

## REVIEW

## Uncertain Beginnings: Acinar and Ductal Cell Plasticity in the Development of Pancreatic Cancer

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The pancreas consists of several specialized cell types that display a remarkable ability to alter cellular identity in injury, regeneration, and repair. The abundant cellular plasticity within the pancreas appears to be exploited in tumorigenesis, with metaplastic, dedifferentiation, and transdifferentiation processes central to the development of pancreatic intraepithelial neoplasia and intraductal papillary neoplasms, precursor lesions to pancreatic ductal adenocarcinoma. In the face of shifting cellular identity, the cell of origin of pancreatic cancer has been difficult to elucidate. However, with the extensive utilization of *in vivo* lineage-traced mouse models coupled with insights from human samples, it has emerged that the acinar cell is most efficiently able to give rise to both intraductal papillary neoplasms and pancreatic intraepithelial neoplasia but that acinar and ductal cells can undergo malignant transformation to pancreatic ductal adenocarcinoma. In this review, we discuss the cellular reprogramming that takes place in both the normal and malignant pancreas and evaluate the current state of evidence that implicate both the acinar and ductal cell as context-dependent origins of this deadly disease. (*Cell Mol Gastroenterol Hepatol* 2022;13:369–382; <https://doi.org/10.1016/j.jcmgh.2021.07.014>)

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Despite recent advances in cancer treatment, overall survival from pancreatic cancer remains dismal with few efficacious therapeutic options. Nearly 80% of pancreatic cancer patients present with unresectable or metastatic disease, and those few who undergo complete resection can expect a still dismal median survival of ~20 months.<sup>1</sup> Overall, the survival at 5 years is approximately 8%, and owing to increasing incidence, pancreatic cancer is projected to become the second-leading cause of cancer-related death in the United States.<sup>2</sup>

Pancreatic ductal adenocarcinoma (PDAC) consists of several hallmark features—an extensive desmoplastic reaction, a hypoxic local tumor microenvironment, a relative restriction of immunologic surveillance, and a pervasive resistance to conventional chemotherapy.<sup>3</sup> On average, PDAC lesions harbor 60–70 mutations and display considerable tumor heterogeneity with variation noted among

several identifiable subclones.<sup>4</sup> Genomic assessments have revealed that the *KRAS* gene is near-universally mutated, occurring in approximately 90% of cases.<sup>5</sup> Three tumor suppressors are as well often mutated—*CDKN2A* (~50%), *TP53* (60%–70%), and *SMAD4* (40%–50%), with *SMAD4* typically associated with increased metastatic burden.<sup>6</sup>

Notably, well-differentiated PDAC carries the morphological appearance of abundant ductal structures with wide expression of the ductal marker cytokeratin 19.<sup>7</sup> Thus, by histology alone, the putative cell of origin was long thought to be the ductal cell. However, the discovery and characterization of precursor neoplastic lesions in the pancreas that are associated with PDAC, either pancreatic intraepithelial neoplasia (PanIN) or intraductal papillary mucinous neoplasms (IPMNs), have shed new light on the beginnings of PDAC. In this review, we assess the current state of evidence regarding the beginnings of these pancreatic premalignant lesions and of malignant PDAC, and offer our perspectives on the uncertainties that remain.

## Lessons from the Endocrine Pancreas

Much of the ambiguity regarding the potential cellular origins of pancreatic cancer rests in our newfound understanding of plasticity among terminally differentiated cells of the pancreas, particularly in the endocrine pancreas (as summarized in Figure 1). In initial diabetes-related studies, experimental models of rodent injury, including pancreatectomy, appear to induce the self-replication of  $\beta$  cells, rather than differentiation of precursor cells or transdifferentiation from another compartment.<sup>8</sup> This mechanism of self-replication appears essential to homeostasis in the setting of pregnancy, obesity, or induced insulin resistance. However, the response to near-complete ablation of the  $\beta$  cell population within the endocrine pancreas using a

**Abbreviations used in this paper:** ADM, acinar-ductal metaplasia; CAC, centroacinar cell; IPMN, intraductal papillary mucinous neoplasm; PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma.

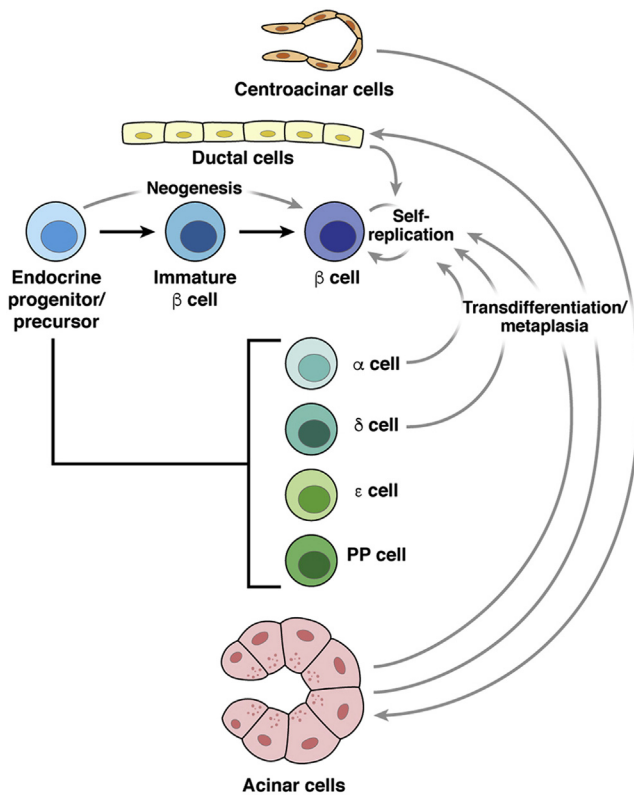


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**Figure 1.** Cellular plasticity within the normal pancreas. Pancreatic progenitors arise from definitive endoderm; ‘Tip’ and ‘trunk’ compartment specialization follows formation of microlumens within the pancreatic bud and gives rise to major cell types as indicated. These cell types can be repopulated by transdifferentiation, self-replication, and neogenesis in the setting of injury or insult.

