

## REVIEW

## On the Origin of Pancreatic Cancer: Molecular Tumor Subtypes in Perspective of Exocrine Cell Plasticity

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## SUMMARY

The ontogeny of molecular subtypes in pancreatic cancer may relate to different cells of origin. This review provides a critical insight into the different exocrine cell types and their differentiation states, with a focus on human studies.

**Pancreatic ductal adenocarcinoma (PDAC) is a devastating type of cancer. While many studies have shed light into the pathobiology of PDAC, the nature of PDAC's cell of origin remains under debate. Studies in adult pancreatic tissue have unveiled a remarkable exocrine cell plasticity including transitional states, mostly exemplified by acinar to ductal cell metaplasia, but also with recent evidence hinting at duct to basal cell transitions. Single-cell RNA sequencing has further revealed intrapopulation heterogeneity among acinar and duct cells. Transcriptomic and epigenomic relationships between these exocrine cell differentiation states and PDAC molecular subtypes have started to emerge, suggesting different ontogenies for different tumor subtypes. This review sheds light on these diverse aspects with particular focus on studies with human cells. Understanding the "masked ball" of exocrine cells at origin of PDAC and leaving behind the binary acinar vs duct cell classification may significantly advance our insights in PDAC biology. (*Cell Mol Gastroenterol Hepatol* 2022;13:1243–1253; <https://doi.org/10.1016/j.jcmgh.2021.11.010>)**

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## Pancreatic Ductal Adenocarcinoma, an Exocrine Cell Party

Pancreatic ductal adenocarcinoma (PDAC) accounts for the vast majority of pancreatic tumors (>90%). Most PDAC patients are diagnosed at late stages of disease with locally advanced or metastatic tumors. Despite continuous efforts to improve PDAC treatment, patient survival remains invariably dismal, with an overall 5-year survival rate below 10% globally.<sup>1,2</sup> Worryingly, the statistics predict that PDAC will soon be the second leading cause of cancer-related deaths. These facts underscore the urgent need to better understand tumorigenesis in the pancreas.

PDAC originates in the exocrine pancreas, a tissue composed of acinar cells, which produce and secrete digestive enzymes, and duct cells, which form interposed tubes (ducts) draining the enzymes into the duodenum. Owing to the typical tumor's ductal morphology and expression of ductal markers, PDAC was historically thought to arise from duct cells. However, transitional states in which acinar cells adopt duct cell features have been shown, opening the debate about the true cell of origin of PDAC tumors. While studying the cell of origin in humans is less evident, in rodents, a plethora of studies have shown evidence of acinar cells as cells of origin for PDAC.<sup>3–8</sup> Regarding duct cells, and their possible phenotypic plasticity, much less is known.

In recent years, advanced analyses diverted us from regarding PDAC as one single entity. Based on (epi)genomic, transcriptomic and metabolic features, different subtypes of PDAC have been described, as reviewed elsewhere.<sup>9,10</sup> From these, there is growing international consensus on the existence of 2 transcriptomically defined subtypes: the classical subtype, characterized by expression of pancreatic progenitor markers and regulated by GATA6, and the basal-like/squamous/quasi-mesenchymal subtype of which  $\Delta Np63$  is a driver and which is associated with worse survival outcome.<sup>10</sup> With these different molecular PDAC subtypes coming to the fore and with the inherent plasticity of exocrine cells, trying to identify a single cellular source of this cancer might even be futile.

Most experimental research on PDAC's cell of origin has, for obvious reasons, been conducted on mouse models. In this review, however, we emphasize the human studies on this topic. We briefly recapitulate the ontogenesis of acinar and duct cells in the embryo, we describe the (epi)genetic

**Abbreviations used in this paper:** ADM, acinar-to-ductal metaplasia; E, embryonic day; GEM, genetically engineered mouse; HPDE, human pancreatic duct epithelial; HPNE, human pancreatic nestin expressing; IPMN, intraductal papillary mucinous neoplasm; iPSC, induced pluripotent stem cell; PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma; TGF, transforming growth factor.

