Review Article

Genetics and biology of pancreatic cancer and its precursor lesions: lessons learned from human pathology and mouse models

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Abstract: Pancreatic ductal adenocarcinoma (PDA) is one of the most fatal malignancies; it has an extremely poor prognosis due to its late diagnosis and limited response to conventional treatments. To improve PDA prognosis, new diagnostic and treatment strategies are urgently required. Recent genomic sequencing analyses revealed several mutated core signaling pathways and transcriptomic subtypes in PDA. A better understanding of PDA biology based on these genetic insights would promote the future development of novel diagnostic methods and treatments. In this review, we summarize our current understanding of PDA genetics and biology, predominantly via insights from mouse model studies.

Keywords: Pancreatic cancer; mouse model; biology; genetics

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Pancreatic ductal adenocarcinoma (PDA) has one of the worst prognoses of human cancers and it is the fourth leading cause of cancer-related deaths in both the US and Japan (1,2). Worldwide, PDA accounts for more than 200,000 deaths per year, with the total number of PDA-related deaths currently increasing and PDA predicted to be the second leading cause of cancer-related deaths in the US by 2030 (3). To improve its prognosis, new diagnostic and treatment strategies are urgently required; thus, a better understanding of the genetics and biology of PDA and its precursor lesions is important. Here, we review the current knowledge regarding the genetics and biology of PDA and its precursor lesions, mainly obtained through mouse model studies.

Genomic events in PDA

Recent genomic sequencing analyses have revealed the mutational landscape of PDA, which has four common oncogenic events in well-known cancer genes (*KRAS*, *TP53*, *SMAD4*, and *CDKN2A*; known as the "Big 4") (4-8). In particular, gain-of-function *KRAS* gene mutations occur in most PDA cases (>90%). Mutations in genes not included in the "Big 4" occur with a relatively low prevalence. Significantly mutated genes can be grouped into several core signaling pathways. The initial whole exome sequencing study of 24 PDA samples identified 12 core signaling pathways involved in PDA: KRAS signaling, TGF-β signaling (including *SMAD4*), JNK signaling, integrin signaling, WNT/NOTCH signaling, Hedgehog signaling, control of G1/S phase transition (including *CDKN2A* and *TP53*), apoptosis, DNA damage control, small GTPase-signaling, invasion, and homophilic cell adhesion (4). The second whole exome sequencing study of 99 PDA samples identified axon guidance, including SLIT/

ROBO signaling, as a novel pathway involved in PDA (5). Resent whole genome sequencing data on 456 PDA samples grouped the mutated genes into ten molecular mechanisms: activating *KRAS* mutations, disruption of G1/S checkpoint machinery, TGF-β signaling, NOTCH signaling, WNT signaling, chromatin modification, the SWI/SNF complex, DNA-damage repair genes, SLIT/ROBO signaling, and RNA processing genes (8). These findings imply that these pathways are important for PDA development and/or progression, although their functions need to be explored further.

Recently, the transcriptomic subtyping of PDA has also been performed. The initial study defined three PDA subtypes: classical, quasi-mesenchymal (QM), and exocrine-like (9). The QM-PDA subtype has a high tumor grade and a worse prognosis, whereas the classical subtype has high GATA6 expression and is KRAS-dependent. Then, computationally microdissecting approach defined two PDA tumor subtypes: basal-like and classical, and two stromal subtypes: normal and activated (10). The basal-like subtype and activated stroma in the classical subtype are associated with poor survival. Moreover, another large-scale analysis of transcriptomic data of 266 PDA samples defined four PDA subtypes: squamous, immunogenic, pancreatic progenitor, and aberrantly differentiated endocrine exocrine (ADEX) (8). The squamous subtype features ΔNp63, an isoform of p63, and is associated with mutations in chromatin modification genes such as MLL2, MLL3, and KDM6A. This subtype also loses endodermal identity via the methylation of endodermal genes, including *HNF4A* and *GATA6*. The squamous subtype has worse prognosis and correlates well with the QM-PDA and basal-like subtypes described above (8,11). The immunogenic subtype is correlated with low tumor cellularity, more pronounced immune response gene expression, and is associated with pancreatic progenitor or classical subtypes. The ADEX subtype and exocrine-like subtype described above are also correlated with low tumor cellularity and recent studies suggest that they may be due to the contamination of normal cells (11,12). Composite analysis of these studies redefined three subtypes: Hedgehog (associated with QM-PDA, basal, activated stroma, or squamous), Notch (associated with exocrine-like, normal stroma, or ADEX), and cell cycle (associated with classical or pancreatic progenitor) (13). Furthermore, a recent study using formalin-fixed paraffine embedded samples redefined the PDA subtypes by tumor cellularity, defining three subgroups in high tumor cellularity samples: pure-basal, pure-classical, and immune-classical, and adding two subgroups by analyzing all the samples: desmoplastic and stroma-activated (12). Although the subtyping details differ between these studies, they largely overlap and define the novel biology of PDA (*Figure 1*). Further details have been well reviewed elsewhere (14).

Genomic instability is another characteristic of cancer. PDA can be classified into four groups on the basis of structural genome variation: stable, scattered, locally rearranged, and unstable. The unstable subtype is associated with DNA damage response (DDR) gene deficiency and therapeutic implications for platinum and poly (ADP-ribose) polymerase (PARP) inhibitors (7). Details are described in later sections of this review.

Precursor lesions of PDA

PDA is generally thought to arise from three representative precursor lesions: pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN), and mucinous cystic neoplasm (MCN), although MCN is a rare precursor population (15).

PanIN is a microscopic, flat (or papillary), noninvasive epithelial neoplasm characterized by varying mucin levels and varying degrees of cytologic and architectural atypia (16,17). According to a previous classification system, PanIN was classified as PanIN1, PanIN2, and PanIN3 (carcinoma *in situ*) by the degree of atypia. In a recent expert consensus meeting, the classification was changed to a two-tiered system consisting of low-grade and high-grade PanINs to improve interpretation in a clinical setting (18). PanIN is the most frequent PDA precursor lesion; in autopsy examinations, PanIN-3 was found in 4% of cases, whereas PanIN-1 and PanIN-2 were present in 77% and 28%, respectively (19). Recent genomic analyses have revealed that PanIN-2 and PanIN-3 can spread throughout the entire ductal epithelium by traveling to distinct ductal epithelia (20). Furthermore, PanIN-derived PDA exhibits worse prognosis than IPMN-derived PDA, suggesting that they differ biologically (21-23). However, it is possible that IPMN-derived PDA can be identified earlier during imaging follow-ups.

IPMN is a grossly visible, predominantly papillary (or rarely flat), noninvasive mucin-producing epithelial neoplasm arising in the main pancreatic duct or branch ducts (17). IPMN has also been organized into a three-tiered classification system by the degree of atypia: low-grade, intermediate-grade, and high-grade IPMNs

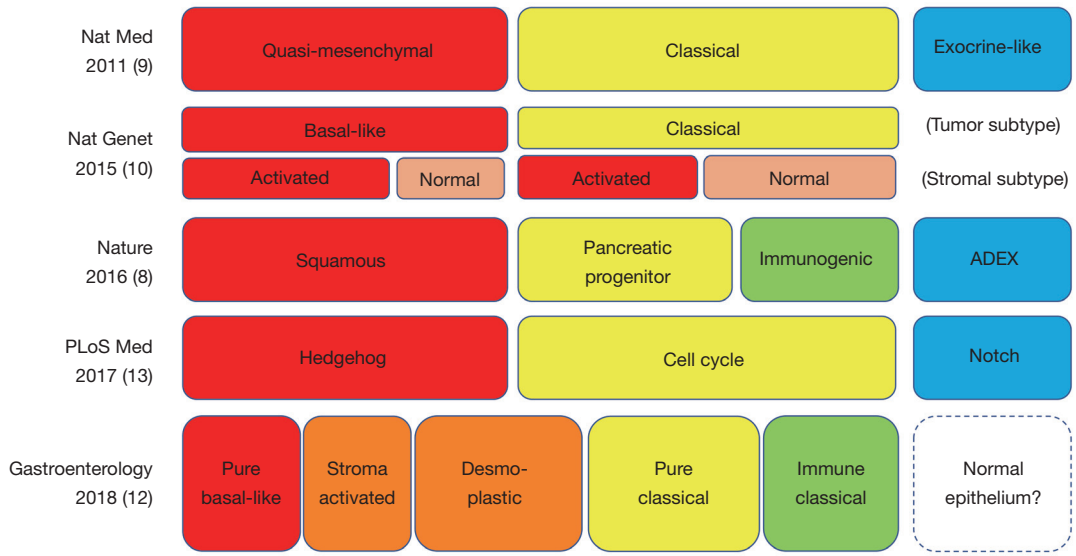

Figure 1 Summary of current transcriptomic subtyping of PDA (8-10,12,13). The transcriptomic subtyping studies of PDA are summarized. The five studies described in this article are listed in a chronological order. Number in parentheses indicates reference number. Red color indicates worse prognosis. Horizontal position shows the similarity of each subtype. PDA, pancreatic ductal adenocarcinoma.