diphtheria toxin-based system is quite different. Lineage-tracing of terminally differentiated cells revealed that glucagon-producing  $\alpha$  cells, rather than a latent progenitor population, are largely responsible for restoration of  $\beta$  cell populations.<sup>9</sup> Interestingly, in young mice, somatostatin-producing  $\delta$  cells (but not  $\alpha$  cells) undergo spontaneous conversion into  $\beta$  cells, proceeding through a dedifferentiated intermediate.<sup>10</sup> This  $\alpha$ -to- $\beta$  cell conversion appears to be inducible by the administration of gamma amino-butyric acid, and arises not from a terminally differentiated  $\alpha$  cell, but a precursor cell lining the duct.<sup>11</sup> The concept of persistent endogenous progenitors in the ductal lining is supported by earlier work suggesting that  $\text{Ngn3}^+$  hormone negative cells are expanded with pancreatic duct ligation, differentiate into  $\beta$  cells, and can be purified from the adult pancreas.<sup>12</sup> Together, these key findings demonstrate that  $\beta$  cell repopulation can occur through a multitude of routes—transdifferentiation of other cell types, self-replication, or neogenesis from a pool of endocrine progenitor cells—depending on the timing and nature of the physiologic insult.<sup>13,14</sup> These studies in the endocrine pancreas make clear that plasticity is an abundant feature of the normal pancreas, and that in disease, one cellular compartment may be repopulated by another.

## Plasticity Within the Exocrine Compartment

Multiple lines of evidence also support abundant cellular plasticity within the exocrine compartment in response to pancreatic injury (see Figure 1). Acute pancreatitis, induced by either pancreatic duct ligation or by the exogenous administration of the cholecystokinin analog caerulein, features release of digestive enzymes from the zymogen granules of acinar cells. In response to this injury, acinar cells lose polarity and flatten somewhat, taking on a cuboidal-columnar morphology and resembling ductal precursors in the embryonic pancreas. Despite not being completely flat and elongated as a typical ductal cell, this process has been termed acinar-ductal metaplasia (ADM), largely owing to the acquisition of a mucinous cytoplasm and surface expression of cytokeratin 19, both of which are characteristic of ductal cells.<sup>15</sup> In addition to ADM, the caerulein-treated mouse pancreas features infiltration by inflammatory cells, expansion of the interstitium, and loss of acinar cell area. In their acquisition of a “duct-like” phenotype, acinar cells specifically lose key features of their cell identity, including transcription factors essential to acinar cell fate (*Ptf1a*, *Mist1*).<sup>15,16</sup> In mouse and human models, acinar cells undergoing ductal metaplasia frequently will simultaneously express acinar-specific enzymes (amylase, trypsin, elastases, carboxypeptidases, etc.) in addition to CK19 and the HMG-box transcription factor *SOX9* that are found in ductal cells.<sup>15,17</sup> At best, then, so-called ADM could be characterized as incomplete given the persistence of acinar-specific gene expression, with several of the key transcription factors and marker proteins summarized in Table 1.

Further complicating matters is that ADM cannot be viewed as a “pure” transdifferentiation event (ie, direct conversion from acinar to ductal), as it is accompanied by features suggestive of a dedifferentiated phenotype (ie, reversion within the pancreatic lineage). Extensive immunohistochemistry of the pancreas following caerulein administration has shown that the pancreatic progenitor marker *Pdx1*, *Notch*, and  $\beta$ -catenin are all reactivated.<sup>18,19</sup> Notably, differentiated ductal cells do not display abundant *Notch* signaling or  $\beta$ -catenin expression, reaffirming that the acquired acinar cell fate is not entirely ductal in nature. In vitro experimentation has demonstrated the activation of the progenitor marker *Nestin* over the course of acinar cell identity loss.<sup>20</sup> More recent work has shown that the expression of pluripotency genes *Oct4*, *Sox2*, *Klf4*, and *Myc* are sufficient to induce dedifferentiation of the acinar cell and potentiate ADM formation.<sup>21</sup> *Klf4* overexpression alone also potentiates loss of acinar cell identity and is required for ADM formation.<sup>22</sup> In elegant studies by Real and colleagues, haploinsufficiency of the acinar-defining transcription factor *Nr5a2* is capable of driving a low-level inflammatory state that destabilizes terminally differentiated acinar cells.<sup>23</sup> Together, these findings suggest that acinar cells readily acquire features of both ductal and progenitor cells in response to pancreatic injury, a process that has been recently described as “paligenosis.”<sup>24</sup>

**Table 1.** Summary of Key Transcription Factors and Markers of Exocrine and Endocrine Cells

Key Protein	Family	Function	Onset of Expression	Expression in			
				Adult Pancreas	ADM	PanIN	PDAC
Amylase	Secretory zymogen	Carbohydrate digestion	e13.5	Acinar	+	-	-
Cpa1	Secretory zymogen	Cleaves C-terminal branched-chain amino acids from dietary proteins	e10.5	Acinar	+	-	-
Dclk1	Doublecortin	Microtubule-polymerizing activity	No embryonic study	Ductal, islets, and acinar (minority of cells)	+	++	++
Elastase	Chymotrypsin-like	Carbohydrate digestion	e12.5	Acinar	+	-	-
GATA4	Zinc-finger transcription factors	Regulators of the expansion and maintenance of MPCs	e9.5-e10.5	Acinar and B cell	+++	+++	++
GATA6	Zinc-finger transcription factors	Regulators of the expansion and maintenance of MPCs	e9.5-e10.5	Endocrine and exocrine	+	+	-
Hnf1 $\beta$	Homeodomain-containing family TF	Require for pancreatic precursors generation	e8.5	Ductal and centroacinar	+	+	+ (variable)
Hnf6	ONECUT class of cut homeodomain proteins	Essential regulator of Pdx1; endocrine cell differentiation	e8.5	Ductal	+	-	-
Krt19	Type I keratin	Intermediate filament protein key to cellular structure	e13.5	Ductal and centroacinar	+++	++++	+++
Mist1	Basic helix-loop-helix TF	Acinus polarity, cell-cell junctions, and the processing of zymogen granules	e12.5	Acinar	-	-	-
Ngn3	Basic helix-loop-helix TF	Initiates endocrine developmental program	e9.5-e10.5	Low expression in islet	+	-	-
Nr5a2	Nuclear hormone receptor family	MPC formation; normal acinar differentiation and function	e7.5	Acinar	+	-	-
Pdx1	TF of ParaHox gene cluster	Determine fate specification of pancreatic MPCs	e8.5-9.0	B cell and low level exocrine cells	++	++	++
Ptf1a	Basic helix-loop-helix TF	Acinar enzyme gene activator; determine fate specification of pancreatic MPCs	e9.5	Acinar	+ (low)	+ (low)	-
Sox9	Member of the SRY/HMG box (Sox) family	Proliferation, survival, and maintenance of pancreatic progenitors	e9.5	Ductal and centroacinar	+++	++++	++

ADM, acinar-ductal metaplasia; MPC, mesodermal precursor cell; PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma; TF, transcription factor.