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differences that play a role in the plasticity of acinar and duct cells in the adult human exocrine pancreas and how this might have an impact on neoplastic transformation, and we highlight recent discoveries on subsets of exocrine cell types and changing differentiation states. Importantly, for each of these aspects we emphasize relevant experimental models with human cells, and we critically reflect on the shortcomings of each model.

## Looking Behind the Scene, Embryonic Development

Before getting into adult exocrine cell differentiation and plasticity, it is critical to understand the developmental origin of the exocrine acinar and duct cells and, inevitably, also the endocrine cells that regulate glucose metabolism. The development of pancreas has been extensively reviewed elsewhere<sup>11–14</sup>; therefore, we only recapitulate the key facts in an exocrine cell-centered way.

Pancreatic embryonic development can be divided into 3 stages. The first transition starts around embryonic day 9.5 (E9.5) in mice, corresponding to 30 days postconception in humans.<sup>15</sup> At this point, the pancreatic bud arises, and the epithelium starts to expand. PDX1, PTFA1, CPA1, HES1, and SOX9 transcription factors, all present in multipotent progenitor cells at this stage, play a critical role during early pancreas development.<sup>16–18</sup> Around E12.5, corresponding to 40 days postconception in humans,<sup>15</sup> the second transition starts and the establishment of a tip-trunk domains occurs.<sup>11</sup> At this stage, tri- and bipotent progenitor cells are still present. They give rise to tip and trunk cells, respectively. Trunk cells are characterized by NKX6.1, SOX9, and HNF1B expression, while tip cells have a unique expression of PTF1A, CPA1, and NR5A2. Interestingly, despite the clear marker separation of the cells, several reports have shown that, at least until E13.5, both tip (CPA+) and trunk (HNF1B+) cells retain tripotent precursor potential. It is only around E15.5 (52dp)<sup>15</sup> that tip cells lose this tripotent progenitor potential to become fully differentiated acinar cells and a lineage separation between acinar cells and (trunk cell-derived) duct cells can be clearly observed (Figure 1).<sup>11,19,20</sup>

In terms of human experimental models, induced pluripotent stem cells (iPSCs) are generally used to model and study developmental processes *in vitro*. Unlike widely adopted experimental models that differentiate human iPSCs to endocrine beta-like cells,<sup>21–25</sup> there are very few reports on the successful differentiation of iPSCs into exocrine cells. Hohwieler et al<sup>26</sup> reported on the development of acinar- and duct-like organoids derived from iPSCs that upon engraftment show exocrine cell type-specific characteristics. The fact that fundamental insights into exocrine differentiation are less well developed than those of endocrine differentiation (being core to diabetes research) may result from the lack of adequate (human) *in vitro* models.