(carcinoma *in situ*) (17). However, the classification system has recently been changed to a two-tiered system of low-grade and high-grade IPMNs (carcinoma *in situ*) (18). IPMN has also been classified into four subtypes based on its architecture and mucin expression patterns: gastric, intestinal, pancreatobiliary, and oncocytic (24). IPMN is a risk factor for PDA, since IPMN itself can progress to PDA (IPMN-derived PDA) and conventional PDA can arise at a different site from IPMN (IPMN-concomitant PDA) (25). IPMN-concomitant PDA patients have more microscopic neoplastic lesions (with *KRAS* mutations) than IPMN-derived PDA patients, which may explain why IPMN is a risk factor for concomitant PDA (26).

Genetic alterations in PanIN

PanIN genomic analysis has revealed that *KRAS* mutations occur in 90% of PanIN1 lesions; however, the variant allele frequency of *KRAS* mutations is relatively low in PanIN1 and higher in high-grade PanIN lesions (27). These data suggest that *KRAS* mutations are the earliest step in PanIN formation and that low-grade PanIN represents heterogeneous lesions including *KRAS* non-mutant cells. This finding was confirmed using a chimeric mouse model of PanIN (28). Furthermore, recent analysis revealed that *KRAS* gene amplification occurs even in low-grade PanIN (29). In contrast, alterations in *CDKN2A*, *TP53*, and *SMAD4* are observed during later stages of human PanIN development (30). These results suggest that PDA develops via the stepwise accumulation of mutations. A recent genetic study provided new insights into this stepwise accumulation, revealing that 65% of PDA cases harbored at least one chromothripsis event, each of which induces thousands of chromosomal rearrangements, and that 16% of PDA cases exhibit a chromothripsis-mediated simultaneous knockout of the "Big 4" genes (31).

In mice, specific $Kras^{G12D}$ allele expression in embryonic pancreatic progenitor cells (PDX1- or PTF1A-expressing cells) led to sporadic PanIN formation that progressed to PDA during long-term observation (32). Moreover, the additional expression of $Tp53^{R172H}$ or *Cdkn2a* knockout in embryonic pancreatic progenitor cells rapidly progresses to PDA with high prevalence (33,34). These genetically engineered mouse models (GEMMs) mimic human PDA formation well and shed light on PDA development.

Genetic alterations in IPMN

Exome sequencing studies have shown that IPMN has recurrent mutations in *KRAS*, *GNAS*, and *RNF43* (35,36). The mutational frequencies of *KRAS* and *GNAS* differ among subtypes, with meta-analysis revealing that the *KRAS* mutation frequency was 73% in gastric, 44% in

intestinal, 72% in pancreatobiliary, and 29% in oncocytic types. The *GNAS* mutation frequency was 53% in gastric, 74% in intestinal, 24% in pancreatobiliary, and 15% in oncocytic types (37). In this pooled analysis, the *RNF43* mutation frequency was 22.9% and was not associated with the clinicopathologic features of IPMN patients (37).

A recent study investigated the molecular mechanisms of IPMN-derived PDA and IPMN-concomitant PDA (26). The target sequence revealed that *TP53* abnormalities are less frequently observed in IPMN-derived PDA than in IPMN-concomitant PDA (33% *vs.* 71%, respectively) and that *GNAS* mutations are observed only in IPMN-derived PDA (67%). In IPMN-derived PDA, IPMN subclones with *RNF43* and *CTNNB1*/β-*catenin* abnormalities were not likely to be selected during tumor progression. Furthermore, IPMN-concomitant PDA can be classified into two subclasses: "*de novo*" and "branch off". The "branch off" type has the same *KRAS* status as coexisting IPMN, shares a common founder clone with coexisting IPMN, and exhibits longer disease-free survival after resection.

In mice, the embryonic pancreatic expression of the $Gnas^{R201H}$ mutation with oncogenic *Kras* resulted in IPMN formation and early death by severe inflammation (38). An inducible $Gnas^{R201C}$ model was recently developed that allows IPMN to develop and progress into PDA (39,40). Pancreatic-specific $Gnas^{R201C}$ induction in 4-week-old mice with an oncogenic *Kras* background led to IPMN formation and rapid death, similar to embryonic *Gnas* activation (39). However, the $Gnas^{R201C}$ induction of 8-week-old mice led to PDA development (40). Moreover, sequential $Gnas^{R201C}$ induction with adult acinar cell-specific oncogenic *Kras* and *Tp53* heterozygous deletion led to IPMN-derived PDA formation (39). These results suggest that *GNAS* mutations have an oncogenic role. Moreover, the loss of *Smad4*, *Brg1*, *Arid1a*, *Acvr1b*, *Tff2*, or *Pten* has been reported to induce IPMN formation (41-48).

Cellular origin of PanIN

The cellular origin of PDA is controversial (*Table 1*). Lineage tracing experiments in adult acinar cell-specific $Kras^{G12D}$-expressing mice using *Ela-CreERT*, $Mist1^{CreERT2}$, or $Ptf1a^{CreER}$ drivers resulted in spontaneous PanIN formation (49,53,55), demonstrating that PanIN is derived from acinar cells. Lineage tracing experiments have also shown that pancreatitis or oncogenic *Kras* activation can dedifferentiate pancreatic acinar cells into oval tubular complexes, known as acinar-to-ductal metaplasia (ADM) (49-51). In the setting of acute pancreatitis, this dedifferentiation is a transient event and acinar cells are regenerated in wild-type mice; however, under oncogenic *Kras* expression, dedifferentiation persists and results in PanIN formation (51). Although direct conversion from ADM to PanIN has not been demonstrated, these studies suggest that acinar cells are precursors of PanIN through ADM.

Other lineage tracing studies in adult ductal cell-specific $Kras^{G12D}$ expressing-mice using $CK19^{CreERT}$ (marker of large ductal cells) and *Sox9-CreER* (marker of ductal and centroacinar cells) drivers showed that ductal cells can give rise to PanIN, albeit at an extremely low frequency (55,58). The latter study found that PanIN from ductal cells is located near large ducts and occurs at a frequency of less than 100-fold compared with PanIN from acinar cells (55). Recent studies have shown that PDA without concomitant IPMN lesions can be developed from ductal cells if there are deletions or mutations in *TP53* or deletion of *Fbxw7* alleles with $Kras^{G12D}$ expression. They concluded that duct-derived PDA was more lethal than acinar-derived PDA (28,54,57); however, early PanIN lesions were not observed in these mouse models, suggesting that PDA of ductal origin has a distinct progression mechanism from the traditional PanIN model.

Another lineage tracing study in insulin-producing cell-specific $Kras^{G12D}$ expressing-mice using a $RIP\text{-}CreER^{TM}$ driver showed that PanIN can originate from extra-islet insulin-producing cells, not spontaneously, but under pancreatic inflammation (59).

