonic stem cells.⁷²

BMP signaling is required for early pancreas development, cellular differentiation, and cell growth, though a thorough understanding of the role of this pathway later in development and in the adult pancreas is lacking. The most prominent BMPs expressed in the developing pancreas are BMP 4, 5, 6, and 7. BMPs 4, 5, and 6 are expressed in the pancreatic epithelium, whereas 4, 5, and 7 are expressed by the pancreatic mesenchyme.^{37,73,74} The receptors for these ligands, BMPR1A and B, and BMPR2 are expressed in both pancreatic epithelium and mesenchyme. As discussed earlier, BMP signaling from the mesoderm to the pancreatic anlage is one of the earliest steps in organogenesis. It has been shown recently that later in development, BMP signaling in the mesenchyme is required for proper pancreas size and branching.⁷³ Interestingly, overexpression of BMP in Pdx-1-expressing cells led to pancreas agenesis, presumably due to stromal and smooth muscle hypertrophy in the overlying duodenal anlage.³⁷

GDF11 is thought to be an important factor for acinar cell development. GDF11 is robustly expressed in the pancreas between E11.5 through E13.5⁷⁵ and is selectively expressed by acinar cells afterward. Two GDF11 null mice have been derived with slightly different phenotypes reported. Harmon and colleagues found that lack of this factor led to an expansion of Ngn3+ endocrine progenitor cells. They suggested that this might have been a result of HNF6 induction.⁷⁶ Dichmann and colleagues found that knockout of GDF11 led to reduced pancreas size due to acinar cell loss, although the number of Ngn3+ cells was also elevated.⁷⁷ Taken together, these experiments suggest that GDF11 expression promotes acinar cell survival and may influence endocrine cell fate.

5. RETINOID SIGNALING—Retinoids are a family of Vitamin A analogues that have a multitude of effects on various types of tissue both during development and in mature organs, including anterior/posterior patterning and cell fate decisions. Retinoids are produced by aldehyde dehydrogenase (ALDH). Multiple isoforms of ALDH exist, but the major isoform is ALDH2. Once synthesized, the RA analogues are secreted and bind in a

mostly paracrine manner to heterodimers composed of a retinoic acid receptor (RAR) and a retinoic acid X receptor (RXR). This complex is then endocytosed into the cell where it mediates transcription via binding to retinoic acid response elements (RARE) in the regulatory domains of various genes.⁷⁸

RA signaling has been shown to be critical to the development of both the endocrine and exocrine pancreas, though not as much is known regarding its role in the homeostasis of the adult pancreas. In zebra fish and mice, RALDH2 is expressed specifically by the mesenchyme; RA released by the mesenchyme serves to aid in the specification of the dorsal pancreatic bud.^{79,80} However, RA signaling was not evident in the ventral pancreatic anlage, even though the lateral plate mesoderm expressed RALDH2.⁸¹ Indeed, RALDH2-null mice fail to form a dorsal pancreas. Later in development, mesenchymal RA induces differentiation in insulin-positive cells in zebrafish,⁸⁰ though this has not yet been fully confirmed in mice.^{79,80} With respect to the exocrine pancreas, the Gittes laboratory showed that RA elaborated by undifferentiated exocrine progenitor cells facilitates laminin upregulation in the surrounding mesenchyme, which then acts reciprocally on the epithelium to elicit ductal differentiation.⁸² Thus, RA signaling occurs in a bidirectional manner in the developing pancreas, underscoring the complexity of epithelial–mesenchymal interactions.

6. EPITHELIAL GROWTH FACTOR SIGNALING—The epithelial growth factor (EGF) family comprises more than 30 ligands that can bind to multiple ErbB tyrosine kinase receptors. Several EGF ligands and receptors are expressed by the pancreatic epithelium and mesenchyme, which likely mediate reciprocal epithelial–mesenchymal interactions through the EGF receptor pathway.⁸³ EGF ligands mediate a variety of biological effects, including proliferation and differentiation, depending upon the particular ligand–receptor pair. During development, the EGF receptors ErbB1 and ErbB4 are expressed throughout the pancreas starting at E12.5. After this time point, ErbB1 is expressed mainly in endocrine cells, whereas ErbB4 is relegated to the exocrine compartment. Exogenous EGF led to proliferation of progenitor cells *in vitro* and *in vivo*.^{84,85} Continued exogenous EGF treatment led to enhanced duct development,⁸⁵ while withdrawal after treatment led to preferential endocrine differentiation.⁸⁴

7. WNT SIGNALING—The Wnt signaling pathway controls a wide variety of cellular outputs depending on the identity and concentration of ligand and cell type studied. Signaling is initiated when soluble Wnt ligand binds to the extracellular portion of a member of the Frizzled family of receptors. In turn, a member of the Dishevelled family—in complex with a Frizzled receptor by way of lipoprotein-related peptide (LRP)—becomes activated. This event frees the protein Axin to inhibit a “ β -catenin destruction complex” comprising GSK-3 and APC. This complex catalyzes the phosphorylation of cytoplasmic β -catenin, targeting this protein for proteolytic degradation, though the precise details of this interaction are still under intense scrutiny. When Wnt ligand is engaged with Frizzled, β -catenin can translocate to the nucleus to interact with the TCF/LEF family of transcription factors.