## Acinar Cells, the Usual Suspects

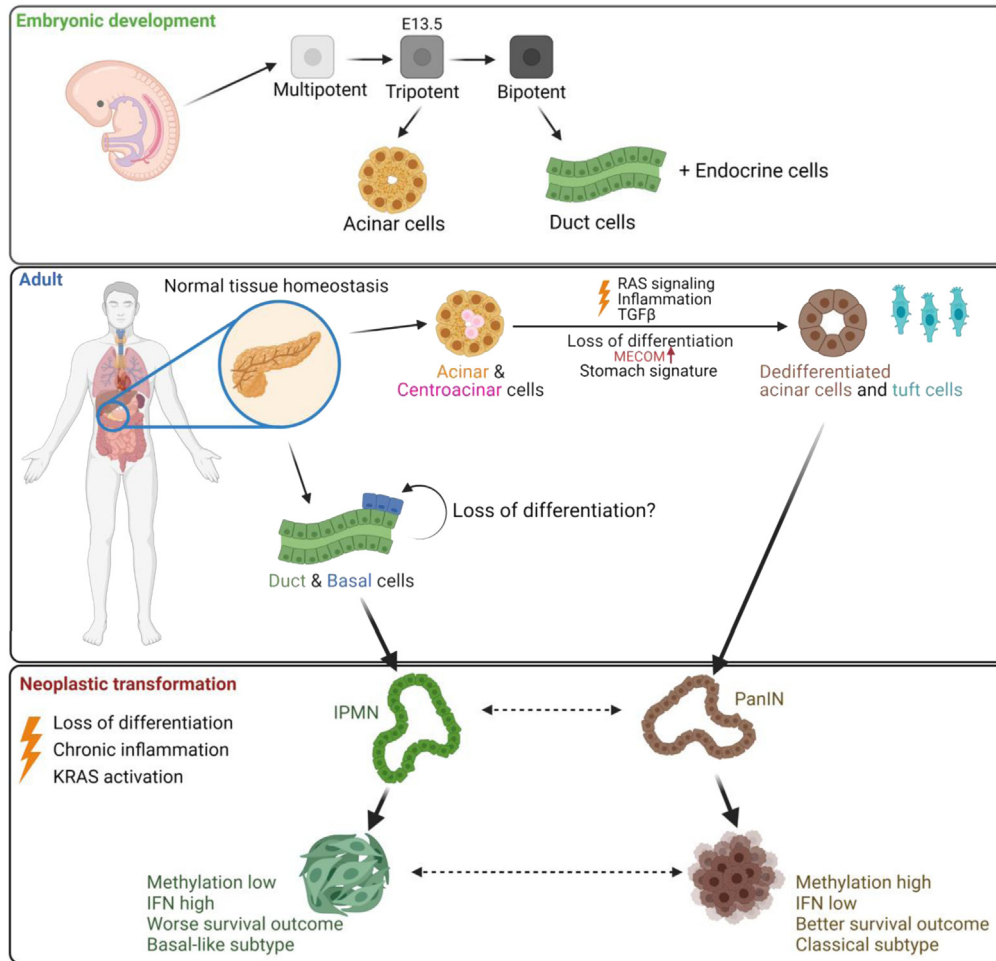
Despite the usual ductal appearance of PDACs, most evidence from genetically engineered mice (GEMs) in the

last 10–15 years has pointed to an acinar cell origin in which acinar cells undergo ductal metaplasia at the onset of tumor formation.

Fully differentiated acinar cells are characterized by the expression of the transcription factors PTF1A, MIST1, GATA4, and NR5A2, which regulate, among others, the expression of digestive enzymes such as amylase and elastase.<sup>19,27,28</sup> It was reported early on that mononucleated and binucleated acinar cells could be observed at the histological level,<sup>29</sup> but it was not until single-cell RNA sequencing was introduced that acinar cell heterogeneity could be better characterized.<sup>30–34</sup> Interestingly, in human studies, a subset of acinar cells (acinar-i cells) shows lower activation of acinar-specific regulatory networks, suggesting that they might have a higher capacity to convert into other cell types of the pancreas.<sup>34</sup> A subpopulation of proliferative *Stmn*<sup>+</sup> acinar cells with similar characteristics was also reported in mice hinting to certain similarities between the 2 species.<sup>31</sup> Moreover, a recent study identified a subpopulation of human acinar cells (acinar-edge cells) with features of progenitor cells or dedifferentiation.<sup>35</sup> New sequencing data and experimental approaches to study acinar cell heterogeneity will continue to shed light on acinar cell heterogeneity.

Importantly, acinar cells are sensitive to experimental injury or stress conditions, rapidly losing their normal phenotype. This dedifferentiation can be a first step toward metaplasia, which is the replacement of one differentiated cell type with another differentiated cell type in the same tissue (by de- or transdifferentiation or by replacement of dying cells) (Figure 1). It is actually essential for an acinar cell to lose the expression of transcriptional regulators such as PTF1A and MIST1 for it to become susceptible to metaplasia.<sup>36,37</sup>

