Review Article

Ductal *vs.* acinar? Recent insights into identifying cell lineage of pancreatic ductal adenocarcinoma

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Abstract: Pancreatic ductal adenocarcinoma (PDAC) is a deadly disease with a 5-year survival rate of less than 8%. To date, there are no early detection methods or effective treatments available. Many questions remain to be answered in regards to the pathogenesis of PDAC, among which, the controversy over the cell lineage of PDAC demands more attention. Ductal cells were originally thought to be the cell of origin for PDAC due to the ductal morphology of most cases of PDAC. However, recent studies have demonstrated that acinar cells are more sensitive to *KRAS* mutation and tend to develop to PanIN and PDAC effectively, very likely by undergoing acinar to ductal metaplasia into a transient state that contributes to PDAC initiation. There is also evidence that both ductal and acinar cells can potentially develop to PDAC when exposed to certain genetic settings and stimuli, suggesting that more scrutiny is required for the identification of the true cell lineage of individual cases of PDAC. In this work, we summarize recent findings in the identification of the cellular origin of PDAC, with the goal of advancing our knowledge on the initiation and progression of the disease. We also discuss various models and techniques for investigating early events of PDAC. Better understanding of these cellular events is crucial to identify new methods for the early diagnosis and treatment of PDAC.

Keywords: Pancreatic ductal adenocarcinoma (PDAC); ductal cells; acinar cells; cell lineage; cell of origin

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most common form of pancreatic cancer, and the fourth leading cause of cancer deaths in the United States. It is a devastating disease with an overall 5-year survival rate of less than 8%, as currently no early detection methods or effective treatments are available (1). Therefore, understanding the initiation and pathological progression of the disease is crucial to the discovery of novel opportunities for the early diagnosis and treatment of PDAC.

Many questions remain to be answered regarding the pathogenesis of PDAC. In particular, the exact lineage of the cell of origin of PDAC remains unclear. Heterogeneities in morphology, cell surface markers, and drug sensitivity are often noted among different subtypes of tumor cells originating from the same organ (2). The most well-established example would be hematological malignancies, which can be derived from myeloid cell lineage or lymphoid cell lineage and have been classified into more than 100 subtypes according to their cell lineage and clinical features (3,4). Identification of these distinct tumor subtypes thus leads to successful diagnosis and provides guidance for choosing specific therapeutic methods. Recent genomic analysis of PDAC samples identified gene expression patterns which defined several PDAC subtypes that correlate with histopathological characteristics (5-7). Unfortunately, although progress has been made in the

identification of the cellular origins of many different cancers, including breast cancer, colorectal cancer, prostate cancer, and brain cancer (2), the cell of origin for PDAC is still controversial, which greatly hinders the diagnosis and treatment of the disease.

Three major precursor lesions for PDAC have been identified and characterized including pancreatic intraepithelial neoplasms (PanINs), intraductal papillary mucinous neoplasms (IPMNs), and mucinous cystic neoplasms (MCNs). It is generally believed that acinar cells may be the cell of origin of PanINs, while IPMNs are derived from ductal cells (8-13). However, these lesions can co-exist with each other (14,15), which adds another layer of complexity to attempts to understand the roles of these precursor lesions in PDAC initiation and progression.

Due to the ductal morphology of most cases of PDAC, ductal cells were originally thought to be the cell of origin for PDAC (16). However, later studies show that acinar cells, the other type of pancreatic exocrine cell, seem to be more sensitive to common pancreatic cancer driver mutations and tend to develop to PanIN and PDAC efficiently, whereas ductal cells are more resistant to mutant *KRAS* and show limited capacity to develop to PDAC (8,11,17,18). In addition, it has been demonstrated that acinar cells can undergo transdifferentiation to form a population of DCLK1+ cells with pancreatobiliary progenitor phenotype (19,20), which then contribute to PDAC initiation and progression. More recent evidence shows that both ductal and acinar cells can potentially develop to PDAC, but react differently when exposed to certain genetic settings and stimuli (21,22), emphasizing that more scrutiny should be placed in the identification of cell lineages as well as their association with subtypes of PDAC.

In the present article, we summarize recent findings in the identification of the cell of origin for PDAC, aiming to advance our knowledge on the initiation and progression of this disease. We also discuss various models and techniques employed for the investigation of early events of PDAC, as well as their advantages and limitations.