Like some of the aforementioned transduction pathways, Wnt signaling is required for normal pancreas development. Wnt8 has been implicated in early endodermal patterning, and *Xenopus* embryos lacking Wnt8 fail to develop pancreas or liver.⁸⁶ Numerous Wnt ligands, Frizzled receptors and soluble inhibitors are expressed in the developing mouse pancreas,⁸⁷ with ligand expression predominantly located in the mesenchyme and receptors and soluble inhibitors located in both compartments. Perhaps more than for any of the other pathway, the spatiotemporal expression of Wnt family members dictates the result of signaling. A multitude of different Cre lines have been used to perturb Wnt signaling in different tissue compartments at different time points during pancreatic development.

When Grapin-Botton and colleagues deleted β -catenin from pancreatic epithelial cells early in development, the resultant mice exhibited both endocrine and exocrine phenotypes.⁸⁸ Later in development, β -catenin deletion led to almost complete absence of exocrine tissue, though the survival of Pdx-1-expressing progenitors was not affected.^{89,90} Constitutive activation of β -catenin at early time points led to pancreatic agenesis through inhibition of FGF10 and subsequent increase in Shh levels.⁴⁴ Postnatally, β -catenin stabilization in Pdx-1-expressing progenitors resulted in a significant increase in the size of the pancreas, with equal effects on all lineages.⁹¹ Thus, the Wnt/ β -catenin signaling pathway exerts diverse and complex effects during pancreas development depending on location and timing.

IV. Genetic Alterations in PDAC

While PDAC can occur in any individual, regardless of sex, race, or geography, there are certain underlying commonalities to all pancreatic tumors that not only aid in the diagnosis of the disease but also contribute to our evolving understanding of its pathogenesis. PDAC is a cancer of the exocrine pancreas which leads to an aggressive disease in which regions of duct-like epithelium are interspersed with less differentiated epithelial cells contained within a sea of proliferative stroma. Metastasis is extremely common. While most patients will succumb to their disease, the genetic events leading to metastatic spread differ from patient to patient. Nevertheless, a few genes are reproducibly mutated in pancreatic cancer, indicating that certain molecular changes are required for the development of this malignancy.

A. Kras

Kras was the first oncogene to be identified as playing a critical role in PDAC. A number of functions have been proposed for the Kras GTPase, including survival, proliferation, and differentiation.⁹² These diverse activities are driven by Kras-mediated activation of three important signaling cascades that also play prominent roles during pancreas development: the RAF/ERK/MAP kinase, PI3 kinase, and RalGDS pathways.^{93,94} Kras also activates the nuclear factor κ B (NF κ B) pathway, which is implicated in the generation of a rigorous inflammatory response.⁹⁵ Pharmacologic suppression of each of these pathways separately effects pancreatic cancer cell growth *in vitro*, though none have resulted in a durable response *in vivo*. An activating mutation at codon 12 in Kras is detected in close to 100% of all PDACs and in many early neoplastic lesions.^{96–98} With the advent of conditional gene mutation in the mouse, the biology resulting from oncogenic Kras expression has been well documented.⁹⁹ Targeted expression of activated Kras in the pancreatic epithelium promotes the formation of the precursor lesion “pancreatic intraepithelial neoplasia” (PanIN; further discussion below; Ref. 100), supporting the notion that Kras mutations are one of the earliest events leading to PDAC.¹⁰¹

Activating Kras mutations in PDAC exhibit the property of “oncogene addiction,”¹⁰² and three studies have shown that genetic silencing of Kras expression can singlehandedly arrest cancer cell growth.^{103–105} As a consequence, pharmacologic inhibitors of Kras have been rigorously pursued,¹⁰⁶ but to date no clinical efficacy has been detected.¹⁰⁷

Activating Kras mutations are not sufficient for PDAC formation. The observation that Kras mutations are common among patients with chronic pancreatitis supports this notion.¹⁰⁸ While expression of oncogenic Kras in genetic mouse models is not sufficient to produce PDAC, the additional insult of chemical pancreatitis by cerulein elicits PDAC in most mice.^{109,110} This experimental model is consistent with the well-documented 16-fold increased risk for PDAC in patients with chronic pancreatitis over the general population.¹¹¹ However, confounding these experiments is the observation that cerulein and other CCK analogues increase Kras expression independently of any induced inflammatory

response.¹¹² Recent work from the Logsdon laboratory suggests that levels of Kras expression also drive the early stages of pancreatic carcinogenesis.¹¹³ In this elegant study, Ji and colleagues showed that forced expression of supraphysiologic levels of wild-type Kras was sufficient to induce inflammation reminiscent of human chronic pancreatitis and, later, produce PanIN lesions. When combined with loss of the tumor suppressor p53 (see below), these lesions progressed to PDAC. Taken together with the observation that many patients with chronic pancreatitis harbor mutations in Kras, without developing PDAC, these data suggest that increased Kras activity in itself is the key mechanism in PanIN initiation but is not sufficient to produce PDAC.

B. 9p21 Locus: Ink4a and ARF Tumor Suppressors

The second most common genetic events in PDAC are mutations occurring at the Ink4a/ARF tumor suppressor locus, occurring in up to 95% of all tumors.^{98,114} Promoter hypermethylation, mutation, and deletion have all been described as Ink4a-inactivating mechanisms. The sequence for Ink4a actually overlaps that of another tumor suppressor, ARF, in the 9p21 locus.¹¹⁵ While both genes have tumor-suppressing functions, they work independently of each other, and Ink4a loss is thought to be the primary event underlying PDAC.