In the pancreas, most knowledge on human acinar cell de- and transdifferentiation stems from cell isolation and *in vitro* culture systems. Purified acinar cell preparations, when put in culture, become amylase negative and gain ductal features such as KRT19 over a few days,<sup>38</sup> hence referred to as acinar-to-ductal metaplasia (ADM). Interestingly, this is accompanied by the upregulation of keratins of simple epithelial tissues (KRT19, KRT7, and KRT8) but not of those typical of stratified tissues (KRT5, KRT10, KRT13).<sup>39</sup> Besides acinar cells obtaining a ductal-type cytokeratin expression profile, we previously showed that, in adherent cultures, acinar cells start to express ductal cell-specific transcription factors such as HNF1B and SOX9, as well as functional ductal markers CAII and CFTR.<sup>40</sup> Slightly differently, when cultured in suspension, human acinar cells rather dedifferentiate toward a progenitor-like state expressing CD142 and MECOM, which are not found in fully differentiated duct cells but are expressed in pancreatic progenitor cells,<sup>41,42</sup> suggesting a regression to a multipotent progenitor-like state. Activation of embryonic pathways has been described in pancreas regeneration after induction of stress, likely preventing them from self-digestion and eventual cell death.<sup>43</sup> It will be interesting to dissect by single-cell analyses if certain acinar cell populations regress further into the progenitor state than others.



**Figure 1. Graphical overview of pancreatic cell plasticity in embryonic development, during normal tissue homeostasis, and in neoplastic transformation.** Pancreatic embryonic development is characterized by multipotent progenitor cells expressing PDX1, which further differentiate into tripotent progenitor cells, giving rise to eventually bipotent trunk cells that differentiate into duct and endocrine cells, leaving behind the “tip” cells that give rise to acinar cells. In adult tissue, exocrine cell plasticity is still present, with acinar cells being able to dedifferentiate under stress conditions. In this process, the transcription factor MECOM is upregulated, permitting dedifferentiation and avoiding cell death of acinar cells. During that same stress insult, tuft cells appear but their function is still rather unknown. Plasticity within the ductal and basal cell population is still under debate, and basal cells may even be a subtype of epithelial ductal cells. During neoplastic transformation, in a context of activation of oncogenes such as KRAS, dedifferentiated acinar cells can further give rise to a tumor, most likely of the classical subtype. Duct cells possibly follow another route and presumably give rise to a basal-like subtype that is characterized with worse survival outcome.

The experimental stress conditions taking place during cell isolation and culture incite RAS signaling, which seems to reproduce the stress conditions of acute and chronic pancreatitis in patients in which loss of acinar cell differentiation and ADM has been described.<sup>7</sup> *In vivo* genetic lineage tracing of rodent cells has extensively supported the concept of ADM upon tissue injury.<sup>3,6–8,44</sup> The metaplastic tissue replaces the normal acini-forming tubular structures with duct cell-specific markers such as *Sox9*, *Hnf1β*, and *Krt19*.<sup>3,5,6,40,45–50</sup> When working with human cells in culture, a genetic tracing method using a fluorescent reporter construct is technically challenging. Therefore, nongenetic lineage tracing methods have been developed (Table 1) of which the use of UEA-1, a lectin that binds to the highly

glycosylated CCK-A receptor on acinar cells,<sup>51</sup> is commonly applied.

Importantly, when combined with a ductal marker (eg, CA19.9), such lineage tracing approaches allow distinguishing between early de-differentiated acinar cells (UEA-1+/CA19.9–) and fully transitioned ADM cells (UEA-1+/CA19.9+).<sup>41</sup> Using this labelling approach, we recently performed the first molecular characterization of the initial steps of human acinar cell dedifferentiation.<sup>42</sup> By profiling dedifferentiated acinar cells and comparing them to the corresponding donor’s duct cells in suspension culture (by RNA sequencing), we uncovered a number of molecular differences. Dedifferentiated acinar cells were enriched for the KEGG pathways “Protein digestion and absorption” and

**Table 1.** Markers Used to Purify Acinar and Duct Cells From Murine and Human Origin

Marker	Purification	References
UEA-1 CA19.9	Acinar, acinar derived Duct	40,41
UEA-1 CD133/CLA	Acinar Duct	50
UEA-1/PNA	Acinar cells	52