Within the exocrine compartment, ductal cells can also alter their identity, as has been observed in mice expressing the transgenic diphtheria toxin receptor under the control of the *Pdx1* promoter. Diphtheria toxin administration in these mice ablates the acinar and endocrine compartments, which is then repopulated by cells of ductal origin adopting the pancreatic progenitor program.<sup>25</sup> Despite this, many of the *in vivo* studies utilizing nonspecific pancreatic injury, however, fail to identify significant injury to or plasticity among ductal cells—a feature that has been attributed to the elevated expression of the antiapoptotic protein Bcl-2—suggesting that the plasticity within the exocrine compartment is most evident in acinar cells.

Loss of acinar cell identity in response to caerulein-induced pancreatitis is reversible, with the exocrine pancreas undergoing “redifferentiation” to regain its normal architecture and function. Regeneration of the acinar compartment is dependent on the reactivation of several developmental pathways, including  $\beta$ -catenin signaling,<sup>26</sup> Hedgehog signaling,<sup>27</sup> and the Notch pathway.<sup>28,29</sup> In addition to developmental signals, bona fide regeneration of the acinar compartment also relies on preservation of acinar-specific transcription factors, including Nr5a2<sup>30</sup> and Ptf1a.<sup>31</sup> In support of redifferentiation as the means of regeneration, the enforced expression of pluripotency genes tends to lead to permanent loss of acinar cell identity, rather than to induction of a transient “facultative progenitor” state.<sup>21,22</sup> Regeneration of the acinar compartment also does not occur in *Kras*-driven metaplasia, likely owing to a similar inability to undergo this process of redifferentiation as *Kras* inactivation allows for reversion of both ADM and PanIN.<sup>32</sup> Regeneration of the acinar cell thus appears to be dependent on the ability to reactivate normal developmental pathways to acquire determinants of the differentiated acinar cell type.

Acinar cell plasticity can be exploited to derive other cell types. Inactivation of c-Myc within the pancreas has been shown via lineage tracing to induce the transdifferentiation of acinar cells into adipocytes.<sup>33</sup> This transdifferentiation was also evident during caerulein-induced injury alone, suggesting that acinar cell fate is permissive of even mesenchymal transdifferentiation. In addition, acinar cells can be used to derive endocrine-derived cells following induction of *Pdx1*, *Ngn3*, and *Mafa*—all key transcription factors in  $\beta$  cell development.<sup>34</sup> In other work, suppression of *Ptf1a* activity is also permissive of an acinar-to-endocrine conversion.<sup>35</sup> Lineage-tracing reveals that acinar cells specifically can undergo a transdifferentiation to an endocrine phenotype following pancreatic duct ligation with or without streptozotocin.<sup>36</sup>

Further plasticity in the exocrine pancreas exists among centroacinar cells (CACs) and specific niche populations. Sox9-positive CACs can differentiate into acinar cells,<sup>37</sup> and can do so in response to pancreatic injury (Figure 1).<sup>38</sup> Similarly, the DCLK1+ subpopulation of acinar cells are expanded with pancreatic injury and are necessary for regeneration following caerulein-mediated injury.<sup>39</sup> Therefore, acinar cells, representing over 90% of the pancreas mass, are the most abundant source of cells highly amenable

to a change in identity in noncancer contexts; it should serve as no surprise then that these cells could give rise to cancer in the appropriate contexts.

## Premalignant Lesions of the Pancreas and Their Origins

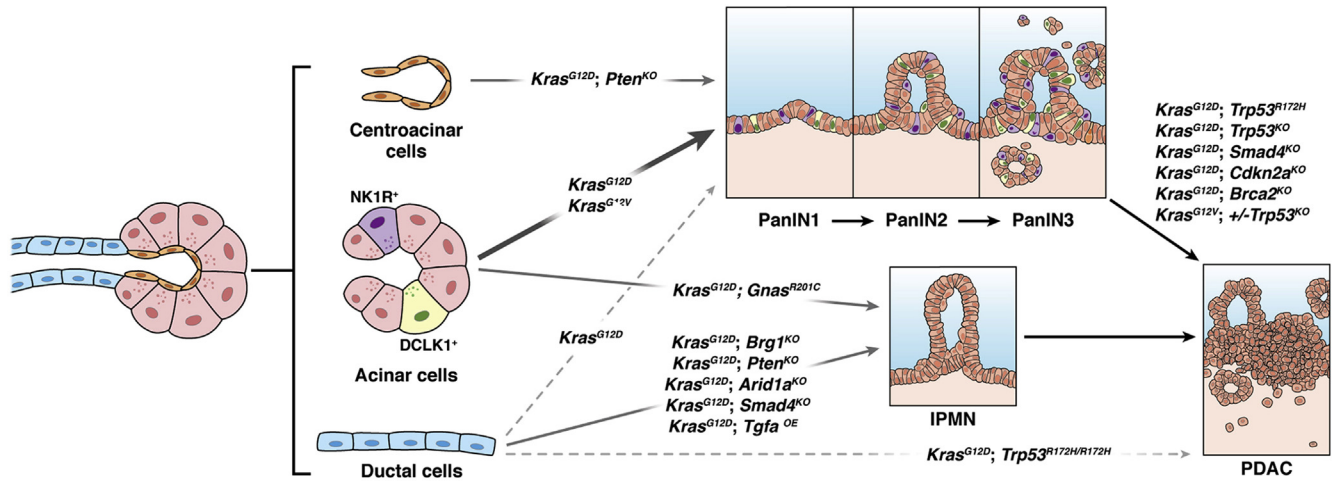
Invariably, PDAC does not appear to arise *de novo*, but instead from progressive dysplasia in precursor lesions derived from the neoplastic transformation of normal cells; the origins of these lesions have thus long thought to be central to understanding the beginnings of PDAC.