Ink4a is involved in the initiation of cell senescence in times of environmental stress and aging, as well as in response to telomere erosion.^{116,117} Specifically, Ink4a halts entry into S-phase of the cell cycle by inhibiting CDK4/6-mediated phosphorylation of Rb. Germline mutations in Ink4a are associated with the Familial Atypical Mole-Malignant Melanoma syndrome (FAMMM). Patients with FAMMM display a 13-fold increased risk for developing PDAC.^{118,119} Interestingly, in addition to telomere shortening, high levels of Kras can induce Ink4a expression, leading to senescence.¹²⁰ Thus, Ink4a loss may be required for cells to bypass the senescence phenotype promoted by activated Kras. Loss of Ink4a expression, in combination with the expression of activated oncogenic Kras, leads to rapid development of PDAC in mouse models.¹²¹ Thus, it is likely that in the setting of a mutation in Kras, deactivation of the senescence mechanisms is sufficient to lead to carcinogenesis in the pancreas.

The ARF tumor suppressor has divergent functions from Ink4a. The best studied function of ARF is the inhibition of MDM2-mediated proteolysis of the tumor suppressor p53, thus stabilizing this protein (see below). Indeed, the concentration of p53 protein in the cell is directly proportional to that of ARF.^{98,122} However, the entirety of ARFs functions has yet to be elucidated. Underscoring the presence of additional functions of the ARF tumor suppressor is the observation that mutations in both p53 and ARF are present in almost half of all PDAC.^{114,123} Indeed, tumor growth and mortality is greatly accelerated in genetic mouse models harboring oncogenic Kras and absence of ARF and p53, compared to mice with oncogenic Kras with either protein alone.¹²⁴ Thus, ARF has tumor-suppressing functions independent of its effects of p53 stability.

C. The p53 Tumor Suppressor Protein

p53 serves as a tumor suppressor by mediating DNA checkpoint responses, usually in the setting of an accumulation of mutations from reactive oxygen species and telomere shortening, both of which take place during PanIN progression. Thus, it is not surprising that p53 mutations, usually in the form of missense mutations,⁹⁸ are seen in later stage PanIN lesions.^{123,125} Following p53 loss, it is conceivable that the rate of genetic aberrancy increases drastically, possibly contributing to the immense heterogeneity of pancreatic tumors and their resistance to multiple chemotherapeutic agents.¹²⁶ Even so, p53 is mutated

in only about half of all PDAC cases,⁹⁸ though p53 levels are suppressed in a much greater fraction.¹²⁵

D. SMAD4/DPC Tumor Suppressor

SMAD4 (DPC) is another commonly mutated gene in PDAC, with mutations present in about 50% of all cases.¹²⁷ Similar to p53, loss of SMAD4 usually occurs late in PanIN progression to PDAC.¹²⁸ As touched upon earlier, SMAD4 is an integral member of the TGF- β signaling cascade. The effect of SMAD4 loss in the exocrine pancreas is further discussed below.

E. LKB1/STK11 Tumor Suppressor

The LKB1 protein product is a serine–threonine kinase involved in a multitude of epithelial cell processes, including the regulation of cell metabolism and establishment of cell polarity.¹²⁹ Many of its diverse functions are mediated through the regulation of AMPK, thereby affecting signaling through the mTOR pathway.^{130–132} Germline mutations in LKB1/STK11 have been identified as the cause of Peutz-Jeghers syndrome, in which patients sustain benign intestinal polyposis as teenagers. These patients have a more than 40-fold increased risk of developing PDAC.¹³³ LKB1 is also mutated in approximately 4% of all cases of spontaneous PDAC, though this rate is higher in intraductal papillary mucinous neoplasms.¹³⁴

F. The BRCA2 Tumor Suppressor Protein

BRCA2, similar in function to BRCA1, is involved in the correction of double stranded breaks in DNA. In this way, BRCA2 is critical for the maintenance of genomic stability. Loss of BRCA2 leads to the rapid accumulation of chromosomal aberrations (Venkitaraman, 2002). Patients with BRCA2 mutations are known to be at elevated risk for developing PDAC, and mutations in BRCA2 account for up to one-fifth of all cases of familial PDAC (Murphy, 2002). BRCA2 mutations are also common in sporadic cases of PDAC.

Recently, the “partner and localizer” of BRCA2 (PALB2) was identified as a possible pancreatic cancer susceptibility gene.^{135–137} PALB2, involved in targeting of BRCA2 to the nucleus as well as in BRCA2-mediated repair of double-strand breaks, was initially identified as a susceptibility gene for breast cancer.¹³⁸ Two years later, Jones and colleagues identified three separate mutations in PALB2 in families with familial pancreatic cancer, thus establishing PALB2 as a susceptibility gene for pancreatic cancer as well. While subsequently confirmed,^{136,137} one group was unable to find an association between polymorphisms in PALB2 and increased pancreatic cancer risk.¹³⁹

G. Palladin

Palladin was identified to be mutated in a family with inherited pancreatic cancer.¹⁴⁰ The protein product is relatively large and is thought to act as a scaffold for other proteins to bind actin. Thus, palladin is the first oncogene in PDAC to encode for a cytoskeletal protein.¹⁴¹ Palladin has also been found to be important for cell motility and differentiation.¹⁴² While previous work focused on the role that such a mutation could play in pancreatic cancer cells, later work identified the bulk of palladin to be expressed in the stroma, and, specifically, in cancer-associated fibroblasts (CAFs; Ref. 143). In the setting of PDAC, CAFs were found to overexpress specific isoforms of palladin, not otherwise seen in normal pancreata. These data, combined with the observation that palladin is not consistently upregulated in cancer cells, suggest a novel, but yet unconfirmed, cell-nonautonomous mechanism for carcinogenesis. Thus, further studies dissecting the role of the stroma, and specifically CAFs, in PDAC formation will likely be very insightful.