Cellular origin of IPMN

IPMN is a neoplasm arising in the main pancreatic duct or branch ducts of humans (17). Several mouse models of IPMN have been developed using duct-specific Cre lines and show that mouse IPMN is derived from ductal cells (42,43,45,48). Another study suggested that IPMN can be derived from pancreatic duct glands (PDG), which are gland-like outpouches budding off the main pancreatic ducts that function as a progenitor niche for the ductal epithelium and express TFF2 (60,61). Embryonic global *Tff2* knockout with pancreatic-specific $Kras^{G12D}$ expression led to PDG hyperplasia and the formation of IPMN-like lesions which progress to PanIN lesions via Smad4 regulation (47). However, a recent study showed that acinar cell-specific *Gnas* mutations can lead to IPMN (39), suggesting that *GNAS* mutations result in IPMN regardless of the cellular origin.

Table 1 Current understanding of cellular origin of PDA and its precursor lesions by lineage-specific studies in mice

Cellular origin	Genotype	Phenotype	Ref.
Acinar cells	*Ela-CreERT*; $Kras^{G12D}$	ADM and PanIN formation	(49-51)
	Ela-CreERT; $Kras^{G12D}$; $Trp53^{R172H/+}$	PDA formation in the setting of pancreatitis	(52)
	$Mist1^{CreERT2}$; $Kras^{G12D}$	ADM and PanIN formation	(49,53)
	$Mist1^{CreERT2}$; $Kras^{G12D}$; $Trp53^{R172H/+}$	PDA formation	(54)
	$Ptf1a^{CreER}$; $Kras^{G12D}$	ADM and PanIN formation	(55)
	$Ptf1a^{CreER}$; $Kras^{G12D}$; $Trp53^{R172H/+}$	PanIN and PDA formation	(56)
	$Ptf1a^{CreER}$; $Kras^{G12D}$; $Trp53^{flox/flox}$	PDA formation	(57)
	$Ptf1a^{CreER}$; $Kras^{G12D}$; $Trp53^{loxP/+}$; $TetO\text{-}GNAS^{R201C}$	IPMN-derived PDA formation	(39)
Ductal cells	*Sox9-CreER*; $Kras^{G12D}$	A few PanIN formation. 112-fold less than $Ptf1a^{CreER}$; $Kras^{G12D}$ mice	(55)
	Sox9-CreER; $Kras^{G12D}$; $Trp53^{flox/flox}$	Rapid PDA formation and few high grade PanIN formation	(57)
	$CK19^{CreERT}$; $Kras^{G12D}$	A few PanIN formation	(58)
	$Ck19^{CreERT}$; $Kras^{G12D}$; $Trp53^{flox/flox}$	Ductal atypia and rapid PDA formation	(28)
	Hnf1b-CreER^{T2}; $Kras^{G12D}$; $Trp53^{R172H/+}$	Normal	(54)
	Hnf1b-CreER^{T2}; $Kras^{G12D}$; $Trp53^{R172H/R172H}$	Rapid PDA formation without PanIN formation	(54)
	Hnf1b-CreER^{T2}; $Kras^{G12D}$; $Brg1^{flox/flox}$	IPMN formation	(42)
	Hnf1b-CreER^{T2}; $Kras^{G12D}$; $Arid1a^{flox/flox}$	IPMN formation	(43)
	Sox9-CreER; $Kras^{G12D}$; $Arid1a^{flox/flox}$	IPMN and PDA formation	(45)
	Sox9-CreER; $Pten^{flox/flox}$	IPMN and IPMN-derived PDA formation	(48)
	Sox9-CreER; $Kras^{G12D}$; $Pten^{flox/+}$	PanIN, Pre-IPMN, IPMN and PDA formation	(48)
	Sox9-CreER; $Kras^{G12D}$; $Pten^{flox/flox}$	Rapid IPMN and IPMN-derived PDA formation. Died within 2–4 weeks	(48)
Insulin producing cells (extra islet)	*RIP-CreER*^{TM}; $Kras^{G12D}$	PanIN formation in the setting of pancreatitis	(59)
	RIP-CreER^{TM}; $Kras^{G12D}$; $Trp53^{flox/flox}$	Undifferentiated PDA formation in the setting of pancreatitis	(59)

PDA, pancreatic ductal adenocarcinoma; ADM, acinar-to-ductal metaplasia; PanIN, pancreatic intraepithelial neoplasia; IPMN, intraductal papillary mucinous neoplasm.

Cellular origin of human PDA

Evidence suggests that PanIN is mostly derived from acinar cells; however, PDA can also originate from ductal cells and insulin-producing cells in mice. Recent studies using an organoid culture system have provided further insight into the cellular origin of human PanIN and PDA. CRISPR/Cas9 enabled oncogenic *KRAS* overexpression and the deletion of *CDKN2A*, *TP53*, and *SMAD4* to be engineered in human duct cell organoids, which subsequently developed PanIN-like lesions after orthotopic transplantation (62). Another group performed oncogenic *KRAS* knock-in and the deletion of *CDKN2A*, *TP53*, and *SMAD4* in pancreatic ductal organoids, which developed PDA after subcutaneous transplantation (63). These data suggest that human ductal cells can give rise to PDA, however, the contribution of human acinar cells towards PDA development remains unclear.

Inflammation and PanIN/PDA

Chronic pancreatitis is a risk factor for PDA (64). Experimental pancreatitis induced by caerulein, a cholecystokinin analog, caused ADM and accelerated PanIN formation in an embryonic $Kras^{G12D}$ expression model. Chronic pancreatitis

in an adult acinar cell-specific *Kras*^{G12V} expressing model with *CDKN2A* or *TP53* deletions induced high-grade PanIN and invasive PDA by overcoming oncogene-induced senescence, even though these mice only spontaneously induce low-grade PanIN formation (65). This study shows that inflammation is a critical step in PanIN/PDA progression, a notion supported by many studies in which the knockout of key inflammatory regulators, including *Nfκb*, *IL-6*, and *Stat3*, induced the loss of PanIN formation in a mutant *Kras* background (66-69). Furthermore, chronic inflammation induced by COX2 or IKK2 activation in a *TP53*-null background produced various pancreatic cancer subtypes, including acinar cell carcinoma, PDA, sarcomatoid carcinoma, and neuroendocrine tumors without mutant *Kras* (70). These findings suggest that chronic inflammation plays an important role in PDA formation independently of *Kras* mutations.

Many studies have been conducted to understand the roles of environmental factors, including stromal cells, hematopoietic cells, and immune cells, in PDA formation. Tumor-stroma and tumor-immune cell interactions are hot topics in pancreatic cancer research and have been reviewed in detail elsewhere (71,72).

Involvement of molecular signaling pathways in PDA development

Resent whole genome sequencing data on 456 PDA samples showed that significantly mutated genes in human PDA can be grouped into ten molecular mechanisms (8). Here, we describe the current understanding of these signaling pathways in PDA and their precursor lesions (*Table 2*).

KRAS and RTK signaling

KRAS mutations are the earliest step in PanIN formation in terms of the mutational landscape of PDA, as described previously (27). *KRAS* is a key player in the RTK/RAS signaling pathway and its mutation causes constitutive Ras-GTP activation leading to downstream signaling.

In inducible *Kras*^{G12D} mouse models which allow reversible and conditional *Kras*^{G12D} expression, *Kras* mutations were shown to be indispensable for PanIN initiation and PanIN and PDA progression by controlling anabolic glucose metabolism (73,74). Although the majority of PDA cells undergo apoptosis upon *Kras* repression, a few PDA cells with stem cell properties can survive via oxidative phosphorylation (75) and in a Yap-dependent manner (76).