“Pancreatic secretion,” indicating an acinar cell origin and conservation of typical acinar cell gene expression. Accordingly, duct cells showed higher expression of ductal genes (Table 2) and enrichment of pathways such as “O-linked glycosylation and ECM-receptor interaction.” Interestingly, while both dedifferentiated acinar and duct cell populations in culture showed an enrichment for “Pathways in cancer,” different genes contributed to this pathway. In the dedifferentiated acinar cell fraction, these were members of the collagen type IV family, WNT pathway family members, fibroblast growth factors, and the transcription factor MECOM. In the duct cell fractions, these genes were transforming growth factor  $\alpha$  (TGF $\alpha$ ), TGF $\beta$ 2, and components of laminin subunits.<sup>42</sup>

Altogether, human acinar cells under stress undergo a process whereby they dedifferentiate and can undergo ADM, regressing into a pancreatic progenitor state.

## Duct Cells, the Underdogs

The other exocrine cells, the duct cells, are by far outnumbered by acinar cells. This stochastic disadvantage may have led to the conclusion that they are less prone to tumor development. Still, other factors may have contributed that can be understood once we get to know more about duct cells.

Fully differentiated duct cells express HNF6, HNF1B, FOXA2, and SOX9. Interestingly, all these transcription factors are also expressed by multipotent pancreatic progenitor cells and later during embryogenesis they become restricted to the bipotent trunk cells.<sup>30,53–55</sup> This underscores the important fact that these widely used markers cannot discriminate adult differentiated duct cells from embryonic duct or trunk cells (and hence dedifferentiated

duct cells). In this respect, distinct functional markers (eg, those involved in bicarbonate secretion such as CAII and CFTR)<sup>56–58</sup> may be best to characterize fully differentiated duct cells.

Very recent single-nucleus and single-cell RNA sequencing studies have shed light on the existence of different subsets of duct cells in both human and mouse tissues.<sup>30,59,60</sup> For example, a small subpopulation with higher expression of genes linked to mucin secretion and a larger subpopulation showing higher expression of typical duct cell markers such as CFTR and SLC4A4 were noticed by Chiang et al.<sup>30</sup> Qadir et al<sup>60</sup> reported high heterogeneity within the duct cell population with a rather continuous change in differentiation states. While new studies continue to arrive,<sup>59</sup> it will be important considering the relationship between the different RNA expression clusters and their relevance in homeostatic and perturbed conditions of the pancreas.

## Squeezing in Centroacinar Cells

The so-called centroacinar cells are a group of cells located within the center of the acinus at the duct terminus. They share characteristics with both acinar and duct cells. The number of studies on centroacinar cells is very limited in rodents and is nonexistent in humans. In mice, centroacinar cells have shown to be enriched in genes previously associated with embryonic pancreatic progenitor cells (*Sca1*, *c-Met*, and *Nestin*).<sup>61</sup> It is unclear if the Aldh-positive cells, which have been attributed to adult cell plasticity, are centroacinar cells, as was suggested.<sup>61</sup> In the context of tumor formation, centroacinar cells have gained particular interest because of continuous activation of the Notch pathway, known to be important in PDAC biology.<sup>62</sup> Their specific location and the stem cell markers make them a good candidate to be at the origin of PDAC.

## Tuft Cells, the Mysterious Guests

Tuft cells are chemosensory cells present in the digestive tract reacting to inflammatory conditions but absent from healthy pancreas. They are marked by DCLK1 expression,<sup>63,64</sup> although this does not seem to be a specific marker, as lineage tracing uncovered *Dclk*+ acinar and duct cells as well.<sup>65</sup> Induction of ADM and chronic pancreatitis in mice stimulates the formation of tuft cells,<sup>66</sup> suggesting that tuft cells might arise de novo under stress conditions in the