H. Temporal Accumulation of Signature Mutations During PanIN-to-PDAC Progression

Three neoplastic, noninvasive precursor lesions to PDAC have been identified: (1) IPMN, (2) mucinous cystic neoplasm (MCN), and (3) PanIN. PanINs are the most prevalent and have been identified in up to 16–28% of individuals based on autopsy data.^{144,145} The prevalence and severity of PanIN lesions increases with age, and, as touched upon previously, with certain medical conditions such as chronic pancreatitis.¹⁴⁴ Consensus conferences have classified PanINs according to grades of increasing abnormality (Fig. 4; Refs. 146,147). PanIN1 lesions exhibit flat (PanIN1A) or papillary (PanIN1B) features, though both are characterized as having a columnar appearance with accumulation of mucinous material and nuclei uniformly located along the basal aspects of the cell. PanIN2 lesions are classified by their loss of cellular polarity and some nuclear pleomorphism. PanIN3 lesions display complex and heterogenous structures within a single lesion, including papillary and cribriform-like structures. Nuclei in these lesions are extremely pleiomorphic, randomly oriented, and show obvious mitotic figures.

The molecular basis of PanIN formation has been partially revealed through the creation of genetically engineered models of PDAC, in which PanIN progression can be observed to occur in a step-wise manner, reflecting the accumulation of signature mutations that are also seen in human samples. To a rough approximation, activating mutations in *Kras* and telomere shortening characterize PanIN1 lesions, loss of *Ink4a* correlates with PanIN2 formation, while *p53*, *SMAD4*, *BRCA2*, and *LKB1* loss are associated with the development of late stage PanIN lesions and frank PDAC.^{123,148} Thus, based on available evidence, PanIN progression to PDAC follows a somewhat predictable genetic-histological sequence, similar to the well-described polyp-to-carcinoma progression scheme described for colorectal cancer.¹⁴⁹

Another important trend during tumor development is the dramatic buildup of stroma as PanIN lesions progress to frank carcinoma. Indeed, one of the distinguishing hallmarks of pancreatic cancer is its exuberant desmoplastic response. The stromal infiltrate is composed of a number of cell types, including fibroblasts, macrophages, and endothelial cells. As the interstitium is flooded with these cells, matrix and fibrotic material become highly abundant, leading to significant changes in the physical characteristics of the pancreas itself. As tumors form, hardened nodules arise as a result of local hyperactivation of matrix-secreting stroma adjacent to cells that have undergone malignant transformation. It is likely that this desmoplastic response is required for the accelerated nature of tumor growth and metastasis seen in this disease and that intercompartmental cross-talk through prominent developmental pathways is required for this conditioning of the neoplastic microenvironment.

V. Activation of Developmental Pathways Driving Cancer Progression

Studies utilizing genetically engineered mouse models have revealed that several developmentally relevant signal transduction pathways that are quiescent in the adult pancreas reemerge during cancer progression. The activation of these pathways seems to be shared by the vast majority of pancreatic tumors, in mice and humans.

A. Hedgehog Signaling

While the suppression of Hedgehog signaling is critical for the development of the embryonic pancreas, sustained activation of this pathway is an important contributor to the pathogenesis of PDAC. Thayer and colleagues were the first to describe the aberrant expression of *Shh* ligand, starting in early PanIN lesions and continuing through carcinoma in human tissue specimens,¹⁵⁰ and this finding has been confirmed by others.^{123,148,151} The involvement of the Hedgehog pathway in PDAC is also supported by the work of Jones and colleagues,¹⁵² in which genetic alterations in Hedgehog pathway genes were found in a

large fraction of pancreatic tumors. In a mouse model in which constitutive expression of Hedgehog signaling via Gli2 was combined with an oncogenic mutation in Kras (Kras^{G12D}), PanIN formation and PDAC development were accelerated compared to Kras mutation alone.¹⁵³ Furthermore, Hedgehog signaling was shown to act synergistically with Kras signaling by protecting pancreatic epithelial cells from apoptosis via PI3 kinase signaling.¹⁵¹ Finally, pharmacologic and/or genetic inhibition of Hedgehog signaling attenuates the growth and metastasis in a variety of mouse models of PDAC.^{154–157} Thus, Hedgehog signaling seems to be consistently activated in PDAC, where it plays an important functional role in tumor growth.