Evidence for the cellular origin for PDAC

PDAC was initially characterized by its ductal, glandular morphology, and so it was conventionally conjectured that PDAC originated from ductal cells (16,23,24). Earlier genetically engineered mouse models (GEMM) of PDAC did not pay attention to cell lineage. The KC (*Kras*/Cre) model, which utilized $Pdx1^{Cre}$ or $p48/Ptf1a^{Cre}$ to activate a conditional knocked-in $Kras^{G12D}$ allele ($LSL\text{-}Kras^{G12D}$) in pancreatic progenitor cells, was the first genetically GEMM to faithfully recapitulate the human PDAC tumorigenesis process in mice (25). Nevertheless, the etiology of this model is still different from that of human PDACs, which more commonly occur in elderly people. More GEMMs have been generated to study gene mutations in PDAC, however, the majority of mouse models use $Pdx1^{Cre}$ or $p48/Ptf1a^{Cre}KRAS$, including the most frequently used KPC ($LSL\text{-}Kras^{G12D/+}$;$LSL\text{-}Trp53^{R172H/+}$;*Pdx-1-Cre*) model (26,27). *Pdx1* and *p48/Ptf1a* are expressed in early progenitor cells during pancreatic development (28). Lineage tracing experiments have shown that both *Pdx1* and *p48/pft1a* expressing cells contribute to all the cell lineages in the pancreas, including both acinar and ductal cells (29-31). The expression of these genes shows relatively restricted pattern in specific cell types in the adult stage; *Pdx1* is expressed abundantly in beta cells with lower levels in acinar cells, while *Ptf1a* is expressed primarily in acinar cells (32,33). Thus, GEMMs of PDAC which use $Pdx1^{Cre}$ or $p48/Ptf1a^{Cre}$ have unspecific cell lineage. This limitation may explain some of the unexplained GEMM phenotypes. For example, using a *Pdx1*- and *Ptf1a-Cre* transgenic mice model, Nabeel Bardeesy *et al.* observed that while $Kras^{G12D}$ alone led to PanIN formation, the combination of $Kras^{G12D}$ and *Smad4* deficiency resulted in the development of IPMN (34). To address this problem, recent efforts have been made in generating GEMMs with Cre or CreER driven by more specific lineage promoters (31).

Unexpectedly, several GEMMs have suggested that, without additional mutations, ductal cells are relatively resistance to oncogenic *Kras*-induced formation of PDAC precursor lesions. Kopp *et al.* used transgenic $Sox9^{CreER}$ to activate the expression of a knocked-in $Kras^{G12D}$ allele ($LSL\text{-}Kras^{G12D}$) in ductal cells at postnatal day 10 (11). Although the $LSL\text{-}Kras^{G12D}$ allele was effectively recombined by $Sox9^{CreER}$ in about 12% of ductal cells, these mice rarely developed PanIN lesions between the ages of 8 and 17 months. In another model, Ray *et al.* used a knockin $Ck19^{CreERT}$ to activate $Kras^{G12D}$ expression in larger pancreatic ductal cells between the ages of 6 and 8 weeks (18). In the six mice examined at 4.5 months post-tamoxifen treatment, only two displayed mucinous ductal lesions. Although all five mice exhibited PanIN lesions at the age of 6 months, the absolute numbers of lesions were still low. In addition, it was found that the duct-derived lesions were primarily limited to the large ducts rather than randomly distributed throughout the

pancreas.

Nevertheless, several recent studies have revealed that oncogenic *Kras* could initiate PDAC tumorigenesis in ductal cells in the presence of additional mutations. Kopp *et al.* used transgenic *SOX9*^{CreER} to delete the tumor suppressor *Pten* in ductal cells at the age of 4 weeks (35). These mice developed intraductal papillary lesions resembling human intraductal papillary mucinous neoplasia between the ages of 6 and 14 months. Some of these IPMNS progressed to invasive PDACs in association with the acquisition of spontaneous KRAS mutation. In line with this observation, ductal depletion of *Pten* in the context of oncogenic *Kras*^{G12D} resulted in development of IPMN-associated invasive PDACs in one month. Another study used the same transgenic *Sox9*^{CreER} to simultaneously activate a knockin *Kras*^{G12D} allele and delete *Trp53* in ductal cells between the ages of 3 and 4 weeks (36). These mice developed microscopic tumors as early as 4 weeks post-tamoxifen injection and reached their humane endpoint within 10–13 weeks. It is noteworthy that only small numbers of high grade PanINs were found in these mice. Ferreira *et al.* introduced the *Kras*^{G12D} mutation and *Fbw7* deletion into ductal cells by use of knocked-in *Ck19*^{CreERT} (22). This model developed *in situ* carcinoma devoid of PanIN lesions within 1 month post-tamoxifen injection. These data imply that either the acquisition of additional mutations in the presence of oncogenic *Kras* results in ductal cells bypassing the PanIN stage or, more likely, that ductal cells might be responsible for PDAC initiated from a low-grade PanIN-independent route.

In the past decade, the potential role of the pancreatic acinar cell lineage as the cell of origin of PDAC has been extensively studied. Guerra *et al.* crossed double transgenic *Elase-tTA/Tet-O-Cre* mice, which express Cre recombinase under the control of the *Elastase* promoter in a tet-off system, with a line carrying a knockin conditional *Kras*^{G12V} allele (*LSL-Kras*^{G12V}) (8). In this model, oncogenic *Kras*^{G12V} expression was turned on in 20% to 30% of acinar cells as well as a low number of centroacinar cells during late embryonic development if doxycycline was not provided in the drinking water. These mice readily developed full spectrum PanIN lesions, and some progressed to PDACs with about 1-year latency. The similarity of the phenotype of this model and that of KC mice suggests that acinar cells might be the origin of PDACs developed in those models.