**Table 2.** Gene Expression in Duct and Dedifferentiated Acinar Cells

Dedifferentiated Acinar Cells	Genes in Common	Duct Cells
PTF1A <sup>7</sup>	CK19, CK7 <sup>7,41,42</sup>	DCDC2 <sup>42</sup>
CD142 <sup>41,42</sup>	SOX9 <sup>7,41</sup>	CFTR <sup>42</sup>
GP2 <sup>41,42</sup>	CD133 <sup>7</sup>	CA19.9 <sup>41,42</sup>
MECOM <sup>42</sup>	HES1 <sup>7</sup>	
CPA1, CPA2, CEL, CTRB1 <sup>42</sup>	HNF6, HNF1B <sup>7</sup>	
	CAII <sup>7</sup>	
	PDX1 <sup>7,41,42</sup>	

pancreas or reflects a novel population of ADM cells (Figure 1). Lineage tracing by Maruno et al<sup>67</sup> recently uncovered that *Dclk1*+ cells continuously give rise to progeny within pancreatic intraepithelial neoplasia (PanIN) lesions, suggesting they are at the origin of tumor formation. Their presence in human pancreatitis and PanINs suggests a role in pancreatic tumor formation,<sup>63,66</sup> while a recent report suggested they have a protective role.<sup>66,68</sup>

## Basal Cells, the Unexpected Visitors

In many epithelia, basal cells are a progenitor cell pool in embryonic development and become reactivated in adult tissues during major injury to restore damage. In the pancreas, these cells were presumed nonexistent until we very recently identified cells in the human pancreatic ducts with a location on the basal lamina and typical expression of  $\Delta$ Np63, KRT5, KRT14, and S100A2, all standard markers of basal cells in other tissues.<sup>69</sup> While  $\Delta$ Np63<sup>+</sup> pancreatic cells express KRT19, they overall lack expression of other specific ductal markers such as SOX9 and HNF1 $\beta$ . However, manipulation of  $\Delta$ Np63 in a human pancreatic ductal epithelial cell line resulted in transitions between duct and basal cell differentiation, strongly indicating that basal cells can arise as a result of duct cell plasticity, possibly duct cell dedifferentiation. This was further reinforced by the fact that in patients with chronic pancreatitis, the prevalence of basal cells highly increases in absence of apparent proliferation of these cells (Figure 1).<sup>69</sup>

Because of the lack of evidence for a similar cell type in the adult mouse pancreas,<sup>69</sup> the importance of adequate human experimental models to study basal cells becomes critical.

## Neoplastic Transformation, a Whodunit

Previously, we provided a grasp of the different exocrine cell types that might be implicated in pancreatic tumor formation. Experimental approaches to gain insights into tumor ontogenesis still mainly rely on mouse models that look into the development of PanIN or intraductal papillary mucinous neoplasms (IPMNs) as the main PDAC precursor lesions. Data generated from human cells de facto are very scarce. While the recent establishment of organoid cultures might pave the way for more experimental analysis on human cells,<sup>70</sup> we here discuss the existing models to study normal duct and acinar cells.

The only existing human pancreatic *in vitro* models for duct cells are the human pancreatic duct epithelial (HPDE)<sup>71</sup> and human pancreatic nestin-expressing (HPNE) cell lines.<sup>72</sup> Interestingly, *in vivo* engraftment of HPDE cells carrying a *KRAS*<sup>G12D</sup> mutation (the most prevalent oncogenic mutation in PDAC) resulted in only limited tumor formation.<sup>73</sup> Our recent discovery that HPDE cells mostly resemble basal cells<sup>69</sup> calls for further investigation. We do not know if a basal cell was at the origin of the cell line and if the basal phenotype of HPDE cells is maintained upon *KRAS* mutation.

For acinar cells, no human cell line has been reported and studies with primary cells isolated from human healthy donors are rare, with the cells rapidly dedifferentiating and

undergoing ADM upon cell culture. For better insights, we thus briefly refer to rodent studies.