### Pancreatic Intraepithelial Neoplasia

First described by Brat et al<sup>40</sup> in the analysis of lesions associated with human PDAC, PanIN represents flat or papillary epithelial lesions that develop progressive dysplasia. PanINs are graded based on the level of atypia as either PanIN-1, -2, or -3, and are almost always associated with *KRAS* mutation<sup>41,42</sup> and, with greater atypia, associated tumor-suppressor loss.<sup>43</sup> Indeed, the majority of human PDAC is found in association with PanIN,<sup>44</sup> suggesting that PDAC occurs either as a transformation of underlying PanIN or requires a concomitant PanIN lesion. Interestingly, recent sequencing of human PanINs not associated with PDAC suggests that tumor suppressor inactivation is infrequent among isolated PanIN.<sup>45</sup>

Mounting evidence clearly demonstrates that acinar cells are the likely cell of origin for PanINs.<sup>46–48</sup> In initial studies, the expression of a *Myc* transgene under the control of the *Ela* (elastase) promoter was found to drive loss of elastase expression and of acinar cell differentiation, as well as the accumulation of duct-like structures.<sup>49</sup> It was not until the subsequent development of *Cre-lox* technology that the ability to direct conditional expression of driver oncogenes allowed for further mouse models of PanIN. In one early study, direction of mutated *Kras* to progenitor cells of the pancreas clearly recapitulated the PanIN histology in mice, suggesting that the rodent model parallels the histological features of malignant precursors to human PDAC.<sup>50</sup> Usage of the cytokeratin 19 promoter to drive mutant *Kras* expression in ductal cells, however, failed to induce neoplasia and showed only a lymphocytic infiltration in the pancreas.<sup>51</sup> In seminal work from Guerra et al,<sup>52</sup> the expression of *Kras*<sup>G12V</sup> was restricted to acinar and CACs using the *Elastase* promoter and coupled to a tetracycline-regulated (tetO) system. In this system, PanINs developed by 2–3 months of age in the absence of doxycycline and progressed to PDAC by 10–12 months of age.<sup>52</sup> In contemporaneous work, however, targeting of *Kras*<sup>G12D</sup> to *Nestin*-expressing cells was shown to generate PanIN with the same frequency as pan-pancreatic drivers such as *p48* or *Pdx1*,<sup>53</sup> suggesting a potential progenitor origin for PanIN. As mentioned previously, *Nestin* upregulation is a hallmark finding of acinar cell dedifferentiation, such that these findings also do not exclude acinar cells as the source of the “progenitor” population.

Additional support for acinar cells as the source of PanIN has arisen from the use of additional promoters specific to



**Figure 2.** Cell-of-origin of pancreatic neoplasia and adenocarcinoma in mouse models. The predominant cell types of the exocrine compartment of the pancreas – acinar, centroacinar, and ductal cells – give rise to pancreatic intraepithelial neoplasia (PanIN) and intraductal papillary mucinous neoplasms (IPMNs) in the mouse in the genetic contexts indicated above. Subsequent pancreatic ductal adenocarcinoma (PDAC) usually requires tumor suppressor loss in addition to oncogene activation.

the acinar cell population, as illustrated in Figure 2. In an early study, cloning of *Kras*<sup>G12D</sup> in-frame in to the locus of *Mist1*—a basic helix-loop-helix transcription factor expressed primarily in acinar cells—generated acinar-ductal metaplasia and PanIN.<sup>54</sup> Usage of a tamoxifen-inducible *Elastase-Cre* (*ElaCreERT*) in conjunction with the LSL-*Kras*<sup>G12D</sup>-allele has also been shown to generate PanIN from acinar cells.<sup>55</sup> Importantly, this study also utilized a Cre-dependent *Notch1* gain-of-function transgene containing green fluorescent protein to label acinar cells and were able to show that PanIN lesions were derived from green fluorescent protein-expressing cells. Habbe et al<sup>56</sup> took these studies one step further by using both *ElaCreERT* and *Mist1CreERT2* alleles coupled with *Kras*<sup>G12D</sup> to show that PanINs arise spontaneously alongside ADM lesions and even “biphenotypic” cells displaying features of both acinar and ductal differentiation. Critically, Cre recombinase induction in the adult using these drivers is specific to the acinar cell population and excludes CACs. Together, these efforts provide strong evidence that acinar cells readily generate PanINs, both with and without superimposed pancreatitis.

Despite these studies, the capacity of ductal cells to generate PanINs was not completely evaluated until a few years ago. In key work from Kopp et al,<sup>57</sup> this question was answered elegantly using *Ptf1aCre<sup>ER</sup>* and *Sox9Cre<sup>ER</sup>* alleles to drive mutant *Kras* expression in acinar and ductal and CACs, respectively. The authors observed mutant *Kras* could not generate significant PanIN derived from *Sox9*-expressing ductal and CACs despite the fact that PanIN lesions express *Sox9*. While other reports suggest that ductal cells can give rise to PanIN,<sup>58</sup> acinar cells are far more efficient at undergoing loss of terminally differentiated identity to express specific ductal markers (including *Sox9*) that are hallmarks of ADM and PanIN, suggesting that *Sox9* (while essential to ADM and PanIN development)<sup>59,60</sup> does not confess cell of origin. Additional evidence showing that inactivation of mutant *Kras* in early and established PanIN restores acinar cell area and

acinar-specific transcriptional programs also highlights the acinar cell as the likely source of PanIN.<sup>32</sup> Data from humans do support this, as ADM found in association with PanIN frequently does contain the same *KRAS* mutations, suggesting that coexistent ADM/PanIN arise from the same preneoplastic acinar cell.<sup>61</sup> Furthermore, ADM but not ductal cells are typically found in proximity to neoplastic precursor lesions, again supporting an acinar origin for PanIN.<sup>62</sup>