Taking advantage of the fact that *Ptf1a* expression is limited to the acinar cell lineage in the pancreas after birth, Kopp *et al.* gave *Ptf1a*^{CreER}*Kras*^{G12D}*R26R*^{YFP} mice tamoxifen injections at postnatal day 10 to activate expression of oncogenic *Kras*^{G12D} and YFP reporter in acinar cells (11). This lineage tracing model displayed abundant YFP+ PanIN lesions in mice between the ages of 8 and 17 months, clearly demonstrating that these PDAC precursor lesions were derived from acinar cells. Similar phenotypes were also observed in several other postnatal GEMMs using *proCPA1*^{CreERT2} or *Mist1*^{CreERT2} to turn on oncogenic *Kras* expression in acinar cells (9,10,37). These data support the notion that at least embryonic or young acinar cells are susceptible to oncogenic *Kras*-induced PDAC initiation via a PanIN-dependent route.

In contrast to embryonic or young acinar cells, *Elase-tTA/Tet-O-Cre/LSL-Kras*^{G12V} mice failed to develop even low-grade PanINs if *Kras*^{G12V} expression in acinar cells was induced at 60 days after birth (8). Under this condition, oncogenic *Kras*^{G12V} was only expressed in adult acinar cells, suggesting that adult acinar cells were highly refractory to oncogenic *Kras*-mediated malignant transformation. These mice still developed full spectrum PanIN lesions and invasive PDACs if they were challenged with induction of pancreatitis, suggesting that inflammatory insults restored the susceptibility of adult acinar cells to oncogenic *Kras*-induced transformation.

The tumor promoting effects of pancreatitis on acinar cells have been observed in several other GEMMs (11,38). These observations have important clinical relevance because chronic pancreatitis is a major risk factor for human PDAC (39). Several clues point to the critical contributions of inflammation induced acinar-to-ductal metaplasia (ADM) to the PDAC promoting effects of pancreatitis (40). Interestingly, Jensen *et al.* found that acinar cells recapitulate elements normally associated with undifferentiated progenitor cells in pancreatitis (41). It is possible that pancreatitis-induced ADM might alter adult acinar cells to a state similar to that of developing acinar cells, thus restoring their susceptibility to oncogenic *Kras*-mediated transformation. Liou *et al.* demonstrated that macrophage-secreted cytokines and tumor necrosis factor α can drive ADM through activation of NF-κB and MMPs, which represents a possible mechanism of pancreatitis-induced ADM (40).

Kopp *et al.* reported that *Kras*^{G12D}-driven PanIN formation from acinar cells proceeds through initiation of a gene expression program similar to ductal cells, suggesting that the transition of acinar cells to ductal-like cells is critical for the early development of PDAC (11). Consistently, it was also observed that SOX9, which is primarily expressed

in ductal cells, was induced in acinar cells with *Kras*^{G12D} mutation, which in turn promoted acinar-to-ductal metaplasia and PanIN formation. In addition to acinar-to-ductal metaplasia, an acinar-to-pancreatobiliary cell conversion was previously proposed to cause the initiation of PDAC. DelGiorno *et al.* and Bailey *et al.* identified a group of Dclk1+ and acetylated tubulin+ tuft cells which showed high similarity with pancreatobiliary epithelium cells in PanIN lesions from *Kras* mutant mouse models (19,20). Further analysis suggested that *Sox17* could induce the conversion of acinar cells to Dclk1+ and acetylated tubulin+ pancreatobiliary cells, which may represent cancer stem cells and contribute to PDAC initiation. More recently, it was reported that Dckl1+ cells are primarily located in the acinar compartment, and low-grade PanINs were infrequently observed in *Dclk1*^{CreERT}*Kras*^{G12D} mice after induction with Tamoxifen, while cerulein treatment of *Dclk1*^{CreERT}*Kras*^{G12D} mice led to the rapid development of multiple PanINs (42).

Genetic variance in acinar vs ductal-derived PDAC

As discussed above, acinar and ductal lineages possess conspicuously different susceptibilities to oncogenic *Kras* insult. This raises the possibility that acinar and ductal cells might require distinct genetic/epigenetic abnormalities to promote *Kras*-induced PDAC tumorigenesis. However, knowledge in this field is still very limited and more questions remain to be answered.

BRG1-SOX9 axis

BRG1 is a component of switch/sucrose non-fermentable (SWI/SNF) chromatin-remodeling complexes. Somatic BRG1-inactivating mutations and deletions were found in human PDACs (43,44). Figura *et al.* showed that knockout of *Brg1* in *Ptf1a*^{Cre}; *Kras*^{G12D}; *Brg1*$^{f/f}$ mice resulted in formation of cystic neoplasms resembling human IPMNs (12). However, it is noted that loss of Brg1 in the developing pancreas results in an abnormal pancreatic development, which may account for the observed cystic pancreas. They further generated *Hnf1*^{CreERT2}*Kras*^{G12D}*Brg1*$^{f/f}$ mice to simultaneously introduce *Kras*^{G12D} and *Brg1* deletion into adult ductal cells. The authors observed atypical duct cells in a subset of pancreatic ducts from 5 out of 5 experimental mice 6 weeks after tamoxifen induction, only 1 out of 5 developed lesions resembling human IPMNs. In comparison, the mice in the control group (*Hnf1*^{CreERT2}*Kras*^{G12D}*Brg1*$^{f/+}$) were normal after tamoxifen injection. These data together may suggest a suppressive role of Brg1 in adult ductal cells in PDAC tumorigenesis. However, the non-pancreatic phenotypes in the *Hnf1*^{CreERT2}*Kras*^{G12D}*Brg1*$^{f/f}$ mouse model limited the conclusions that could be drawn from this study regarding cellular origin.