The distribution of Hedgehog signaling within the neoplastic pancreas has been a point of controversy, but it is likely that both stromal and epithelial compartments are subject to pathologic Hedgehog signaling in cancer. As stated previously, Hedgehog signaling during development occurs in a paracrine manner whereby epithelially expressed ligand acts upon the mesenchyme. Consistent with this, Shh ligand is exclusively secreted by the epithelial compartment in both PanIN and PDAC lesions.^{150,151,158} Furthermore, *in vivo* reporter assays have shown that “canonical” Hedgehog signals act exclusively in the stromal (mesenchymal) compartment in PanINs and PDACs.¹⁵⁸ These results support the notion that paracrine Hedgehog signaling leads to profound phenotypic changes in the stroma, including diversification and activation of matrix production (Bailey, 2008). Indeed, inhibition of Hedgehog-mediated conditioning of the microenvironment has been associated with the restriction of growth and metastasis in orthotopic transplantation models of PDAC.^{154,156,157}

Even so, other studies suggest that downstream components of the Hedgehog pathway are activated in the neoplastic epithelium as well.^{123,148,150,151,159} Nolan-Stevaux and colleagues showed that genetic depletion of Smo in a genetically engineered mouse model (p48-Cre; LSL-Kras^{G12D/+}; Trp53^{F/+}; Smo^{F/F}) developed PDAC with the same rate and time course as control mice.¹⁶⁰ However, further analysis of the resultant tumors showed that Gli target genes continued to be expressed in ductal structures, suggesting that the downstream mediators of Hedgehog signaling—Gli transcription factors—were activated by a noncanonical, Smo-independent mechanism. Thus, it is likely that downstream components of the Hedgehog pathway are active in both the epithelium and stroma of pancreatic cancers.

An important contribution to our understanding of the role of Hedgehog signaling in the pathogenesis of PDAC comes from the work of Tuveson and colleagues.¹⁵⁷ This group utilized the well-established mouse model of PDAC, the KPC model (Pdx-1-Cre; LSL-Kras^{G12D/+}; Trp53^{F/+}). In this model, tumors were highly impermeable to the delivery of fluorescently tagged drugs; indeed, impermeability may be one reason that pancreatic tumors are so exceptionally refractory to standard chemotherapeutic agents. Remarkably, upon treatment with a small molecule inhibitor of Smo (IPI-926), the stromal compartment reorganized and condensed, leading to increased vascularization. While tumor angiogenesis is commonly viewed as having a protumorigenic effect, in this experimental model the expanded vasculature permitted enhanced delivery of the chemotherapeutic agent gemcitabine, resulting in some tumor cell death. These data provide an important proof of principle that targeting stromal Hedgehog signaling can enhance chemotherapeutic delivery and efficacy in PDAC.

B. FGF Signaling

The FGF pathway is one of the first signaling pathways to be associated with PDAC, beginning with observations from Murray Korc’s laboratory. FGFs are upregulated in approximately 60% of all PDAC samples, and the degree of FGF expression correlates with

advanced tumor stage and shorter patient survival at the time of diagnosis.^{161,162} However, the evidence supporting a functional role for FGFs in PDAC progression comes mainly from *in vitro* studies. PDAC cells express high levels of FGFR3b and FGFR3c, which signal through the MAP- and Jun-kinase pathways to affect changes in cell proliferation, adhesion, and/or motility.^{163–165} The FGFR3b isoform has also been associated with cells that secrete SPARC (secreted protein acidic and rich in cysteine), a molecule that has been implicated in proliferative signals and aggressive phenotypes in PDAC.¹⁶⁶ Moreover, FGF10 signaling through FGFR2 has been shown to induce cancer cell migration and invasiveness.¹⁶⁷ A role for FGF1 and 2 signaling in modulation of epithelial-to-mesenchymal transition (EMT) and motility has also been proposed.¹⁶⁸ It is surprising, given the high levels of FGFs in PDACs, that these tumors are relatively hypovascular, since one of the major effects of FGF is the induction of angiogenesis.¹⁶⁹

C. Notch Signaling

Given its role in maintaining pancreatic progenitor cells in an undifferentiated state, it is not surprising the Notch pathway is active in PanIN progression and PDAC. The first description of Notch pathway activation during pancreatic carcinogenesis came from the laboratory of Steven Leach.⁵⁹ In that study, comparison of 26 human PDAC specimens with 29 normal pancreas samples revealed overexpression of a variety of downstream Notch effectors and upregulation of several Notch receptors and ligands. Many of these findings have since been confirmed in other studies,^{170–173} suggesting that the Notch pathway is broadly activated in PDAC. More recently, global genomic analysis of 24 human pancreatic cancer specimens revealed a high frequency of mutations affecting Notch pathway constituents.¹⁵²

Several studies suggest that Notch promotes PDAC cell growth through autocrine signaling. Repression of Notch signaling in cultured pancreatic cancer cells, either through pharmacologic or genetic inhibition, leads to reduced proliferation *in vitro*.^{59,171,174–176} Notch activation causes pancreatic epithelial cells to adopt a more undifferentiated, duct-like phenotype.⁵⁹ Furthermore, Notch signaling exhibits synergy with the Kras pathway, and the combination of a constitutively active Notch transgene with activated Kras dramatically increased the rate of PanIN formation in genetically engineered mouse models, though these lesions did not progress to PDAC.^{175,177} Finally, a recent study suggests that Notch 2 signaling as a consequence of Jagged1 ligand binding was associated with the acquisition of a mesenchymal phenotype, featuring expression of the EMT markers Zeb1, Slug, and Snail.¹⁷⁶

Given these data, investigators have sought to inhibit Notch signaling as a way to constrain cancer progression in the pancreas.¹⁷⁸ Indeed, using the PKC model of spontaneous PDAC, treatment with a small molecule inhibitor to the gamma-secretase enzyme, an obligatory step in Notch signaling, led to arrest of PanIN progression with no carcinoma detected in any of the treated mice.¹⁷⁴ Similar results have been seen using other gamma-secretase inhibitors in xenograft models.¹⁷¹