### Intraductal Papillary Mucinous Neoplasms

Unlike PanINs, IPMNs are macroscopic lesions that are characterized by cystic appearance radiographically, intraluminal mucin, and papillary growth. Insofar as IPMNs resemble large ducts and can involve the main pancreatic duct, these precursor lesions are thought to arise from ductal cells more proximally in the arborization of the pancreatic ductal tree. Indeed, ductal cell populations are heterogeneous and consist of 2 groups—those connecting to the acini and those forming the terminal duct—with spatial localization impacting gene expression and morphology.<sup>63,64</sup> In humans, IPMN appears to be associated with 15% of pancreatic cancer, but the vast majority of clinically identified IPMNs do not progress to malignancy despite bearing many of the same oncogenic drivers (activating *KRAS* mutations in particular) To what degree IPMNs and PanINs are related remains unknown, but the former also features frequent mutations to *GNAS* that are quite rare in “conventional” PanIN-associated PDAC,<sup>65</sup> suggesting distinct origins. Moreover, intestinal-type IPMNs tend to proceed to histologically distinct colloid carcinoma that is associated with more favorable outcome.<sup>66</sup> Tubular variant adenocarcinomas arising from gastric and pancreatobiliary histologic subtype IPMNs, however, are indistinguishable from classical PanIN-derived PDAC—whether this reflects convergent mechanisms of tumorigenesis between PanIN and IPMN or if

there is concomitant PanIN that progresses to PDAC is unknown.

Mouse models have shed light on the origins of IPMN, with ductal cells historically considered the likely precursor. Early studies utilizing *Kras*<sup>G12D</sup> as the oncogene coupled with transgenic overexpression of *Tgfa* generated large cystic and papillary structures mimicking IPMN (in addition to PanIN); these were seen using *p48-Cre* mice but not when using *Elastase-Cre*<sup>ER</sup> mice—suggesting that adult acinar cells do not readily give rise to IPMN.<sup>67</sup> The ability of ductal cells to specifically serve as cells of origin for IPMNs has been observed with mutant *Kras* activation coupled with loss of *Brg1*, the catalytic subunit of SWI/SNF chromatin remodeling complexes. Loss of *Brg1* in acinar cells using the *Ptf1a-Cre*<sup>ER</sup> driver abrogates the development of PanIN, whereas loss in ductal cells (using *Hnf1b-Cre*<sup>ERT2</sup> mice) potentiates the development of rare *Kras*-driven IPMN-like lesions.<sup>68</sup> Both *Brg1* deletion and loss of *Arid1a* (another SWI/SNF subunit) in ductal cells has been shown to drive dedifferentiation of these cells in vitro.<sup>69,70</sup> Similarly, development of macroscopic, cystic intraductal papillary lesions has been demonstrated with loss of the tumor suppressor phosphatase *Pten* or *Liver kinase B1 (Lkb1)* directed specifically to ductal cells using the *Sox9-Cre*<sup>ERT2</sup> allele.<sup>71,72</sup> Together, these findings certainly demonstrate the competency of ductal cells to give rise to IPMN-like lesions in mice, as summarized in Figure 2.

The aforementioned mouse models invariably have utilized mutant *Kras* as the driver oncogene for IPMN development. However, approximately 60% of IPMNs feature mutations to *GNAS*, which encodes a protein that functions downstream of several G-protein coupled receptors to activate adenylyl cyclase to generate cyclic adenosine monophosphate.<sup>65,73,74</sup> Coupling of one of the frequent *Gnas* mutations (R201H) to *Kras*<sup>G12D</sup> in the pancreas using *Ptf1a-Cre* generated cystic tumors consisting of marked dilated ducts lined with papillary dysplastic epithelia, resembling IPMN.<sup>75</sup> Interestingly, a more recent study coupled another hotspot *Gnas* mutation (*Gnas*<sup>R201C</sup>) to *Kras*<sup>G12D</sup> to generate IPMNs but discovered that the same phenotypes were observed even with usage of the *Ptf1a-Cre*<sup>ER</sup> allele to direct recombination events solely in adult acinar cells.<sup>76</sup> This study calls into question the notion that acinar cells generate PanIN and ductal cells generate IPMN, and suggests that neoplastic fate may be as dependent on the specific driver mutation as it is on the cell type in which the mutation arises.

## Perspectives on the Origins of PDAC

Beginning with the initial mouse models using oncogenic *Kras* in Pdx1+ pancreatic progenitors,<sup>50</sup> there have been a multitude of studies describing specific murine allelic configurations to generate PDAC.<sup>77–85</sup> However, it is only with the advent of in vivo lineage tracing that specificity regarding the ability of a particular cell type to yield invasive disease has been addressed (and is summarized in Figure 2 and Table 2). In the following, we address the state of the evidence regarding each potential cellular precursor of PDAC.

## Acinar Cells

Given the evidence in the mouse model outlined previously to show that acinar cells give rise to PanIN, it would appear evident that these cells likely serve as the cell of origin for PDAC. Indeed, several lineage-tracing mouse models that incorporate mutant p53 with acinar-directed *Kras* activation largely support this possibility, with mice developing invasive PDAC.<sup>93,95</sup> Usage of the more mature acinar-specific driver *Mist1-Cre*<sup>ERT2</sup> coupled to *Kras*<sup>G12D</sup> and mutant *p53* also leads to invasive PDAC and relatively rapid demise among mice that mirror early nonselective *Pdx1-Cre* and *p48-Cre* models.<sup>89,87</sup>

However, several critical caveats remain. First, the bulk of evidence is derived from cell type-specific mouse models and therefore addresses only the competence of murine cell types to give rise to a histology similar to that seen in associated with human PDAC. In all of the available genetic mouse models, oncogenic *Kras* is directed to all cells of the corresponding lineage; this does not completely mimic what is likely a sporadic event in humans, as acinar cells adjacent to PanINs largely bear wild-type *KRAS*.<sup>96</sup> It is therefore quite possible that the phenotypes derived from widespread *Kras* activation in acinar cells, which represent 95% of pancreatic exocrine cells, might be quite different from the human scenario wherein presumably only few cells acquire oncogenic mutations. On the other hand, many of the mouse models do employ tamoxifen- or doxycycline-inducible oncogenic *Kras*, with efficiencies of recombination far less than the total acinar cell population. In addition, oncogenic *Kras* is necessary but not sufficient for the development of neoplasia (as injury/inflammation is frequently necessary), such that widespread *Kras* activation in these models should not necessarily be viewed as a limitation of these studies.