Interestingly, *Brg1* seems to play a supporting role in oncogenic KRAS-induced PDAC initiation in acinar cells. *Ptf1a*^{CreER}*Kras*^{G12D} mice formed full spectrum of PanINs after tamoxifen injection, while the number of PanINs formed in *Ptf1a*^{CreER}*Kras*^{G12D}*Brg1*$^{f/f}$ mice after tamoxifen injection was significantly lower. Moreover, depletion of *Brg1* in established PanINs with an elegant dual recombinase system induced regression of the lesions *in vivo*, strongly suggesting that Brg1 has a critical role in acinar cells-derived PDAC progression (45).

The different expression levels of SOX9 in acinar and ductal cells may partially explain the seemly paradoxical roles of *Brg1* in these two lineages for PDAC tumorigenesis. Adult acinar cells express little to no SOX9 under normal conditions. However, it is ectopically induced to an "intermediate" level in acinar-derived ADM and PanIN lesions. In fact, knockout of *Sox9* in adult acinar cells substantially inhibited oncogenic *Kras*-induced ADM and PanIN formation (11). Tsuda *et al.* found that BRG1 directly bound to the promoter of SOX9 to induce its expression (45). Depletion of *Brg1* in acinar cells prevented SOX9 up-regulation and thus inhibited *Kras*-induced ADM and PanIN. In line with this observation, overexpression of SOX9 rescued the *Kras*-driven PanIN phenotype in *Brg1* knockout acinar cells, highlighting the importance of the BRG1-SOX9 axis in acinar lineage, PanIN-dependent PDAC progression. On the other hand, a high level of SOX9 expression is shown to play a role in maintaining ductal cell morphology and regulating the expression of duct-specific genes (46-48). It is possible that down-regulation of SOX9 to an "intermediate" level in ductal cells might enhance their susceptibility to oncogenic *Kras* insult. Indeed, in *Hnf1*^{CreERT2}*Kras*^{G12D}*Brg1*$^{f/f}$ mice, SOX9 expression was reduced in the dysplastic epithelium of the IPMN-like lesions (12), and overexpression of SOX9 reduced IPMN formation in an *ex vivo* model in a follow-up study (49). Nevertheless, it has not been directly tested whether SOX9-haploinsufficiency in adult ductal cells will increase their susceptibility to oncogenic insults. Whether SOX9 regulates different targets in a dose-dependent manner in

adult ductal cells and PDAC precursor lesions also remains unanswered, and any exploration of this question may improve our understanding of PDAC tumorigenesis.

ARID1A

ARID1A is another frequently mutated component of the SWI/SNF complex in human PDAC. In a recent study, Wang SC *et al.* demonstrated that *Arid1a* deletion along with oncogenic *Kras* led to pancreatic mucinous cysts in *Ptf1a*^{Cre}*Kras*^{G12D}*Arid1a*$^{f/f}$ mice which resemble human IPMN (50). They further introduced *ductal cell-specific Arid1a loss in Sox9*^{CreER}*Kras*^{G12D}*Arid1a*$^{f/f}$ mice, which displayed duct dilation 3–4 months after induction and 1 out of 5 mice developed a cystic lesion after 1 year of induction, in association with elevated level of c-MYC expression. In comparison, the authors observed that heterozygous Arid1a deletion in acinar cells promoted inflammation, PanIN, and PDAC formation in *Ptf1a*^{CreER}*Kras*^{G12D}*Arid1a*$^{f/+}$ mice and *Ptf1a*^{CreER}*Kras*^{G12D}*Tp53*$^{f/+}$*Arid1a*$^{f/+}$ mice, compared to wild type or null Arid1a controls.

In another recent study, Kimura *et al.* reported that *Ptf1a*^{Cre}*Kras*^{G12D}*Arid1a*$^{f/f}$ mice developed both PanINs and IPMNs after birth (51). More specific lineage tracing analysis showed that acinar specific *Ptf1a*^{CreERT2}*Kras*^{G12D}*Arid1a*$^{f/f}$ mice developed PanINs but not IPMNs after tamoxifen injection. *In comparison*, while oncogenic *Kras*^{G12D} in adult ductal cells (*Hnf1*^{CreERT2}*Kras*^{G12D}) alone did not induce any sign of ductal atypia, *Hnf1*^{CreERT2}*Kras*^{G12D}*Arid1a*$^{f/f}$ mice (1 out of 6) formed IPMN lesions after tamoxifen injection.

Although these two reports seem to suggest that loss of *Arid1* promotes PDAC tumorigenesis both in the acinar and ductal cell lineages, the limited number of IPMN formation from ductal cells with *Arid1a* deletion makes it difficult to identify the role of *Arid1a* in ductal derived PDAC initiation. In addition, similarly to *Brg1* knockout, *Arid1a* deletion down-regulates SOX9 in adult ductal cells to facilitate a dedifferentiation process, highlighting the critical role of SWI/SNF complex-mediated chromatin modifications to the maintenance of ductal cell identity.