D. TGF- β Signaling

The TGF- β signaling pathway is frequently dysregulated in PDAC. Indeed, as discussed above, loss of TGF- β signaling via Smad4 loss is seen in approximately half of all PDAC tumors. Mutations in the TGF- β receptor are present in a number of patients lacking Smad4 mutations, and collectively the vast majority of PDACs exhibit genetic alterations in a component of the TGF- β pathway by global genomic analysis.¹⁵² The general effect of these mutations is the silencing of signals that suppress proliferation and maintain an epithelial phenotype.⁶³

Given space constraints, only a few of the many studies detailing alterations in TGF- β signaling in PDAC will be described here. One of the earliest studies to examine TGF- β signaling in PDAC was performed almost 20 years ago by the laboratory of Murray Korc.¹⁷⁹ Sixty human pancreatic cancers were analyzed, showing that the three TGF- β ligands were expressed in about 40% of all tumors. The expression pattern of the isoforms was found to differ compared to controls with preferential expression of TGF- β 2 by aggressive tumors of advanced stage. The relative expression of TGF- β receptor subtypes was also altered in PDAC compared to controls in another study.¹⁸⁰ PDAC specimens exhibited a fivefold increase in TGF- β R2 transcript, which was expressed in virtually all cancer cells. However, TGF- β R3 was not expressed in cancer cells, but rather, was confined to the stromal compartment. Indeed, later studies showed that TGF- β 1 binding to this receptor induced a particularly aggressive desmoplastic response in a mouse model of PDAC.¹⁸¹ These and other data point to the existence of both paracrine and autocrine TGF- β signaling during PDAC progression.

A number of specific effects have been documented for TGF- β signaling in the cancer cell itself. Recent work has detailed an important role for TGF- β ligand-dependent signaling in the acquisition of a mesenchymal phenotype late in cancer development. Reflecting data seen in colon cancer, TGF- β signaling led to activation of NF- κ B and the downregulation of PTEN, which caused cells to proliferate and acquire motile and invasive properties.^{182,183} The induction of EMT in PDAC cells by TGF- β signaling was also shown to be potentiated by Ras signaling. In elegant biochemical studies, Horiguchi and colleagues revealed that cooperation between the Ras and TGF- β pathways via Smad signaling induces the EMT marker Snail and represses the epithelial marker E-cadherin.¹⁸⁴

However, despite the aforementioned studies, the precise role for TGF- β signaling in PDAC is still quite controversial. The frequent loss of TGF- β signaling components in PDAC suggested that this pathway was acting through a tumor suppressor mechanism. This has been confirmed in studies using genetically engineered mouse models, as loss of Smad4 in mice expressing oncogenic Kras led to rapid development of PDAC through PanIN precursors in the majority of mice.^{185,186} Such results underscore the complexity of the effects mediated the TGF- β pathway, and it is not surprising that many other pathway components have been implicated in the development of pancreatic cancer, including the inhibition of activins,¹⁸⁷⁻¹⁹⁰ increased expression of mitogenic BMPs,¹⁹¹ and the induction of the GDF family member macrophage inhibitory cytokine-1.¹⁹²

E. Retinoic Acid Signaling

Studies of the role of retinoid signaling in PDAC have taken a somewhat circuitous path. Early work suggested that retinoids could have antiproliferative and prodifferentiation effects on PDAC cell lines.¹⁹³⁻¹⁹⁵ Transgenic mice engineered to overexpress cytoplasmic retinoid binding protein (CRBP) exhibited aggressive, poorly differentiated pancreatic cancer that was associated with inhibition of retinoid signaling.¹⁹⁶ These and other studies served as the basis for a number of clinical trials using synthetic retinoids in combination to standard therapy, with results that have not been encouraging.¹⁹⁷

Recent work, however, has prompted a reinterpretation of previous studies of the role of retinoids in PDAC. Rasheed and colleagues examined 269 specimens of human PDAC for expression of ALDH, the rate limiting enzyme in endogenous RA synthesis.¹⁹⁸ In their analysis, patients with tumors harboring ALDH+ cells had a significantly shorter survival. Furthermore, ALDH+ tumors were found to have a greater capacity for clonogenic growth *in vitro*, and ALDH+ cells were found to express classical EMT and stem cell markers. Additional analysis showed that ALDH+ cells were enriched after exposure to chemotherapeutic agents and continued to exhibit tumorigenicity,^{199,200} thus prompting the

authors to label these cells as candidate pancreatic cancer stem cells. While more work is needed to substantiate this claim, it is perplexing that the *de novo* expression of the machinery required for RA synthesis is associated with PDAC progression. Interestingly, recent work from the Leach laboratory identified ALDH1 expression as a marker for centroacinar cells,⁶⁰ a cell that has been theorized not only to be a putative stem cell within the pancreas, but also the cell of origin for PDAC.⁶¹

F. EGF Signaling

EGF ligands (EGF and TNF- α) and receptors (ERBB3 and EGFR) are overexpressed in PDAC cells compared to normal pancreatic epithelium.^{201–203} One mechanism of enhanced EGF signaling within pancreatic tumors stems from the fact that EGF endocytosed by PDAC cells tends to be recycled and secreted as opposed to degraded.²⁰⁴ The high level of expression of both ligands and receptors in PDAC cells would thus permit autocrine EGF signaling. Since EGF signaling is complex and leads to diverse effects, it is hard to make broad statements regarding the effect of signaling through the EGF pathway in PDAC. Nevertheless, multiple studies have shown that pharmacologic inhibition of EGF signaling inhibits cancer cell growth *in vitro* and tumorigenesis in implantation models,^{205,206} in part due to decreased angiogenesis and fibroblast activation.^{205–207}