As for HPDE ductal cells, normal differentiated acinar cells from mice do not readily form tumors upon introduction of oncogenic mutant *Kras*.<sup>74</sup> They need additional insults to progress. For example, chronic or repetitive acute pancreatitis can induce dedifferentiation in acinar cells, which become susceptible for neoplastic transformation.<sup>75,76</sup> Chuvin et al also reported a “spontaneous” induction of ADM by activation of the TGF $\beta$  pathway in acinar cells with formation of PanIN under active oncogenic *Kras* expression<sup>77</sup>. In all these cases, though, a dedifferentiation of acinar cells happened prior to PDAC development. The fully differentiated state of the acinar cells can thus be perceived as a tumor suppressor mechanism. Indeed, by maintaining the expression of *Ptf1a* in acinar cells, their ability to form a tumor is suppressed. Moreover, re-expression of *Ptf1a* in PDAC precursor lesions reverts their status to fully differentiated exocrine cells.<sup>78–80</sup> Similar observations have been published for other acinar cell transcription factors, *Mist-1*,<sup>81</sup> *Nr5a2*,<sup>82</sup> and *Gata6*.<sup>83</sup> In conclusion, while studies using human tissue are lacking, there is vast evidence for mouse acinar cell derived PDAC formation.

A handful of studies have shown that, when using duct cell-specific mutant models, a pancreatic tumor can form as well, mostly through formation of IPMNs.<sup>4,47,48,84–86</sup> Mutant *Kras* activation in duct cells alone does not lead to tumor formation, but it does in combination with homozygous p53 deletion.<sup>87</sup> Although there is sparse evidence for it, loss of functional duct cell differentiation may be a prerequisite for tumor formation, similar to what was observed in acinar cells.<sup>88</sup> A report showed that loss of BRG1 induces the decline of duct cell identity and drives tumor formation from duct cells.<sup>89</sup> This observation calls for better study of ductal cell dedifferentiation.

In the absence of human tumorigenesis studies, indirect evidence from transcriptomic and epigenetic profiling has been building. A recent study<sup>90</sup> searched for the putative cancer cell of origin of different tumor entities by analyzing whole genomes of 32 human cancer types. The mutational landscape was matched to the regional patterns of chromatin modifications from more than 100 normal tissue types. It was found that, despite the mutations, the chromatin structure of the cell of origin is maintained as shown for several cancer types in which the cell of origin is very well established, such as colorectal and lung adenocarcinoma. The predicted matching “normal tissue” for PDAC was stomach mucosa, which is not entirely surprising for several reasons. First, pancreatic acinar cells can be at the origin of pancreatic cancer through dedifferentiation and metaplasia, and dedifferentiated acinar cells express stomach-specific genes, as explained previously.<sup>91</sup> Second, in terms of cellular plasticity, the stomach and pancreas are very much alike, as reviewed in Hibdon et al.<sup>92</sup> Third, Schlesinger et al<sup>32</sup> uncovered a specific subpopulation of ADM cells that acquire a gene signature specific to stomach epithelial cells. Fourth, using *Ptf1a-dtTomato-LSL-Kras*<sup>G12D</sup> mice at time points of spontaneous induction of PanIN

formation and performing single-cell RNA sequencing, researchers have recently identified the existence of different subpopulations of metaplastic acinar cells, including stomach-like cell types.<sup>32</sup> Altogether, said study<sup>90</sup> points to a predominance of acinar cells at the origin of PDAC, while it did not distinguish between PDAC subtypes.