One important signal downstream of KRAS is the RAF-MEK-ERK pathway. RAF kinases, including A-RAF, B-RAF, and C-RAF, are direct KRAS effectors that mediate MAPK activation. Activated RAF phosphorylates MEK1 and MEK2, which in turn activate the serine-threonine kinases ERK1 and ERK2. *BRAF* mutations are found in approximately 3% of human PDA cases and are mutually exclusive with *KRAS* mutations (6). The pancreas-specific expression of a *Braf*^{V600E} mutant allele initiated PanIN formation without a *Kras* mutation and resulted in lethal PDA with a *Tp53*^{R270H} mutation in mice (77). The effect of the genetic inactivation of the RAF-MEK-ERK pathway in *Kras*^{G12V}-mediated lung tumors was investigated. The deletion of MEK or ERK by combining *Mek1*$^{lox/lox}$ and *Mek2*$^{-/-}$ or *Erk1*$^{-/-}$ and *Erk2*$^{lox/lox}$ significantly improved survival; however, systemic elimination in adult tissues led to early mouse mortality (133). Interestingly, whereas *Kras*-driven lung tumor formation is blocked in the absence of *C-Raf* in mice without lethality by systemic elimination in adult tissues (133,134), pancreas-specific *C-Raf* ablation had no suppressive effects on PDA development in mice (78). However, additional *Egfr* ablation critically inhibited PDA formation and led to the complete regression of 6/12 established PDA tumors in mice without lethal effects (89).

Another important signal downstream of KRAS is the PI3K-PDK-AKT pathway. Embryonic pancreatic or adult acinar cell-specific expression of the *Pik3ca*^{H1047R} mutant allele induced PanIN formation and PDA progression (78). Furthermore, the embryonic pancreatic deletion of *Pik3ca* or the central downstream factor *Pdk1* abolished *Kras*^{G12D}-mediated PanIN formation in mice (78,79). A recently developed dual recombinase system, which combines flippase-FRT (Flp-FRT) and Cre-loxP recombination technologies to sequentially manipulate gene expression, revealed that PDK1 is also indispensable for PanIN progression (80). The embryonic pancreatic deletion of *Pik3ca* with oncogenic *Kras* led to AKT phosphorylation but RAC1 downregulation, which is another factor downstream of PIK3CA. Embryonic pancreatic *Rac1* deletion abolished PanIN formation, indicating that RAC1 is a critical factor downstream of PIK3CA in PanIN formation (79). Moreover, the loss of mTRAIL-R, an ortholog of the human TRAIL-receptor, delayed PDA growth, inhibited metastasis, and prolonged the survival of PDA-bearing mice in *Kras*^{G12D} and *Trp53*$^{+/-}$ backgrounds via direct RAC1 inhibition (81). The tumor suppressor gene PTEN is a negative regulator of the PI3K pathway. The embryonic pancreatic loss of *Pten* led to the expansion of centroacinar

Table 2 Functionally validated genes using GEMMs in each pathway

Pathway	Gene	Initiation (Solely)	Initiation and progression (with *Kras* mutation)	Progression of lesions	Ref.
KRAS and RTK signaling	*KRAS*	Oncogenic	–	Oncogenic (in PDA)	(32,73-76)
	BRAF	Oncogenic	–	–	(77)
	CRAF	–	Oncogenic	–	(78)
	PIK3CA	Oncogenic	Oncogenic	–	(78,79)
	PDK1	–	Oncogenic	Oncogenic (in PanIN)	(78,80)
	RAC1	–	Oncogenic	–	(79)
	TRAIL-R	–	Oncogenic	–	(81)
	PTEN	Tumor suppressive (IPMN)	Tumor suppressive	–	(48,82-84)
	RICTOR	–	Oncogenic	–	(85)
	EGFR	–	Oncogenic	–	(86,87)
	PTPN11 (SHP2)	–	Oncogenic	Oncogenic (in PDA)	(88)
	CRAF + EGFR	–	Oncogenic	Oncogenic (in PDA)	(89)
Cell cycle	*CDKN2A*	–	Tumor suppressive	–	(34)
	TP53	Tumor suppressive (under inflammation)	Tumor suppressive	–	(33,65)
	RB	–	Tumor suppressive	–	(90)
	p21 (CDKN1A)	–	Tumor suppressive	–	(91)
	MYC	Oncogenic	Oncogenic	–	(92-97)
TGF-β signaling	*SMAD4*	–	Context dependent[1)]	–	(41,98-100)
	TGFBR2	–	Tumor suppressive	–	(101)
	ACVR1B	–	Tumor suppressive (IPMN)	–	(102)
NOTCH signaling	*NOTCH1*	–	Context dependent[2)]	–	(50,96,103)
	NUMB	–	Tumor suppressive	–	(104)
	NOTCH2	–	Oncogenic	–	(96)
	LFNG	–	Tumor suppressive	–	(105)
	DNMAML	–	Tumor suppressive	–	(106)
	SOX9	–	Oncogenic	–	(55)
	HES1	–	Context dependent[3)]	–	(52,107)
	FBXW7	–	Tumor suppressive	–	(28,108)
WNT signaling	*β-catenin*	Oncogenic (SPN)	Context dependent[4)]	–	(51,109-111)
	DKK1	–	Oncogenic	Oncogenic (in PanIN)	(110)
	ATDC	–	Oncogenic	–	(112)
	WNT1	–	Oncogenic (PanIN, MCN)	–	(111)
	NFATc1	–	Oncogenic	–	(113-115)

Table 2 (*continued*)

Table 2 (*continued*)

Pathway	Gene	Initiation (Solely)	Initiation and progression (with *Kras* mutation)	Progression of lesions	Ref.
Chromatin modification	*KDM6A*	–	Tumor suppressive	–	(116)
	BMI1	–	Oncogenic	–	(117)
	RING1B	–	Oncogenic	Oncogenic (in PDA)	(118)
	EZH2	–	Tumor suppressive	–	(119)
SWI/SNF complex	*SMARCA4 (Brg1)*	–	Context dependent[5]	Oncogenic (in PanIN)	(42,56,120)
	ARID1A	–	Tumor suppressive	–	(43-45,121)
	SMARCB1	–	Tumor suppressive	Tumor suppressive (in PDA)	(122)
DNA-damage repair genes	*BRCA1*	–	Tumor suppressive	–	(123,124)
	BRCA2	–	Context dependent[6]	–	(125-129)
	ATM	–	Tumor suppressive	–	(130,131)
SLIT/ROBO signaling	*ROBO2*	–	No effect	–	(132)

[1], mainly a tumor suppressive role but heterozygous mutation with *TP53* mutation inhibits metastasis; [2], knockout models show a tumor suppressive role, whereas overexpression model shows an oncogenic role; [3], embryonic pancreatic knockout model shows a tumor suppressive role, but adult acinar specific knockout model shows an oncogenic role; [4], mainly an oncogenic role but simultaneous β-catenin stabilization with Kras mutation leads ITT formation and impairs PanIN formation; [5], a tumor suppressive role in ductal cell-derived IPMN formation but an oncogenic role in acinar cell-derived PanIN formation. PDA, pancreatic ductal adenocarcinoma; IPMN, intraductal papillary mucinous neoplasm; PanIN, pancreatic intraepithelial neoplasia; MCN, mucinous cystic neoplasm; ITT, intraductal tubular tumor.

cells and papillary carcinoma (82), whereas adult acinar cell-specific *Pten* deletion does not lead to PanIN formation, but adult duct cell-specific *Pten* deletion does lead to IPMN formation (48). Furthermore, the embryonic pancreatic heterozygous loss of *Pten* with *Kras* mutations accelerated PanIN formation and PDA progression by activating downstream mTOR signaling via the mTORC1 and mTORC2 complexes (83,84). The embryonic inactivation of mTORC2 by *Rictor* deletion, which is an essential subunit of mTORC2, profoundly prolongs the survival of PDA-bearing mice in $Kras^{G12D}$ and $Trp53^{+/-}$ backgrounds (85). Serotonin (5-HT) was recently identified as an upstream regulator of the PI3K/AKT/mTOR pathway; its stimulation promotes HTR2B (a 5-HT receptor that has a selective antagonistic drug)-LYN-p85 complex formation, and activates the PI3K/AKT/mTOR pathway (135).