The functions of Arid1a in acinar and ductal derived PDAC initiation were also investigated in several other studies (52,53). For example, Livshits *et al.* reported that acute *Arid1a* knockdown in acinar cells in the context of mutant *Kras* resulted in increased PanIN formation associated with loss of acinar identity (52). Using *Ptf1a*^{Cre}*Arid1a*$^{f/f}$ mice, Wang W *et al* reported that *Arid1a* deletion led to progressive loss of the acinar cell mass, ADM, and progression of ductal cystic lesions (53). In the context of oncogenic *Kras*, *Arid1a* deletion was found to accelerate IPMN progression and PDAC formation in *Ptf1a*^{Cre}*Kras*^{G12D}*Arid1a*$^{f/f}$ mice, compared to *Ptf1a*^{Cre}*Kras*^{G12D} control. In addition, while PanINs were the most common lesions in *Ptf1a*^{Cre}*Kras*^{G12D}*p53*$^{f/+}$ mice, concurrent *Arid1a* deletion led to a shift to predominant IPMNs in *Ptf1a*^{Cre}*Kras*^{G12D}*p53*$^{f/+}$*Arid1a*$^{f/f}$ mice. These observations suggest that *Arid1a* is important for maintaining acinar homeostasis, while suppressing *Kras*-driven IPMN formation. Lineage specific Cre lines are needed to dissect the distinct contributions of *Arid1a* to acinar and ductal derived PDAC formation.

SMAD4

Inactivating-SMAD4 mutations are rarely found in low grade PDAC precursor lesions, but they are common in PDACs as well as high grade PanINs and IPMNs (54,55). Thus, *Smad4* mutations have been conventionally considered to associate with late stage progression of PDACs, and its potential roles in early PDAC development have largely been ignored. Both *Ptf1a*^{Cre}*Kras*^{G12D}*Smad4*$^{f/f}$ and *Pdx1*^{Cre}*LSL-Kras*^{G12D}*Smad4*$^{f/f}$ *mice showed similar phenotypes to those observed in Ptf1a*^{CreER}*Kras*^{G12D}*Brg1*$^{f/f}$mice (34,56). These mice did not develop PanIN lesions, but rapidly formed IPMNs/MCN after birth, raising the possibility that inactivation of Smad4 at an early stage may impair PDAC tumorigenesis from acinar lineage. To date, *Smad4* has not been specifically deleted in acinar or ductal cells to investigate its contributions to PDAC tumorigenesis in a lineage specific context. The SMAD proteins have been shown to interact with BRG1 to activate transcription via orchestration of chromatin remodeling. It is possible that SMAD4 is required for BRG1-mediated ADM induction in the early stages of PDAC tumorigenesis of acinar cell lineage, consistent with our previous report that SMAD4 was required for TGFβ induced ADM of human primary acinar cells *in vitro* (57). The possibility that SMAD4 may play complicated roles in a stage- and cell lineage-dependent manner emphasizes the demand for new models to carefully evaluate its functions in PDAC tumorigenesis.

PTEN

PTEN is a well know tumor suppressor that plays critical roles in controlling the PI3K pathway. Although PTEN

inactivation mutations have not been identified in the majority of PDACs, loss and down-regulation of PTEN expression are common (58). *Pten* loss in adult ductal cells enables oncogenic *Kras*-induced PDAC development via the IPMN route, similarly to *Brg1*, but the underlying mechanisms are likely different (35). While *Brg1* deletion only induced IPMN formation in the presence of oncogenic *Kras* mutation, depletion of *Pten* in ductal cells alone was sufficient to induce formation of IPMN lesions. Moreover, some IPMNs subsequently gained spontaneous *Kras* mutations and progressed to invasive PDACs, suggesting that *Pten* deficiency might result in chromosomal instability in ductal cells.

The effects of PTEN on PDAC tumorigenesis in acinar cells have not been directly tested. The PDAC progression in $Pdx1^{Cre}Kras^{G12D}Pten^{f/+}$ mice was significantly accelerated when one copy of *Pten* was deleted in pancreatic progenitor cells (59). Interestingly, the PDACs in this model were mainly developed in a PanIN-dependent manner, suggesting that tumors were most likely initiated from the acinar lineage. In line with this speculation, a large number of ADMs were observed before PanIN formation in this model. Mechanistic analysis revealed that *Pten* deletion synergized with oncogenic *Kras* to induce strong stromal response and NF-κB signaling activation, which likely contribute to ADM induction in this model. Nevertheless, the potential involvement of ductal cells in this model cannot be disregarded. The roles of *Pten* in acinar-derived PDAC tumorigenesis need to be tested more rigorously with appropriate lineage tracing models.

P53

Tumor suppressor p53 is frequently mutated in PDAC. It is not surprising to see a large body of evidence showing inactivation of *Trp53* (either heterozygous or complete loss) in acinar or ductal cells in the presence of oncogenic *Kras* contributed to PDAC formation in genetically engineered mice models (8,12,22,36,50). Interestingly, evidence suggests that gain-of-function mutation of *Trp53* along with oncogenic *Kras* can also promote both acinar- and ductal-derived PDAC formation. For example, Baily *et al.* reported that oncogenic activation of *Kras* along with introduction of one allele of mutant $Trp53^{R172H}$ is sufficient to induce transformation of acinar cells in their mouse model ($Mist1^{CreERT2}$) (21). In comparison, simultaneous activation of oncogenic Kras and two alleles of mutant $Trp53^{R172H}$ in adult ductal cells ($Hnf1b^{CreERT2}$) are required to generate PDAC as early as 2 months after tamoxifen induction. While the mechanism by which the gain-of-function mutation of *Trp53* could promote PDAC development remains unclear, a possible explanation is that the gain-of-function mutation may suppress wild type p53 function through a dominant negative effect (60).