Second, acinar cells are a highly heterogeneous population of cells that may feature variable differentiation states. Single-cell analysis of acinar cells has identified a progenitor-like subpopulation with long-term self-renewal capacity that expresses the progenitor transcript *STMN1*.<sup>97</sup> *STMN1*<sup>+</sup> populations are expanded with pancreatic injury, suggesting a potential role in regeneration. Therefore, the possibility remains that it is not acinar cells as a whole that represent the cell of origin, but rather is a subpopulation of acinar cells whose behavior and function are quite different from the bona fide acinar population. On the other hand, expansion of *STMN1*<sup>+</sup> populations could simply reflect that this is another gene whose expression is induced in injury, and not necessarily outgrowth of an acinar subset.

Third, many of the mouse models also employ caerulein, which appears to selectively injure acinar cell populations. Intriguingly, mouse models that employ pancreatic duct ligation as an alternative means of inducing pancreatic inflammation show different exocrine cell predispositions to tumorigenesis. In this model, acinar cells are surprisingly recalcitrant to tumorigenesis undergoing apoptosis after ADM, suggesting that the specific mode of acinar cell injury may impact the likelihood of tumor information.<sup>88</sup>

Regardless, the enormous plasticity and heterogeneity of acinar cells can thus be seen as either one reason it is the

**Table 2.** Development of Premalignant Lesions and PDAC From Varying Cells of Origin

Mutation	Embryonic Expression		Acinar Expression		Ductal Expression	
	Pathology	Cre Driver	Pathology	Cre Driver	Pathology	Cre Driver
Kras <sup>G12D</sup>	PanIN/PDAC	Ptf1a-Cre <sup>32,50</sup> Pdx1-Cre <sup>50</sup>	PanIN/late PDAC	Ptf1a-Cre <sup>ER57,86</sup>	No lesions or cancer Early PanIN PDAC	Sox9-Cre <sup>ER57,86</sup> CK19-Cre <sup>ERT258</sup> Sox9-Cre <sup>ERT2</sup> with PDL <sup>88</sup>
			Sporadic PanIN	Mist1-Cre <sup>ER56,87</sup> Elastase-Cre <sup>ERT255,56</sup> Dclk1-Cre <sup>ERT39</sup>		
	sporadic PanIN	Pdx1-IRES-Cre <sup>21</sup> Nestin-Cre <sup>53</sup>	PanIN	Elastase-Cre <sup>ERT2</sup> with caerulein <sup>59</sup> Mist1-Cre <sup>ER</sup> with caerulein <sup>87</sup> Dclk1-Cre <sup>ERT</sup> with caerulein <sup>39</sup> Elastase-Cre <sup>ERTM</sup> with PDL <sup>88</sup>	PDAC	CK19-Cre <sup>ERT2</sup> with PDL <sup>88</sup>
			No lesions or cancer			
Kras <sup>G12V</sup>	PanIN/PDAC	Elas-tTA; tetO-Cre <sup>52</sup>	No lesions or cancer PanIN/PDAC	Elas-tTA; tetO-Cre <sup>52</sup> Elas-tTA; tetO-Cre with caerulein <sup>52</sup>		
Kras <sup>G12D</sup> ; p53 <sup>R172H</sup>	PanIN/PDAC	Pdx1-Cre <sup>77</sup>	PanIN/PDAC	Ptf1a-Cre <sup>ER86</sup> Mist1-Cre <sup>ER89</sup>	No lesions or cancer	Hnf1b-Cre <sup>ERT289</sup>
Kras <sup>G12D</sup> ; p53 <sup>flox/+</sup>	PanIN/PDAC	Pdx1-Cre <sup>80</sup>	PanIN/PDAC	Ptf1a-Cre <sup>ER86</sup>		
Kras <sup>G12D</sup> ; p53 <sup>flox/flox</sup>			PanIN/PDAC	Ptf1a-Cre <sup>ER90,86</sup> Elastase-Cre <sup>ERT2</sup> with caerulein <sup>91</sup>	PDAC Nonmucinous tubular lesions/PDAC	Sox9-Cre <sup>ER90,86</sup> CK19-Cre <sup>ER91</sup>
Kras <sup>G12D</sup> ; p53 <sup>flox/-</sup> or flox/R270H or flox/R172H			PanIN/PDAC	Ptf1a-Cre <sup>ER86</sup>	PDAC	Sox9-Cre <sup>ER86</sup>
Kras <sup>G12D</sup> ; p53 <sup>R172H/R172H</sup>					PDAC	Hnf1b-Cre <sup>ERT289</sup>
Kras <sup>G12V</sup> ; p53 <sup>+/-</sup>	PanIN/PDAC	Elas-tTA; tetO-Cre <sup>52</sup>				
Kras <sup>G12D</sup> ; p16 <sup>-/-</sup>	PanIN/PDAC	Pdx1-Cre <sup>80,78</sup>				
Kras <sup>G12D</sup> ; p16/Arf <sup>flox/flox</sup> or -/-	PanIN/PDAC	Pdx1-Cre <sup>80,78</sup>				
Kras <sup>G12D</sup> ; Smad4 <sup>flox/flox</sup>	IPMN/PDAC	Pdx1-Cre <sup>79</sup>				
Kras <sup>G12D</sup> ; Tgfb2 <sup>flox/flox</sup>	PanIN/PDAC	Ptf1a-Cre <sup>92</sup>				
Kras <sup>G12V</sup> ; p16/Arf <sup>flox/+</sup>			PanIN	Elas-tTA; tetO-Cre <sup>93</sup>		
Kras <sup>G12V</sup> ; p16/Arf <sup>flox/lox</sup>			PanIN/PDAC	Elas-tTA; tetO-Cre <sup>93</sup>		
Kras <sup>G12D</sup> ; Lkb1 <sup>lox/lox</sup>	PanIN/PDAC	Pdx1-Cre <sup>84</sup>			IPMN/premature death	Sox9-Cre <sup>ERT272</sup>
Kras <sup>G12D</sup> ; Brg1 <sup>ff</sup>	IPMN/PDAC	Ptf1a-Cre <sup>68,70</sup>	No lesions or cancer	Ptf1a-Cre <sup>ER68</sup>	Few IPMN	Hnf1b-Cre <sup>ERT268</sup>
Kras <sup>G12D</sup> ; Arid1a <sup>f/+</sup>	PanIN	Ptf1a-Cre <sup>70</sup>	PanIN	Ptf1a-Cre <sup>ERT2</sup> with caerulein <sup>70</sup>	No lesions or cancer	Hnf1b-Cre <sup>ERT270</sup>
Kras <sup>G12D</sup> ; Arid1a <sup>ff</sup>	PanIN and IPMN/PDAC	Ptf1a-Cre <sup>70</sup>	PanIN	Ptf1a-Cre <sup>ERT2</sup> with caerulein <sup>70</sup>	Few IPMN	Hnf1b-Cre <sup>ERT270</sup>
Kras <sup>G12D</sup> ; Fbw7 <sup>F/F</sup>			PanIN/PDAC	Elastase-Cre <sup>ERT2</sup> with caerulein <sup>91</sup>	Nonmucinous tubular lesions/PDAC	CK19-Cre <sup>ER91</sup>
GNAS <sup>R201H</sup>	Early IPMN	Ptf1a-Cre <sup>75</sup>				
Kras <sup>G12D</sup> ; GNAS <sup>R201H</sup>	IPMN/cystic tumor	Ptf1a-Cre <sup>75</sup>				
Kras <sup>G12D</sup> ; GNAS <sup>R201C</sup>	IPMN	Ptf1a-Cre <sup>76</sup>	IPMN	Ptf1a-Cre <sup>ER76</sup>		