Signals upstream of *Kras* are also important for *Kras*-mediated pancreatic tumorigenesis. Loss of *Egfr* or the EGFR ligand sheddase *Adam17* in the embryonic pancreas blocked the formation of ADM and PanIN with *Kras* mutations and reduced the formation of PDA with additional *Trp53* deletions (86,87). The loss of *Ptpn11* (also known as SHP2) significantly inhibited the formation of PanIN with *Kras* mutations and inhibited PDA formation and progression with additional *Trp53* deletions (88). These findings suggest that signals upstream of KRAS are also required to initiate pancreatic tumorigenesis. Consistently, other studies have shown that pancreatic tumorigenesis is strongly dependent on a minimal threshold of KRAS activity which is not achieved simply by a single mutant *Kras* allele, but by a positive feedback loop with inflammatory stimuli (67) and by amplification (29).

Cell cycle

CDKN2A, TP53, and TP53BP2 are involved in the cell cycle; the roles of CDKN2A and TP53 in pancreatic tumorigenesis are described above. CDKs and RB signaling

are also key regulators of the cell cycle, with the embryonic pancreatic loss of *Rb* with oncogenic *Kras* shown to accelerate PanIN formation, increase the frequency of cystic neoplasms, and promote rapid PDA formation by inhibiting the senescence response and reducing Tp53 activity (90). P21, also known as CDKN1A, is a CDK inhibitor (136). The embryonic pancreatic heterozygous loss of *p21* with oncogenic *Kras* was shown to accelerate PanIN and PDA formation by overcoming oncogene-induced senescence (91).

MYC stimulates the cell cycle via several parallel mechanisms (92). *Myc* overexpression in the embryonic acinar compartment under control of the *Elastase* promoter (Ela-MYC model) resulted in the development of acinar cell carcinomas or mixed carcinoma with acinar and ductal differentiation (93). Embryonic pancreatic MYC activation in an inducible *Myc* overexpression model led to the development of PDA with liver metastasis, whereas *Myc* downregulation in established PDA led to cell death and lesion regression (94). However, a recent *Myc* knock-in model showed that embryonic pancreatic *Myc* expression does not form tumors by itself, although it significantly reduced survival in an oncogenic *Kras* background (95). Embryonic pancreatic *Myc* deletion with oncogenic *Kras* resulted only in PanIN1 formation (96), whereas embryonic pancreatic *Myc* heterozygous deletion with oncogenic *Kras* and *TP53* mutation reduced PDA progression and increased survival (95). An ESC-based mouse model using an inducible RNA interference-based approach showed that *Myc* knockdown in adult pancreatic epithelium in an oncogenic *Kras* background decreased, but did not abolish, ADM and PanIN formation (97). Mechanistically, MYC regulates ductal-neuroendocrine lineage plasticity in PDA and induces gemcitabine resistance (95). A subset of PanIN cells expressing neuroendocrine markers have been shown to promote PanIN progression in connection with sensory neurons, which are known to cause PanIN and PDA progression (137,138).

TGF-β signaling (including SMAD4)

TGF-β signaling is known to have both pro-tumorigenic and tumor suppressive roles in a context-dependent manner. SMAD4, the central mediator of TGF-β, is inactivated in approximately 55% of human PDA cases (139), whereas other TGF-β signaling members are mutated in less than 10%. Furthermore, *SMAD*4 inactivation is highly correlated with *TP53* inactivation (140).

In mice, the loss of *Smad4* in the embryonic pancreas with *Kras* mutations led to IPMN or MCN formation and PDA progression (41,98,99). Moreover, in the context of *Kras* and *Tp53* mutations, the heterozygous loss of *Smad4* in the embryonic pancreas inhibited metastasis compared to *Smad4* wild-type mice, whereas the homozygous loss of *Smad4* exhibited a similar metastatic burden as in *Smad4* wild-type mice, although proliferation was inversely correlated with *Smad4* gene dosage (100). When looking at other members of TGF-β signaling, loss of *Tgfbr2* (the TGF-β receptor) in the embryonic pancreas with *Kras* mutations led to highly aggressive (with dense stroma but less metastatic) PDA formation with a short latency time (101). Furthermore, the inhibition of CXCR2 disrupted tumor-stromal interactions and prolonged survival in this mouse model (141). Loss of *Acvr1b* (activin receptor and another ligand of the TGF-β superfamily) in the embryonic pancreas with *Kras* mutations led to IPMN formation (46). Additionally, the pharmacological inactivation of *TGF*-β accelerated PDA initiation and progression in mice (102). These studies demonstrate the tumor suppressive role of TGF-β during the early stages of PDA development. Conversely, TGF-β is known to induce epithelial-mesenchymal transition (EMT), which promotes invasion and metastasis (142). TGF-β also promotes cancer stem cell heterogeneity and drug resistance in squamous cell carcinoma (143). These two papers highlight the context-dependent pro-tumorigenic and tumor suppressive roles of TGF-β signaling in cancer initiation and progression. A recent study revealed that SMAD4 is a critical regulator of this context-dependency in PDA. TGF-β treatment in *Smad4*-normal PDA promoted apoptosis via SMAD2/3-mediated SOX4 expression and SMAD4-mediated KLF5 repression through SNAIL-driven EMT, whereas TGF-β treatment in *Smad4*-mutated PDA promoted tumorigenesis via SOX4 and KLF5 cooperation (144).

NOTCH signaling

The NOTCH signaling pathway is a highly evolutionarily conserved pathway that mediates cell-to-cell communication (145) and plays a critical role in cell proliferation, differentiation, development, and homeostasis (146). NOTCH signaling (including *JAG1*, *NF2*, *BCORL1*, and *FBXW7*) is frequently mutated (8) and multiple components are upregulated in human PDA (147). NOTCH signaling has four receptors: NOTCH1-4. NOTCH1 is expressed in acinar cells, whereas NOTCH2 is expressed in ductal and centroacinar

cells (96). Embryonic pancreatic *Notch1* deletion with oncogenic *Kras* accelerated PanIN formation and progression and reduced survival via β-catenin activation (96,103). These results suggest that NOTCH1 has a tumor suppressive role in PDA formation. Interestingly, embryonic pancreatic or adult acinar cell-specific *Notch1* activation with oncogenic *Kras* accelerated ADM and PanIN formation/progression (50). Consistently, the embryonic pancreatic and adult acinar-specific deletion of *Numb* [a multifunctional protein that negatively regulates NOTCH signaling, especially NOTCH1 (104)] with $Kras^{G12D}$ also accelerated ADM and PanIN formation although it reduced cell viability during PanIN progression (148). These data suggest that the tumor suppressive role of NOTCH1 is dose-dependent. Furthermore, adult acinar cell-specific *Notch1* deletion increased the number of PanIN lesions without changing their severity, supporting the tumor-suppressive role of NOTCH1 (149).