FBW7

The tumor suppressor FBW7 is found to associate with PDAC biology, and its protein expression is downregulated in PDAC patients (61). Ferreria *et al.* reported that *Fbw7* deletion together with oncogenic $Kras^{G12D}$ in adult acinar cells ($Elastase\ 1^{CreER}$) and adult ductal cells ($Ck19^{CreER}$) both resulted in formation of carcinoma in mouse model (22). However, the ductal lineage derived PDAC seemed to develop independently of low grade PanIN. Interestingly, the authors also demonstrated that embryonic deletion of *Fbw7* in $Kras^{G12D}Pdx1^{Cre}$ mice only promoted proliferation and transformation of ductal cells, but not acinar cells.

Taken together, the abovementioned studies make it clear that acinar and ductal cells can respond to certain genetic contexts differently, which adds another layer of complexity for elucidating PDAC initiation (*Figure 1*). On the other hand, the cell lineage-specific characteristics may provide opportunities for the development of new diagnostic and treatment strategies for the disease.

Clinical implications of PDAC cell of origin

The fact that PDACs can be derived from both acinar and ductal lineages raised the question of whether the cell origin affects the properties of PDAC. Figura *et al.* compared IPMN-PDAC from $Ptf1a^{Cre}Kras^{G12D}Brg1^{f/f}$ mice with PanIN-PDAC from $Ptf1a^{Cre}Kras^{G12D}p53^{f/+}$ mice (12). Ductal derived IPMN-PDAC developed with shorter latency than acinar derived PanIN-PDAC. However, IPMN–PDAC mice survived longer, and most of them died due to pancreatic exocrine insufficiency without any indications of death from malignant disease. Similar to this study, Kimura *et al.* compared IPMN-PDAC and PanIN-PDAC derived from $Ptf1a^{Cre}Kras^{G12D}Arid1a^{f/+}$ and $Ptf1a^{Cre}Kras^{G12D}Arid1a^{f/f}$ mice, respectively (51). They also found that IPMN-PDAC developed faster than PanIN-PDAC, but no significant difference in survival was observed. It needs to be pointed out that these tumors carried different mutations and were not generated from specifically adult acinar or ductal cells, and thus the contributions of cell origin to the cancer