Table 2. Continued

Mutation	Embryonic Expression		Acinar Expression		Ductal Expression	
	Pathology	Cre Driver	Pathology	Cre Driver	Pathology	Cre Driver
<i>Kras</i> <sup>G12D</sup> , <i>GNAS</i> <sup>R201C</sup> , <i>p53</i> <sup>LoxP/+</sup>			IPMN/PDAC	<i>Ptf1a-Cre</i> <sup>ERT2</sup>		
<i>Pten</i> <sup>fllox/fllox</sup>	PanIN and papillary adenocarcinoma/PDAC	<i>Pdx1-Cre</i> <sup>94</sup>	No lesions or cancer	<i>Elastase-CreER</i> <sup>Tm34</sup>	IPMN/PDAC—spontaneous <i>Kras</i> mutation	<i>Sox9-Cre</i> <sup>ERT271</sup>
<i>Pten</i> <sup>fllox/fllox</sup> , <i>p53</i> <sup>-/-</sup>	Papillary adenocarcinomas	<i>Pdx1-Cre</i> <sup>94</sup>				
<i>Kras</i> <sup>G12D</sup> , <i>Pten</i> <sup>fllox/fllox</sup>			IPMN/PDAC		IPMN/PDAC	<i>Sox9-Cre</i> <sup>ERT271</sup>

Note: Blank cells indicate models that have not been evaluated.

ADM, acinar-ductal metaplasia; IPMN, intra-ductal papillary mucinous neoplasm; PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma; PDL, pancreatic duct ligation.

likely cell of origin for PanIN-associated PDAC, or a potential confounding variable in studies targeting genetic events to this diverse cell population. The previous caveats notwithstanding, the preponderance of evidence shows that acinar cells are highly capable of generating PanIN and IPMN in response to oncogenic *Kras* and co-occurring mutant *Kras/Gnas*, respectively, and that precursor lesions derived from acinar cells can progress to invasive disease. The transcriptional and epigenetic determinants of acinar cell plasticity, particularly in the context of oncogenic stress, thus appear to be worthwhile avenues for further investigation.

### Ductal Cells

With recent data, the capacity of ductal cells to function as the cell of origin for PDAC has become increasingly unclear. Certainly, the capacity of these cells to undergo a cell identity change outside of cancer contexts appears limited, particularly as compared with acinar cells. Accordingly, it should be noted that while ductal cells may not effectively produce PanIN, there are multiple contexts in which these cells can give direct rise to PDAC. Specifically, Bailey et al<sup>89</sup> demonstrated the ability of ductal cells (selected using the *Hnf1b-Cre*<sup>ERT2</sup>) allele to generate PDAC in the setting of 2 copies of mutant *p53* but without associated PanIN lesions. In a similar study employing deletion of *p53* using a floxed allele, a duct-specific driver of Cre recombinase (*Sox9-Cre*<sup>ER</sup>) combined with *Kras*<sup>G12D</sup> was observed to generate PDAC more rapidly than with an acinar-specific driver (*Ptf1a-Cre*<sup>ER</sup>).<sup>90</sup> Further work by Flowers et al<sup>86</sup> employing a comprehensive panel of genetically engineered mice largely recapitulate these findings, namely that *p53* mutation or deletion is required for PDAC development from pancreatic ductal cells (in the absence of superimposed injury). Interestingly, duct- and acinar-origin tumors appear molecularly distinct, and map closely to basal and squamous and classical and progenitor transcriptional subtypes, respectively.<sup>86</sup> In all of these aforementioned studies, there is a paucity of low-grade PanIN that accompanies ductal-driven PDAC, reinforcing the notion that ductal cells are not competent to generate PanIN but may give rise to PDAC. These findings thus raise the distinct possibility that in humans a greater proportion of PDAC may in fact arise from ductal cells, and that coincident PanIN found in patients might arise from separate mutational events in distinct acinar cells. However, genetic sequencing of PanINs and associated PDAC suggest more shared than distinct drivers, indicating that independent coevolution of acinar-derived PanIN and ductal-derived PDAC is likely infrequent.<sup>87</sup> On the other hand, Ferreira et al<sup>91</sup> also demonstrated using *Fbw7* deletion that ductal-driven PDAC arises independent of PanIN and that, importantly, orthotopic transplantation induces the development of bystander PanIN, suggesting that not all PanIN lesions are precursors of PDAC.