Interestingly, EGF signaling exhibits significant cross-talk with other pathways that have been implicated in PDAC pathogenesis. For example, transgenic overexpression of the EGF ligand TGF- α can induce acinar-to-ductal metaplasia, resulting in the formation of early PanIN lesions.²⁰⁸ Underlying this phenomenon is the activation of two pathways discussed above: Notch and Kras.^{59,209} Activation of Erk, Cdk4, and cyclin D also occur with TGF- α stimulation.²¹⁰ Based on these data, it would be tempting to predict that pharmacologic inhibition of EGF signaling would have clinical efficacy against PDAC. However, administration of biologics (cetuximab, erlotinib) to antagonize EGF signaling have failed to produce any durable response in clinical trials.²¹¹

G. Wnt Signaling

Abnormal Wnt/ β -catenin signaling has been documented in PanIN and PDAC lesions in humans.^{212–214} Indeed, Wnt signaling was one of four signaling pathways in which genetic alterations were found in a large percentage of PDAC specimens by global genomic analysis.¹⁵² While numerous papers have been published on the subject, a study from the Hebrok group provides unique insight into how β -catenin is involved in PanIN and PDAC formation.²¹⁵ As reviewed previously, genetically engineered mice that express oncogenic Kras in the pancreatic epithelium will form PanIN lesions at a slow rate, but few, if any, tumors will form.¹⁰⁰ Treating such animals with cerulein induces experimental pancreatitis that leads to rapid PanIN formation and eventual progression to PDAC in a large fraction of mice.²¹⁶ During regeneration after injury, Wnt signaling, as measured by Axin2 and Lef1 expression, is transiently increased in control mice, and signaling is completely abolished after regeneration. However, in mice with oncogenic Kras, Wnt- β -catenin signaling continues to be active as PanINs form. To test whether β -catenin signaling was required for Kras-induced PanIN formation, compound mutants were bred to express oncogenic Kras and a transgenic allele of β -catenin that was resistant to degradation. When pancreatitis was induced in these mice, PanIN lesions did not form. Thus it was shown that PanIN formation required β -catenin signaling in concert with oncogenic Kras expression.²¹⁵ Moreover, Morris and colleagues provide a possible molecular explanation for the clinical observation that chronic pancreatitis is associated with a significant increase in the risk for PDAC.¹¹¹ Wnt/ β -catenin signaling has been shown to interact with other developmental pathways during neoplastic progression. TGF- β signaling through Smads has been shown to lead to decreased β -catenin levels.²¹⁷ Similarly, Notch signaling serves to lower β -catenin protein

levels.²¹⁸ Finally, β -catenin signaling, in itself, is known to activate the Gli transcription factors, the downstream effectors of the Hedgehog pathway.²¹⁹ Thus, the Wnt/ β -catenin pathway acts as a sort of node interconnecting multiple developmental pathways that have reemerged during PanIN and PDAC formation.

VI. Concluding Remarks

In addition to their lethal efficiency, most pancreatic cancers share major molecular pathways that drive their formation. As illustrated here, a handful signaling pathways are reproducibly activated during the initiation of PanIN formation and progression to carcinoma. However, these molecular pathways elicit broad effects that combine to produce the pathology unique to PDAC. Thus, while some of these deregulated pathways are shared by other tumors, the unique developmental history of the pancreas accounts for the distinct behavior and natural history of this malignancy. Some of these signals relate to developmental pathways utilized to maintain the undifferentiated state (e.g., Notch) while others are involved in the growth of the tissue (e.g., EGFs and FGFs). Innovative approaches to therapy will likely stem from targeting of those pathways that have little role in normal adult tissues and which are specifically utilized by cancer cells as a result of their exploitation of developmental signals.

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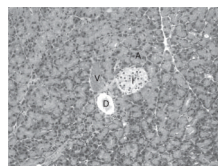
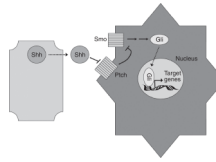


FIG. 1. Histology of the pancreas. Hematoxylin and eosin staining of a normal pancreas, depicting an Islet of Langerhans (I), pancreatic duct (D), and blood vessel (V) surrounded by acinin(A). 10 \times .

**FIG. 2.**

Hedgehog signaling. Paracrine Hedgehog signaling occurs when ligand (depicted here as Sonic Hedgehog, Shh) is secreted. Shh then binds to the Patched receptor (Ptch) on the target cell. When unbound by ligand, Ptch inhibits the transmembrane protein Smoothened (Smo). However, once ligand binds to Ptch, Smo is relieved of inhibition, leading to the activation of the Gli family of transcription factors and target gene expression.