In contrast to *Notch1* deletion, embryonic pancreatic *Notch2* deletion with $Kras^{G12D}$ was shown to decrease PanIN progression and prolong survival by downregulating MYC, suggesting that NOTCH2 has an oncogenic role in PDA formation (96). In addition, MCN and late-occurring anaplastic PDA were found in these mice. The role of NOTCH3 in pancreatic tumorigenesis was investigated indirectly; the embryonic pancreatic deletion of *Lfng*, which regulates the ligand binding of the NOTCH receptor, with $Kras^{G12D}$ accelerated PDA formation by upregulating NOTCH receptors, especially NOTCH3 (105). These studies suggest that each NOTCH receptor has a distinct role in pancreatic tumorigenesis. The role of NOTCH signaling in PanIN development has also been investigated by inducing the dominant negative form of MAML1 (DNMAML), which represses all canonical Notch-mediated transcription in a cell-autonomous manner. Embryonic pancreatic *DNMAML* expression with $Kras^{G12D}$ delayed PanIN formation, suggesting tumor suppressive role of NOTCH signaling in broad sentence (106). Pharmacologically, the γ-secretase inhibitor (GSI) broadly inhibits NOTCH signaling. Embryonic pancreatic $Kras^{G12D}$ and $Tp53^{+/-}$ expressing mice treated with GSI were resistant to PDA development, supporting an oncogenic role for NOTCH signaling (150). Furthermore, combining GSI and gemcitabine treatments after PDA formation synergistically and significantly improved anti-tumor efficacy and median survival in mice (151); however, due to the strong gastrointestinal side effects of GSI (152,153), a more specific therapy is warranted. SOX9, a master regulator of pancreatic development, is also a factor downstream of NOTCH in pancreatic development. Loss of *Sox9* in adult acinar cells with a $Kras^{G12D}$ background prevented PanIN formation (55). Recently, the role of HES1, a critical factor downstream of NOTCH, was also investigated. Embryonic *Hes1* deletion, which is involved in the terminal differentiation of adult acinar cells, with $Kras^{G12D}$ promoted ADM and PDA formation, and decreased survival, while prevented high-grade PanIN formation (107). On the other hand, adult acinar cell-specific *Hes1* deletion with $Kras^{G12D}$ and/or $Tp53^{R172H}$ significantly inhibited pancreatitis-induced PanIN formation and blocked PanIN progression with downregulating SOX9 (52). Since the loss of HES1 in adult ductal/centroacinar cells led to ductal-to-acinar metaplastic expansion (154,155), it remains unclear whether the immaturation of acinar cells or recombination of ductal cells in embryonic models can explain this phenotypic discrepancy.

FBXW7 is a component of the SKP1-CULLIN1-Fbox E3 ubiquitin ligase complex, which targets multiple well-known oncoproteins, including NOTCH1, by ubiquitination-mediated destruction (156). In the "Sleeping Beauty" transposon-insertional mutagenesis model to cause random mutations, *Fbxw7* mutations accelerate $Kras^{G12D}$-induced PDA formation with a high frequency (24%) (157). The embryonic pancreatic heterozygous deletion of *Fbxw7* with $Kras^{G12D}$ accelerated PanIN formation, whereas the homozygous deletion of *Fbxw7* with $Kras^{G12D}$ significantly accelerated PDA formation at 2–3 weeks of age via YAP activation (108). Moreover, the adult duct cell- or acinar cell-specific homozygous deletion of *Fbxw7* with $Kras^{G12D}$ resulted in PDA formation, with AGR2 defined as a putative marker of duct-derived PDA (28).

WNT signaling

The WNT signaling pathway is an important embryonic signaling pathway that is required for the proliferation, morphogenesis, and differentiation of several organs (158). Mutations in the *RNF43* gene, which inhibits Wnt/beta-catenin signaling by ubiquitinating the Frizzled receptor and targeting it to the lysosomal pathway for degradation (159), occur in 5% of PDA cases (8). *RNF43* mutations are also associated with IPMN, as described above. Initial functional study of *RNF43* inactivating mutations in PDA showed that *RNF43*-mutated PDA is dependent on WNT signaling and is effectively inhibited by Porcupine inhibitor (160). Recently, genome wide CRISPR/Cas9 screening showed

that FZD5 is a druggable target for PDA with *RNF43*-inactivating mutations (161). The role of WNT signaling in PDA development has been explored. WNT signaling has a canonical pathway, including β-catenin, and a non-canonical pathway (158,162). The initial studies for understanding the role of WNT signaling, especially β-catenin, have been summarized in another review (163). In brief, embryonic pancreatic β-catenin activation led to the formation of pseudo-papillary neoplasm, a rare pancreatic tumor histopathologically characterized by β-catenin activation. Embryonic pancreatic β-*catenin* activation with $Kras^{G12D}$ inhibited PanIN formation but led to intraductal tubular tumors (109), whereas acinar-specific β-*catenin* activation with $Kras^{G12D}$ also impaired PanIN formation after acute pancreatitis (51). On the other hand, embryonic pancreatic β-*catenin* knockout inhibited the regeneration of acinar cells from pancreatitis-induced ADM (51) and, in the context of *Kras* mutations, the loss of β-*catenin* inhibited ADM and PanIN formation (110). These results demonstrate the oncogenic role of β-catenin and suggest that precise β-catenin dosage control is critical for ADM and PanIN formation. Further studies confirmed the necessity of WNT signaling in *Kras*-induced tumorigenesis. The overexpression of *Dkk1*, an endogenous inhibitor secreted in WNT signaling, using an inducible *Dkk1* allele impaired PanIN formation and progression in *Kras* mutated pancreatic epithelium, furthermore, it inhibited the proliferation of PanIN and induced apoptosis of surrounding stroma in established PanIN (110). Unlike the initial studies on β-catenin dosage impact, recent studies have shown that the overexpression of WNT signaling has a tumor promoting role in the PanIN-PDA sequence. The embryonic pancreatic activation of *Atdc* (also known as *TPRM29*) with $Kras^{G12D}$ promoted PanIN progression and PDA formation by inducing EMT via CD44 upregulation after β-catenin upregulation (112). The sequential postnatal activation of *Wnt1* or β-*catenin* in an elastase-tva-based RCAS-TVA system with embryonic *Kras* activation resulted in PanIN progression and PDAC formation (111). Additionally, female mice with the sequential postnatal activation of *Wnt1*, but not β-*catenin*, with embryonic *Kras* activation developed MCN via paracrine β-catenin activation in stromal cells (113).

The importance of the non-canonical WNT pathway has also been clarified recently. Non-canonical WNT signaling is mediated by intracellular calcium ions and JNK, which leads to NFAT signaling activation (162). The embryonic pancreatic activation of *Nfatc1* with $Kras^{G12D}$ promoted PDA formation by controlling gene expression via enhancer-to-promoter communication through NFATc1-STAT3 complex formation. The embryonic deletion of *Nfatc1* with $Kras^{G12D}$ inhibited pancreatitis-induced ADM formation, although it did not affect ADM and PanIN formation without pancreatitis (114). The following studies helped to clarify the precise role of NFATc1 in ADM formation. The EGFR-mediated activation of NFATc1 induced ADM development by forming a complex with c-JUN on the *Sox9* promoter and activating SOX9 (115). *Nfatc1* expression was silenced by EZH2-mediated histone methylation during acinar cell recovery from ADM (164). Moreover, the embryonic pancreatic activation of *Nfatc1* with $Kras^{G12D}$ and $Tp53^{R172H}$ resulted in the progression of dedifferentiated PDA, demonstrating that NFATc1 drives EMT reprogramming and maintains PDA in a stem cell-like state via the SOX2-dependent transcription of EMT and stemness factors. NFATc1-SOX2 complex-mediated PDA dedifferentiation and progression is opposed by antithetical p53-miR200c signaling and inactivation of the tumor suppressor pathway is essential for tumor dedifferentiation and dissemination (165).

Chromatin modification

Histone modification enzymes are mutated in 24% of human PDA cases. Mutations have been observed in the *KDM6A*, *SETD2*, and ASCOM complex members *MLL2* and *MLL3* (8). KDM6A, MLL2, and MLL3 exist in the same complex and drive transcriptional activation via H3K4 methylation and H3K27 demethylation (166). KDM6A is a SWI/SNF-interacting partner involved in the demethylation of lysine residues on histones. Transcriptomic analysis revealed that *KDM6A* mutations or loss occur in 18% of PDA samples and correlate with the squamous subtype, which has the worst prognosis of the four PDA subtypes (7,8). The embryonic pancreatic loss of *Kdm6a* with oncogenic *Kras* induced aggressive and metastatic squamous-like PDA in female mice, whereas in male mice the concomitant loss of the Y chromosome-encoded KDM6 family member UTY, which lacks demethylase activity, resulted in a similar phenotype (116).