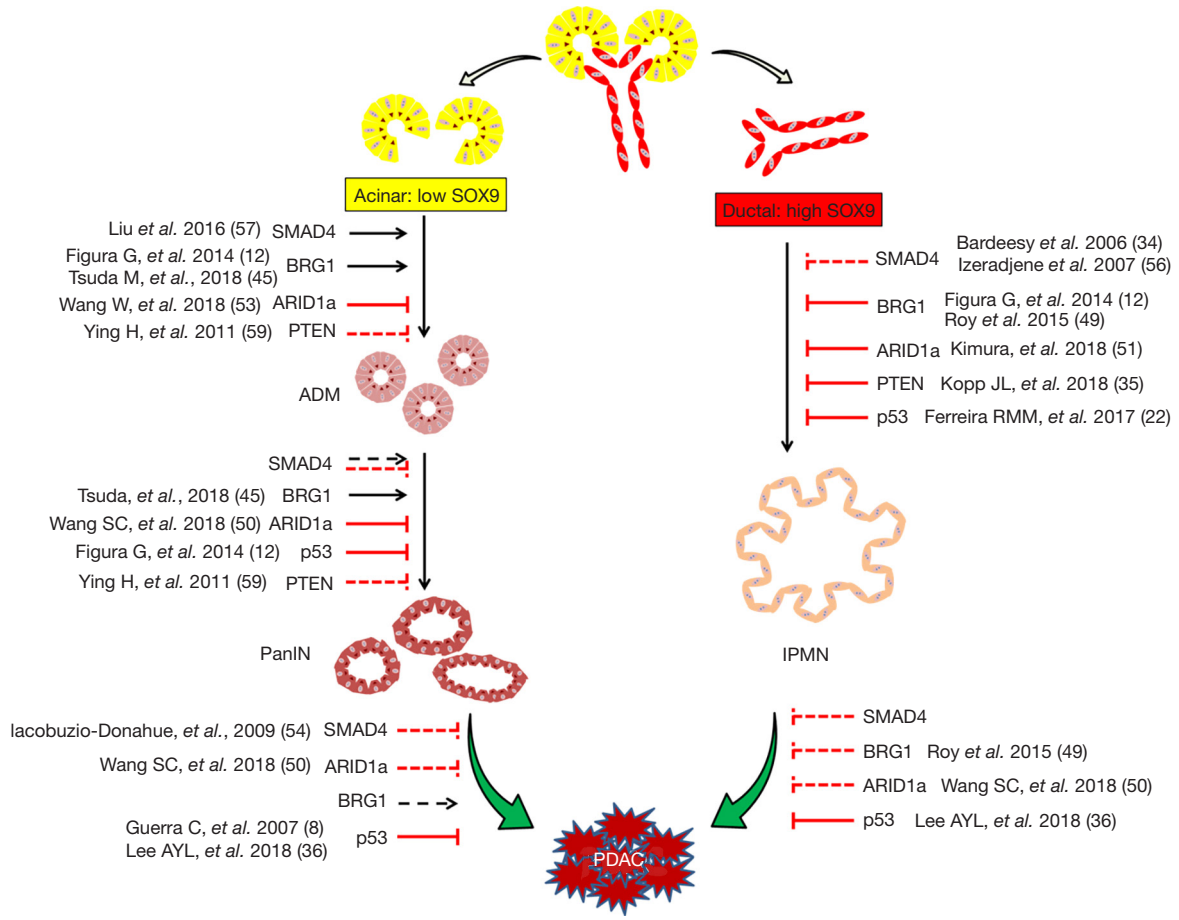

Figure 1 Summary of recent findings on the genetic context in favor of acinar *vs.* ductal-derived tumorigenesis (8,12,22,34-36,45, 49-51,53,54,56,57,59). Evidence suggest that acinar and ductal cells are capable of transformation and progression to pancreatic ductal adenocarcinoma (PDAC) in response to different genetic abnormalities. For example, BRG1 may suppress ductal-derived PDAC tumorigenesis wile playing supporting roles in acinar-derived PDAC initiation. The effects of SMAD4 on PDAC development are rather complicated, and seem to act in a stage- and cell lineage-dependent manner. At early stages, SMAD4 is believed to inhibit ductal-derived PDAC initiation while being essential for ADM. However, at later stages, SMAD4 may play a tumor suppressive role in both acinar and ductal-derived PDAC formation. In comparison, consistent findings have been reported in regard to the suppressive role of ARID1a, p53 and PTEN in both acinar and ductal-derived tumorigenesis. The solid lines in the figure indicate demonstrated functions of BRG1, SMAD4, PTEN, p53 and ARID1a in PDAC development, while the dashed lines indicate proposed effects from these genes inferred from current evidence.

phenotype should be interpreted carefully.

Two recent studies showed that PDAC initiation could be triggered in both acinar and ductal cells with an identical combination of oncogenic drivers (*Kras*^{G12D} and *TP53* deletion) (22,36). The invasive tumors derived from both lineages were histologically indistinguishable, but they developed via two distinct PanIN-dependent and independent manners. Both groups found that the ductal-derived invasive PDACs formed faster than acinar-derived invasive tumors. The mice with ductal-derived PDACs had shorter median survival than mice with acinar-derived tumors. Additionally, Lee *et al.* reported that distant metastasis only happened with ductal-derived PDACs (36). At the molecular level, a few markers including Keratin 20 and AGR2 were found to be differentially expressed between acinar- and ductal-derived PDACs (22,36). Still, a more comprehensive analysis is essential to evaluate the contributions of cell of origin to the heterogeneity

of PDAC.

Together, these data suggest that the cell of origin can affect PDAC development and phenotype, and emphasize the importance of the comprehensive considerations of genetic context when evaluating the contributions of cell origin to PDAC phenotypes.

Different models used to study PDAC cell lineage

Upon careful examination of previous reports, it is evident that different genetic environments are critical to the determination of the fate of acinar and ductal cells. Therefore, it is crucial to develop proper models and methodologies to identify the distinct sets of driver mutations for each cell lineage during PDAC tumorigenesis.

To date, the most compelling data regarding PDAC origination were generated using genetically engineered mouse models (13,31), with the *Kras*^{G12D} being the most frequently introduced mutation. The mutation was introduced by using the Cre/LoxP system guided by a cell type-specific gene promoter, a so-called Cre driver. The most commonly used Cre for acinar cells is driven by the promoters of *Elastase*, *Mist1*, *Ptf1a*, etc., while *Sox9*, *Ck19*, and *Hnf1* promoters are most frequently used for targeting ductal cells. Despite the advantage of cell type specificity to study of PDAC initiation, engineered mouse models using the Cre/LoxP system may not properly recapitulate the disease mechanisms in humans. For example, SMAD4 is found to be frequently inactivated in human PDAC (~50%), and thus efforts have been made to create *Smad4* knockout mice models to mimic PDAC progress in human. However, results showed that *Smad4* knockout in mice led to formation of IPMN or MCN-like lesions, which are different from those observed in humans, raising questions about the reliability of mouse models in the study of human PDAC (62).

Given the convincing evidences from lineage tracing studies using GEMM, it would be reasonable to speculate that PDACs can also originate from both acinar and ductal cells in human patients. It is practically infeasible to apply *in vivo* lineage tracing technique to human beings, and thus robust *ex vivo* PDAC tumorigenesis models would be valuable tools to investigate this question.

Huang *et al.* induced the differentiation of human pluripotent stem cells (PSCs) into pancreatic exocrine progenitor organoids (63). These organoids could be further polarized towards acinar and ductal lineages in 3D culture. Expression of mutant *KRAS* and *TP53*, a combination which is sufficient to induce mouse PDAC initiation, in these human pancreatic progenitor organoids only induced abnormal ductal architecture and nuclear morphology consistent with neoplastic transformation both in culture and *in vivo*. Thus, more work is required to recapitulate the full spectrum of human PDAC tumorigenesis with this model. It is noteworthy that pancreatic progenitor organoids could be polarized towards acinar and ductal lineages in 3D *ex vivo* culture, as it indicates that this model has potential to investigate human PDAC tumorigenesis in specific exocrine lineages.