As mentioned previously, there are multiple genetic mouse models in which the development of IPMN can be driven specifically from a ductal origin. It should be noted, however, that the available models clearly demonstrating ductal-derived IPMNs utilize additional genetic events to



either transforming growth factor  $\beta$  signaling pathway components (*Tgfa*, *Smad4*), chromatin remodelers (*Brg1*, *Arid1a*), to *Pten* or to *Lkb1*, all of which are infrequently mutated in either premalignant or invasive IPMN. The findings by Bardeesy et al<sup>76</sup> that co-occurring *Gnas/Kras* mutations—which actually recapitulate the driver mutations found in human disease—are associated with a non-ductal origin of IPMN are therefore highly compelling. While specific mouse models can generate ductal-derived IPMN, it remains unclear if ductal cells are frequent cells of origin in the human setting. Nevertheless, the frequency of basal and squamous subtype PDAC, which might be more likely to be ductal-derived, and the emergence of mouse models employing duct-specific inactivation of p53 or utilizing alternative forms of pancreatic injury (eg, pancreatic duct ligation) demonstrate that ductal-driven tumorigenesis is quite possible from common driver mutations but not with an apparent low-grade IPMN or PanIN precursor.

### Centroacinar Cells

Several studies do point to CACs, which are present at the junction between terminal acini and ductal cells, as potential precursors to PDAC. Stanger et al were able to identify an expansion and proliferation of these cells in *Pten*-deficient mice, and a development of ductal metaplasia and malignant transformation in *Pdx1-Cre* but not *Elastase-Cre<sup>ERTM</sup>* mice.<sup>94</sup> Notably, *Sox9-Cre Kras<sup>G12D</sup>* mice, wherein mutant *Kras* is expressed in both ductal and centroacinar cells, do not demonstrate robust PanIN. Perhaps these 2 contradictory findings can be reconciled by the fact that the driver mutations are different, and that *Pten* deletion is a sensitivity specific to the ductal-CAC compartment.<sup>71,94</sup> As a putative contribution of CACs appears restricted to only rare models of pancreatic neoplasia, and utilizing *Pten* deficiency that is typically not observed in human disease, there appears to be little evidence to implicate CACs as the cell of origin of PDAC.

### Cancer Stem Cells and Niche Populations

As to the role of niche populations within the exocrine compartment, there are increasing data to point to specific neuroendocrine cells (among acinar cells) responsive to neuronal input and important to PanIN progression<sup>98</sup> such that ablation of sensory neurons impacts the development of PanIN and PDAC.<sup>99-101</sup> Cancer stem cells have also been proposed to exist in the pancreas, as defined by enhanced tumorigenicity of the CD44<sup>+</sup> CD24<sup>+</sup> ESA<sup>+</sup> or CD133<sup>+</sup> CXCR4<sup>+</sup> populations of epithelial cells.<sup>102,103</sup> Largely, these cancer stem cells have been shown to have enhanced clonogenic growth in vitro and heightened metastatic potential in vivo, and also bear substantial overlap with the niche population of so-called tuft cells in the pancreas that express *Dclk1* and acetylated tubulin (AcTub) on their cell surface.<sup>104</sup> AcTub<sup>HI</sup> *Dclk1*<sup>+</sup> cells bear the same “stem cell” markers (CD133, CD24, ESA, etc.) and have been shown to be important to PanIN development and progression.<sup>104,92</sup> Direction of oncogenic *Kras* to the *Dclk1*<sup>+</sup> population appears as efficient at initiating PanIN as usage

of the *Mist1* promoter, and deletion of *Dclk1* diminishes PanIN formation substantially.<sup>92</sup> Importantly, lineage tracing suggests the vast majority of *Dclk1*<sup>+</sup> cells, which represent a rare population (0.1%-0.5% of all cells) in the normal pancreas belong to the acinar lineage, such that these data reinforce the notion that acinar cells are the cell of origin for pancreatic neoplasia and cancer. Whether these are subsets of acinar cells from which premalignant lesions and PDAC exclusively arise remains a subject for further investigation. In addition, there are no data to our knowledge that demonstrate that these cancer stem cells are a fixed population of acinar cells and not a stochastically acquired phenotype among acinar cells exhibiting transcriptional plasticity.

### Conclusions

As the usage of genetically engineered mouse models of PanIN, IPMN, and PDAC arising from both precursor lesions has evolved, so too has the current state of knowledge regarding the cell of origin of pancreatic cancer. The body of literature yields a complex, perhaps muddled, picture that should be no surprise given the abundant plasticity within the normal endocrine and exocrine pancreas. In keeping with the observations from pancreatic injury, acinar cells are easily reprogrammed to give rise to neoplastic PanIN and IPMN lesions. Though human PDAC appears to arise largely in the context of PanIN and IPMN, genetically engineered mice can seemingly bypass premalignant lesions, particularly in the context of PDAC that is generated from the ductal lineage. Overall, pancreatic cancer can be seen as arising from 3 routes—from underlying PanIN, from IPMN, and perhaps from bypassing either of these intermediates. The data suggest that PanIN is most easily generated in the mouse through the expression of oncogenic *Kras* in acinar cells; IPMN, by contrast, can variably arise from acinar cells (in the context of mutant *Kras* and *Gnas*) or ductal cells (in the context of chromatin remodeling or transforming growth factor  $\beta$  signaling mutations). PDAC that arises in the background of a normal pancreas is observed using ductal drivers and specific modes of injury or tumor suppressor loss. The specific roles of CACs, as well as that of niche populations within the acinar and ductal compartments that bear heightened tumor-initiating potential, remain yet to be fully elucidated. Regardless, the multitude of models have made clear that driver mutations may function differently depending on the affected population. In the end, there may not be a cell of origin per se of pancreatic cancer, but rather there may be susceptibilities that are dependent on the intersection between cell type, niche population, mode of injury, and the precise mutational events that destabilize the normal cell.

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**Conflicts of interest**

The authors disclose no conflicts.

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