Recently, when we assessed the DNA methylome and transcriptome landscapes of human PDAC neoplastic cells at the genome-wide level, 2 subgroups of tumors were distinguished.<sup>93</sup> One with a Methylation<sup>low</sup>/IFNsignature<sup>high</sup> pattern associated to worse prognosis, and one with a Methylation<sup>high</sup>/IFNsignature<sup>low</sup> profile and better outcome. Interestingly, comparison of these tumor cells to healthy human duct and acinar or dedifferentiated acinar cells at the methylome and transcriptome level, hinted to different ontogenies for the 2 groups of tumors. The more aggressive Methylation<sup>low</sup>/IFNsignature<sup>high</sup> related to a ductal origin, while the less aggressive Methylation<sup>high</sup>/IFNsignature<sup>low</sup> linked to an acinar cell of origin. Moreover, these methylation/IFN groups partially overlapped with the previously reported PDAC molecular subtypes, thus suggesting that the more aggressive basal-like tumors preferentially originate from pancreatic duct cells, while the less aggressive classical tumors may mainly derive from acinar cells. Remarkably, these findings derived from human data concur with several recent observations made in GEMs. In line with a different survival of the patients, for example, duct-derived PDAC GEMs have been reported to show worse survival than acinar-derived models.<sup>4,45</sup> Additionally, when we analyzed tumor cells of mouse PDACs derived from duct cells (*Sox9CreER*; *Kras*<sup>LSL-G12D</sup>; *Trp53<sup>ff</sup>*), a higher expression of the IFN signature was found compared with acinar-derived (*Ptf1aCreER*; *Kras*<sup>LSL-G12D</sup>; *Trp53<sup>ff</sup>*) mouse PDACs.<sup>93</sup> More recently, Flowers et al<sup>94</sup> further showed, at the transcriptomic level, that the basal-like signature is enriched in ductal-derived GEM PDACs, while the classical signature is enriched in acinar-derived GEM tumors. These and additional studies in other tumor entities argue that the intrinsic characteristics of the cell of origin can greatly influence the fate of the neoplastic cells. Altogether, epigenomic and transcriptomic overlays between normal human cell types and PDAC subtypes hint to a relationship between the less aggressive (classical subtype) tumors and acinar cells and the poorer prognostic (basal-like subtype) tumors and duct cells (Figure 1). Basal-like tumors are also linked to worse prognosis in other tumor entities such as skin,<sup>95</sup> prostate,<sup>96–98</sup> and lung.<sup>99,100</sup> Interestingly, all these tumors originate from an epithelium in which basal cells reside, and indeed, evidence exists that basal cells can directly generate basal tumors.<sup>101–107</sup> Now that we have recently identified basal cells in the human pancreas,<sup>69</sup> the question arises if these cells have a role in pancreatic tumor formation.

The growing evidence of the existence of different cell types within the normal acinar and duct compartment, as well as the transitions within and between these fully differentiated states, will necessarily need to be considered in future studies on the ontogeny of PDAC subtypes and the reported plasticity between the cancer subtypes.<sup>70,108,109</sup>

## Concluding and Pondering

Apart from solitary studies on the HPDE cell line that in the end may not recapitulate normal duct cells, most experimental data on cell of origin of PDAC have been gathered from mice. With minimal genetic hits in *Kras*, mouse acinar cells seem more susceptible than duct cells for PDAC formation, provided that there is additional stress that provokes loss of cell differentiation and ADM, which are the origin of PanIN lesions. Sparser studies showed that transformed duct cells give rise to IPMNs rather than PanINs.<sup>84</sup> Because duct cells are a numeric minority in the adult pancreas, it remains, however, uncertain whether this cell type is truly more resistant to tumor formation or if it is a matter of the number of cells that are transformed in these GEM models. Recently, a link has emerged between duct cell of origin and basal-like PDAC tumors. Still, the role of the newly identified basal cells or duct cells dedifferentiating into a basal cell-like state has not yet been considered. Finally, some groups have also reported that tumors might skip the stage of precursor lesions, casting some doubt on the suggested course of acinar-ADM-PanIN-classical PDAC or duct-IPMN-basal-like PDAC.<sup>110–112</sup>

While new subsets of acinar and duct cells have started to be identified, duct cells are much less understood than acinar cells. The discovery of basal cells in the human ducts only reinforces this. Together with the existence of tuft cells and centroacinar cells, it stresses that a binary acinar-duct cell classification in the pancreas has become futile when studying PDAC ontogenesis. The prominent plasticity in exocrine cell differentiation likely also reflects in the plasticity of tumor subtypes. Next to the available GEMs on which most findings have been relying, more experimental model systems with human cells are needed to draw final conclusions on the development of human PDAC, laying an essential foundation for earlier detection and better therapeutic strategies.

## Outstanding Questions Box

Is dedifferentiation per se (of any given exocrine cell type) a prerequisite for tumor development?

To what extent is there a direct relationship of the cell of origin with the transcriptional subtype of a tumor?

Is there one cell of origin for a certain tumor subtype or is the remarkable plasticity of (some) exocrine cells retained in the plasticity of tumor subtypes?

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#### Conflicts of Interest

The authors disclose no conflicts.