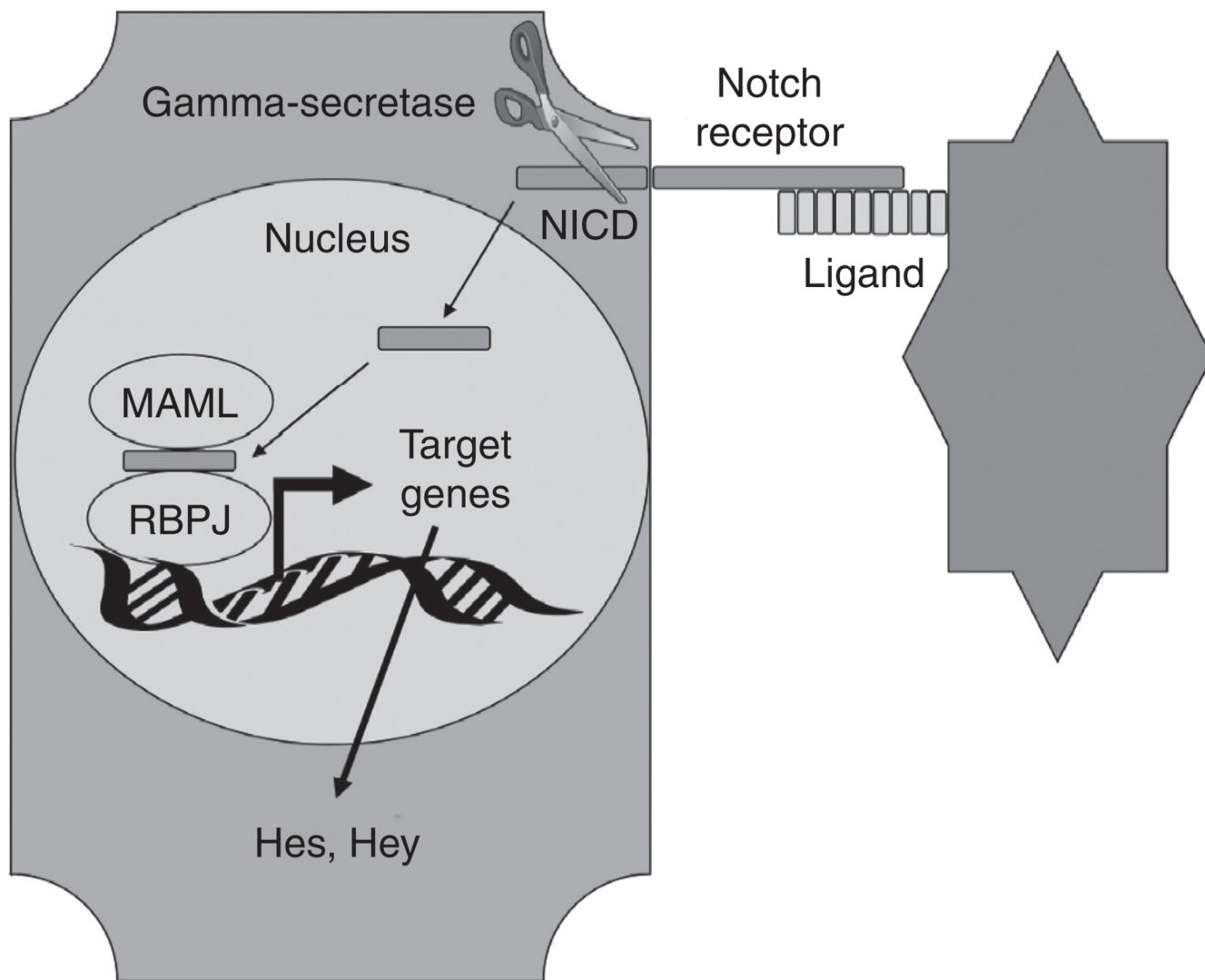


FIG. 3. Notch signaling. Notch signaling is initiated when the extracellular portion of a Notch receptor contacts a Notch ligand from a signaling cell. This triggers the gamma-secretase-mediated cleavage of the intracellular domain of the engaged Notch receptor. This Notch intracellular domain (NICD) translocates to the nucleus where it complexes with Mastermind-like protein (MAML) and recombining binding protein suppressor of hairless (RBPJ κ). This complex then mediates transcription of Notch target genes such as the Hes and Hey family of genes.

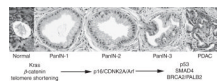


FIG. 4. PanIN-carcinoma sequence. Most PDAC is thought to arise from the sequential progression of PanIN lesions, as depicted. The earliest event underlying PanIN formation is thought to be an oncogenic mutation in Kras. Recent evidence suggests that Wnt- β -catenin is required for this early step. PanIN2 formation is thought to coincide with deactivating mutations in the p16/p19 tumor suppressor locus. Late stage PanINs exhibit mutations in p53, SMAD4, BRCA2, and PALB2.

TABLE I

Reemergent developmental pathways in PDAC

Signaling pathway	Effects in development	Effects in carcinogenesis
Hedgehog	Suppression required for pancreas specification	Canonical: Desmoplasia Noncanonical: Unclear
FGF	Pancreas specification, proliferation, differentiation, cell fate	Epithelial-to-mesenchymal transition (EMT), proliferation
Notch	Differentiation, cell fate	EMT, proliferation
TGF- β	Pancreas specification, proliferation, differentiation, cell fate	EMT, proliferation, desmoplasia
Retinoic acid	Pancreas specification, differentiation, cell fate	Unclear; putative marker of cancer stem cells
EGF	Proliferation, differentiation	Proliferation, desmoplasia
Wnt/ β -catenin	Pancreas specification, cell fate	PanIN formation