Boj *et al.* recently developed a Wnt signaling-dependent 3D culture method for primary PDACs from human patients (64). Importantly, these PDAC organoids retained similar phenotypes to the original PDACs in patients, suggesting that a 3D organoid culture system might be a robust *ex vivo* system for study of human PDAC. Importantly, primary normal exocrine pancreatic cells, probably ductal cells, from human organ donors could also be propagated with the same method. This system is compatible with genetic engineering techniques, highlighting its potential to model human PDAC tumorigenesis. Indeed, Seino *et al.* showed that normal human exocrine pancreatic cells could be engineered to malignant cells which recapitulated human PDACs by introducing oncogenic *KRAS*^{G12D} and deletion of *TP53*, *SMAD4* and *P16* (65). Thus, genetically engineering human primary exocrine pancreatic cells represents another appealing strategy to investigate the mechanisms of human PDAC tumorigenesis.

Efforts have been made to use flow-cytometry to sort primary human pancreatic ductal cells and genetically modify them to carry the four most common PDAC driver mutations (*KRAS*^{G12V} and deletions of *TP53*, *SMAD4* and *P16*) (66). Unexpectedly, these cells failed to give rise to invasive PDAC when transplanted into the pancreases of NSG (NOD/SCID/gamma, NOD.Cg-Prkdcscid IL2R tm1Wjl/SzJ) immune-deficient mice. These data raised the possibility that additional abnormalities might be required for human PDAC initiation from ductal lineage. It should be noticed that these cells were expanded without Wnt protein. Given the fact that *KRAS*, *TP53*, *SMAD4*, and *P16* mutations did not confer Wnt-independency to PDACs, the *ex vivo* expansion under Wnt-free condition might have impaired the tumorigenicity of these genetically modified cells. Thus, well-controlled experiments are still required to draw a solid conclusion.

To further examine the roles of cellular origin in human PDAC tumorigenesis, our lab has developed flow

cytometry-based methods to separate and isolate viable primary acinar and ductal cells from normal human exocrine pancreatic tissues (57,67). We found that a combination of UEA/CD133/CLA surface markers can be used to separate viable acinar and ductal cells from the adult human exocrine pancreas by flow cytometry, which can be used in *ex vivo* lineage tracing experiments. Transient TGF-β exposure accelerated the ADM process in sorted primary human acinar cells, which synergized with oncogenic *KRAS* to promote the growth of acinar-derived ductal-like cells. These data suggest the importance of ADM in human PDAC initiation from acinar lineage, but the underlying mechanisms need to be further investigated. More recently, we found that both primary acinar and ductal cells could be expanded in 3D culture (unpublished data), which enabled us to directly compare human PDAC tumorigenesis in these two lineages. We have generated invasive PDAC from both acinar and ductal cells (unpublished data). Our ongoing studies are focused on identifying lineage-specific oncogenic drivers for human PDAC and evaluating their effects on PDAC phenotypes such as drug resistance and metastatic potential.

Despite recent promising findings from genetically engineered primary human primary pancreatic cells, theses *ex vivo* models are also associated with several concerns. For example, transplantation of primary cells to immunodeficient mice does not allow us to investigate cell fate in the context of the immune system, and so it does not fully recapitulate human disease. Due to cellular plasticity, the *in vivo* cultured exocrine cells may change their cell fate after isolation from endogenous content. Therefore, caution should be taken when interpret the data generated from these models.

Conclusion and summary

PDAC remains one of the deadliest diseases in the world, however, its pathogenesis is still unclear and the cell lineage of PDAC is controversial. Identification of the cellular origins and the associated driver mutations of PDAC will offer opportunities for cancer prevention and provide insight into the development of diagnostic methods and new therapeutic treatments.

Due to technical advances in genetic engineering, researchers have been extensively interrogating the cell of origin for PDAC using *in vivo* murine models. It has now become clear that acinar cells are more sensitive to *KRAS* mutation than ductal cells, and can undergo acinar-ductal metaplasia to effectively develop PanIN and eventually PDAC. By comparison, although ductal cells seem refractory to single *KRAS* mutation, they still can transform to PDAC by other mechanisms when exposed to certain genetic settings. These findings suggest that more scrutiny should be placed on the identification the actual cell lineage of individual cases of PDAC.

A major obstacle that remains is that current *in vitro* and *in vivo* models suffer from critical drawbacks in the identification of PDAC cell lineage, which questions the reliability of the research outcomes. Therefore, there is an urgent need to develop new methodologies to investigate PDAC initiation. Models employing normal human pancreatic acinar and ductal cells allow for identification of the cell lineage of human PDAC. In addition, Shen *et al.* recently developed a sensitive tumor detection and classification method using plasma cell-free DNA methylomes (68), suggesting the possibility of identifying cancer cell lineage using lineage specific epigenetic alterations. The development of new models and techniques for investigating the early events of PDAC will help to advance our knowledge on the initiation and progress of this disease.

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Footnote

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