SETD2 is a methyltransferase known to mediate H3K36 methylation. Its ability to regulate splicing, DNA methylation, chromosome segregation, and DNA-damage repair suggest a tumor suppressive role of SETD2 (167). Furthermore, the loss of SETD2 caused mRNA processing defects in 25% of genes expressed across the genome (168).

Polycomb repressive complexes (PRC) are epigenetic gene silencers involved in the maintenance of a stem cell state and cancer development. PRC1 mediates H2AK119Ub1 and PRC2 mediates H3K27me3 (169). The embryonic pancreatic loss of *Bmi1*, a PRC1 component, almost completely abrogated PanIN formation in $Kras^{G12D}$ with or without an $Ink4a^{-/-}$ background (117). RING1B, another component of PRC1, is also critical for $Kras^{G12D}$ mediated tumorigenesis from adult acinar cell, whereas the CRISPR/Cas9 mediated knockout of *Ring1b* in mouse PDA cells reduced tumorigenicity after orthotopic transplantation (118). These data demonstrate the oncogenic function of PRC1. In contrast, EZH2, a PRC2 component, has a tumor suppressive function, since the embryonic pancreatic loss of *Ezh2* with $Kras^{G12D}$ accelerated PanIN formation and progression by enhancing COX2-mediated chronic inflammation (119).

SWI/SNF complex

The SWI/SNF chromatin remodeling complex permits DNA-protein contacts to regulate gene expression (170). SWI/SNF complexes contain 12–15 subunits and comprise two main groups, the BRM/BRG1-associated factor (BAF) (SMARCA2/SMARCA4-associated factor in humans) and the Polybromo-associated (PBAF) complexes. Recent whole exome and genome sequences have shown that at least one subunit of the SWI/SNF complexes, including *ARID1A*, *ARID1B*, *SMARCA4*, *SMARCA2*, *PBRM1*, and *SMARCB1* are mutated in 14% of human PDA cases (5,7,8).

The initial *in vivo* functional analyses of SWI/SNF complexes in pancreatic carcinogenesis focused on BRG1 (human; SMARCA4), 1 of the 2 catalytic subunits in SWI/SNF complexes. The embryonic pancreatic deletion of *Brg1* with $Kras^{G12D}$ led to the formation of cystic neoplasm that highly resembled human pancreatobiliary type IPMN. Under this background, the IPMN lesions progressed to invasive PDA (42). This IPMN-derived PDA model has better prognosis than the classical model of PanIN-derived PDA with $Kras^{G12D}$ and $Tp53^{+/-}$, mirroring prognostic trends in human PDA patients. *Brg1* null IPMN-derived PDA possesses a distinct molecular signature that supports less malignant features than PanIN-derived PDA. Additionally, the adult duct or acinar-specific deletion of *Brg1* with $Kras^{G12D}$ revealed that IPMN lesions were derived from ductal cells. Another study showed that BRG1 blocks the initiation of ductal tumorigenesis by inhibiting the dedifferentiation of ductal cells via SOX9 regulation, whereas BRG1 promotes tumorigenesis in IPMN-derived PDA by supporting a mesenchymal-like landscape (120).

In contrast, our recent work showed that the acinar-specific deletion of *Brg1* with oncogenic *Kras* impaired ADM and PanIN formation independently of $Tp53^{R172H}$ and inhibited PanIN-derived PDA formation in the presence of $Tp53^{R172H}$ via the direct downregulation of SOX9. Furthermore, the ablation of *Brg1* in established PanINs using a dual recombinase system resulted in their regression (56). These data demonstrate that the BRG1/SOX9 axis is critical for PanIN-derived PDA development, highlighting the cell-specific and context-dependent roles of BRG1 in PDA initiation and progression.

ARID1A is a subunit of the BAF complex and the most frequently mutated gene in multiple human cancers (171), demonstrating that its protein loss and mutations correlated with the poor survival of PDA patients (6). Recently, several *in vivo* functional analyses have been performed on ARID1A (43-45,121). Our initial report showed that the embryonic pancreatic deletion of *Arid1a* with $Kras^{G12D}$ led to IPMN formation that progressed to PDA. The adult duct- or acinar-specific deletion of *Arid1a* with $Kras^{G12D}$ revealed that IPMN caused by *Arid1a* deletion was also derived from ductal cells. Functionally, *Arid1a* loss led to the dedifferentiation of ductal cells and pancreatic ductal dilation by suppressing SOX9 expression. These results highly resembled to those of *Brg1*-deleted mice; however, the incidence of PDA formation in *Arid1a*-deleted mice was significantly lower than in *Brg1*-deleted mice, likely since mTOR pathway activation is lower in *Arid1a*-deleted IPMN than in *Brg1*-deleted IPMN, and PanIN was formed from adult acinar cell-specific *Arid1a*-deleted mice (43). These results confirmed the tumor suppressive role of ARID1A in the pancreas. Recent reports from three other groups have provided additional insights. Firstly, the sequential knockdown of *Arid1a* using an inducible sh*Arid1a* model in adult pancreatic epithelium with $Kras^{G12D}$ resulted in rapid and irreversible PanIN formation, but did not increase PDA formation. ARID1A depletion reduced chromatin accessibility at acinar-specific enhancers, limiting the transcriptional output of acinar master transcription factors (121). Secondly, the embryonic pancreatic deletion of *Arid1a* with $Kras^{G12D}$ and $Tp53^{+/-}$ led to the formation of IPMN and fully invasive, poorly-differentiated adenocarcinomas with increased EMT gene expression and stem cell identity (44). Thirdly, the ductal cell-specific deletion of *Arid1a* with $Kras^{G12D}$ and $Tp53^{+/-}$ resulted in PDA formation, whereas the acinar-specific heterozygous

deletion of *Arid1a* with $Kras^{G12D}$ and $Tp53^{+/-}$ accelerated PDA formation. This study showed that *Arid1a* deletion in ductal cells activates MYC and increases protein synthesis (45).

Another SWI/SNF partner, SMARCB1, has a strong tumor-suppressive function. The deletion of *Smarcb1* in the embryonic pancreas in oncogenic *Kras* with or without a *p53*-null background markedly accelerated tumorigenesis and increased metastatic spread and mesenchymal reprogramming. The loss of *Smarcb1* activated MYC, which drives the anabolic switch and adaptive activation of the ER stress-induced survival pathway (122).

Recent reports have shown that several cancers with mutations in SWI/SNF complex genes have synthetic lethal partners (172). Thus, future studies in PDA with alterations in SWI/SNF complex components are required.

DNA-damage repair genes

DNA-damage repair genes are mutated in 17% of PDA cases, including *BRCA1*, *BRCA2*, *ATM*, and *PALB2* mutations (5% germline, 12% somatic) (8). Germline mutations in DNA-damage repair genes are important familial pancreatic cancer susceptibility genes (173). Germline and somatic mutations in DNA-damage repair genes are also important since they have homologous repair deficiencies and are potential targets of platinum therapies and PARP inhibitors based on the BRCAness concept (174). PDA is associated with four mutational signatures, of which 20 were identified from mutational analysis in over 7,000 cancers (175) (BRCA signature, aging, DNA mismatch repair deficiency, and APOBEC family). A recent genomic study showed that 14% of PDA cases had *BRCA1*, *BRCA2*, or *PALB2* mutations, all of which are associated with a BRCA signature or an unstable genome. Potential DNA-damage repair genes are mutated in up to 24% of PDA cases, with PDA defined by a BRCA signature or an unstable genome thought to have potential DNA-damage repair gene mutations (7).

The function of BRCA genes has been well characterized using mouse models. BRCA1 forms various complexes that function in many biological processes (176). Embryonic pancreatic *Brca1* deletion with $Kras^{G12D}$ and $Tp53^{-/-}$ significantly reduced tumor latency. The BRCT domain mutation that disrupts BRCA1 function in the DNA-damage response also significantly reduced tumor latency, however loss of E3 ligase activity, which is thought to regulate multiple tumor suppressive pathways, did not reduce tumor latency. This fact indicates that the central role of BRCA1 in pancreatic tumorigenesis is in DNA-repair (123). Moreover, the DNA-demethylating drug 5-aza-2'-deoxycytidine was shown to enhance the survival of mice harboring embryonic pancreatic *Brca1*-deletion with $Kras^{G12D}$ and $Tp53^{R270H}$ (124).

BRCA2 is a core mediator of homologous repair in the DNA-damage response (176), with embryonic pancreatic *Brca2* knockout leading to pancreatic insufficiency (125). Germline heterozygous *Brca2* mutations with embryonic pancreatic $Kras^{G12D}$ expression and *Brca2* LOH, or embryonic pancreatic *Brca2* knockout with $Kras^{G12D}$ led to reduced tumor burden and pancreatic insufficiency (126,127); however, additional *Tp53* mutations or knockout in these backgrounds significantly accelerated PDA formation compared to *Brca2* wild-type mice (125-127). These studies clearly demonstrate the context-dependent role of BRCA2 in PDA formation. Furthermore, these studies suggest that germline heterozygous *BRCA2* mutations are a potential tumor suppressor. Germline heterozygous *Brca2* mutations with embryonic pancreatic $Kras^{G12D}$ expression accelerated tumor burden (126,127). Nonetheless, PARP inhibitors including olaparib, cisplatin, and mitomycin C, are efficient in *Brca2*-null PDA but not in *Brca2*-heterozygous PDA (126,127). A recent report using embryonic pancreatic *Brca2* knockout mice with $Kras^{G12D}$ and $Tp53^{+/-}$ confirmed the tumor suppressive role of BRCA2 and revealed a mechanism whereby reactive nitrogen species-induced DNA lesions caused genomic instability in the absence of *Brca2* (128). Another study showed that PD-L1 and IL-6 combination therapy increased the survival of mice harboring embryonic pancreatic *Brca2* knockout with $Kras^{G12D}$ and $Tp53^{R270H}$ (129).

ATM is initially characterized by one of the DDR genes (177) and has a broader ability to integrate and direct various signaling cues to maintain cellular homeostasis (178). Embryonic pancreatic heterozygous *Atm* knockout with $Kras^{G12D}$ enhanced highly stromal infiltrated ADM and PanIN formation via the activation of BMP4 signaling and reduced survival by enhancing EMT and stem cell signaling (130). *Atm* deletion leads to chromosomal instability, complex structural rearrangements including chromothripsis, and deregulated DNA integrity checkpoints. Thus, *Atm* deficiency exhibits synthetic lethality with PARP inhibitors and ATR inhibitors (131). PALB2 has also been confirmed as a tumor suppressor in a mouse model of breast cancer (179,180).

SLIT/ROBO signaling

The Roundabout (ROBO) receptors and their secreted SLIT glycoprotein ligands were originally identified as axon guidance molecules that mediate precise axon path finding and neuronal migration during development (181,182). Recent studies have shown that SLIT/ROBO signaling also acts as a tumor suppressor by suppressing WNT signaling activity and downregulating MET signaling activity (183). Aberrations in SLIT2 and/or ROBO1/2 are observed in 23% of human PDA cases (6% mutation and 18% copy number loss) and low ROBO2 expression levels are correlated with poor survival (5). When sequentially comparing normal mouse pancreas, ADM, and PDA, a progressive decrease in *Robo2*, progressive increase in *Robo1*, and no change in *Robo3* mRNA expression is observed. A recent study showed that the loss of *Robo2* in the embryonic pancreas led to stromal activation and immune cell infiltration via the activation of TGF-β signaling after acute pancreatitis; however, under the $Kras^{G12D}$ background, the loss of *Robo2* had no effect on PanIN formation, stromal expansion, or immune cell infiltration (132).

RNA processing genes

RNA splicing is an essential process carried out by major and minor spliceosomes to remove noncoding regions (introns) in pre-mRNA before protein translation (184). The RNA splicing genes *SF3B1*, *U2AF1*, and *RBM10* are mutated in 16% of human PDA cases (8). SF3B1 and U2AF1 are associated with the U2 snRNP of major spliceosomes. In PDA, *SF3B1* mutations aggregate as K700E hotspot mutations (8); K700E hotspot mutations in breast cancer are associated with CDH1 mis-splicing (185), whereas *U2AF1* mutations aggregate as S34F hotspot mutations; S34F hotspot mutations in lung adenocarcinoma are associated with the mis-splicing of TSH2, a tumor suppressor of the mTOR pathway (185,186). RBM10 is an RNA-binding protein that exclusively represses splicing (187). *RBM10* is located on the X chromosome, and its loss-of-function mutations are the cause of the X-linked recessive disorder TARP syndrome, which mainly affects males (188). *RBM10* mutations in PDA are associated with longer survival despite the histological features of aggressive disease (6).

Organoids *vs.* mouse models

So far, we have described the current understanding of PDA revealed by a number of studies using GEMMs which are valuable for understanding PDA biology. The classical GEMMs have a limitation in studies of PDA development, since genes of interest are activated or deleted at the same time as oncogenic *Kras* induction using a single Cre-based genetic engineering system. The recent development of a dual recombinase system using Flp-FRT enables the role of genes of interest in established PDA to be investigated. Furthermore, the dual recombinase system enables the investigation of tumor-stroma or tumor-immune interactions by manipulating genes of interest in stroma or myeloid cells using their specific Cre line (189,190).

Although GEMMs are faithful models for determining PDA biology, they have several problems. Firstly, engineering multiple alleles increases the time and cost required for the bleeding and maintenance of GEMMs; however, the recently developed CRISPR/Cas9-mediated *in vivo* genetic manipulation technique may resolve this problem (191). Secondly, GEMMs are mice, not humans. Recent advances in 3D organoid culture models can mimic and maintain mouse and human epithelial organ structures in a dish (192). Organoid cultures are not only useful for normal epithelial tissue research but for also cancer research; organoids from normal pancreatic ductal cells (193) and PDA (194) have been already established and bring various benefits to PDA research. PDA organoids can rapidly generate and be maintained not only from surgical specimens, but also from small amounts of endoscopic ultrasound-fine needle aspiration (EUS-FNA) samples taken during initial human PDA diagnosis (194-196). Genetic and transcriptomic analyses of advanced cancers could be performed using PDA organoids, which were consistent with those from matched primary PDA (196-198). Organoids are a good platform for drug testing prior to the treatment of patients, since their treatment response correlates well with that of the patients (196,198,199). An initial platform for high-throughput drug screening using organoids has also been developed (200). Furthermore, organoids can be used for biological investigation; for example, the genetic manipulation of key driver mutations in normal ductal organoids could mimic PDA development *in vitro*, as described above (62,63). Co-culture with fibroblasts revealed that cancer-associated fibroblasts have two sub-populations with distinct roles (201,202).

Current problems in PDA therapy resistance and future aspect

PDA has a weak response to current chemotherapeutic

agents. Overcoming the therapeutic resistance of PDA is critical for improving the survival of PDA patients. The therapeutic resistance of PDA is attributed to both cell intrinsic and extrinsic factors (203). The PDA transcriptome can predict drug response and clinical outcome, but its mutational status cannot (8-10,196). Intrinsic factors such as aberrant signaling pathways, epigenetics, post transcriptional modifications, altered metabolism, and tumor heterogeneity, and extrinsic factors such as the extracellular matrix, cancer associated fibroblasts, immune cells, inflammation, cancer stem cell niche, hypoxia, and EMT plasticity can all cause transcriptomic changes and induce therapeutic resistance (204).

In this review, we summarized the recent advances in our understanding of PDA genetics and biology, with a particular focus on tumor cell intrinsic factors. We believe that a better understanding of PDA biology and genetics could lead to the future development of novel diagnostic methods and treatments and could improve survival. The development of next generation GEMMs and organoids could improve our understanding of PDA biology and genetics. We believe that further investigations using these novel tools could lead to overcome the therapeutic resistance of this devastating disease.

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Footnote

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