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Genetics and Biology of Pancreatic Ductal Adenocarcinoma

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Abstract

Pancreatic ductal adenocarcinoma (PDA) is an aggressive malignancy that carries a poor prognosis with a 5-year survival on the order of 6%¹; new and innovative treatments are needed. Several factors underlie its aggressive nature and resistance to treatment: the genetic framework, early metastasis, a dense stroma, propensity for growth in a nutrient-deplete environment, and immunomodulation have all made therapeutic progress a challenge.² This article focuses on recent advances in understanding the tumor genetics and cell biology of pancreatic cancer. It reviews the established genetic hallmarks, examines more recently described mutations and altered pathways, and highlights key biological principles identified in PDA with a focus on those most likely to lead to future therapeutic targets.

Keywords

Pancreatic Cancer; KRAS; Microenvironment; Stroma; Immunomodulation; Autophagy

GENETICS

Pancreatic adenocarcinoma shows genetic homogeneity on one level with mutations in *KRAS*, found in anywhere from 90% to 95% of advanced pancreatic cancers, and additional frequent and well-characterized mutations in the key tumor suppressor pathways *TP53/p19ARF*, *RB/CDKN2A/INK4A*, and *TGFBeta/SMAD4*.³ The biological significance of many of these mutations has been investigated in numerous contexts and model systems and the main impact of each on the disease is briefly highlighted later. Beyond this element of genetic homogeneity, PDA is broadly characterized by general genetic instability with widespread mutations and chromosomal translocations, including the recent discovery of many additional mutated loci and classes of genes whose full cancer-specific functions have yet to be fully explored. These genes include members of the SWI/SNF family, MLLs, and the DNA damage repair system, including *ATM*. The genetic hallmarks of PDA are described below and the broad implications of recent tumor genetic analyses are discussed later with regard to understanding of the natural history of the disease (Box 1).

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KRAS

KRAS is a member of the RAS family of GTP-binding proteins that controls cellular proliferation and survival. Inactivation of RAS occurs via GTP hydrolysis with the aid of GTPase-activating proteins (GAPs). Activating *KRAS* point mutations at codon 12, the most common in PDA, occur near the nucleotide binding site, desensitizing RAS to GAPs and inhibiting GTP hydrolysis,⁴⁻⁶ resulting in a constitutively active RAS. This process leads to a unique genetic/biochemical/signaling paradigm compared with other oncogenes, because *KRAS* is not so much biochemically turned on via mutation, but rather cannot be turned off. Consequently, mutant RAS is a difficult therapeutic target because the loss of its GTPase enzymatic activity is not easily pharmacologically restored. This challenge has shifted efforts from targeting *KRAS* directly to focusing on downstream signaling pathways of *KRAS*.

KRAS mutation leads to constitutive activations of key mitogenic and survival signaling pathways, including RAF/MEK/ERK and phosphatidylinositol 3 kinase (PI3K). The relative importance of each of these has been evaluated in several *in vitro* and *in vivo* systems. *In vitro* studies revealed that RAS activates RAF and mitogen-activated protein (MAP) kinases, crucial for DNA synthesis, in the absence of other growth factors,^{7,8} and that its activities could also be abrogated with PI3K dominant negatives.⁹ Mouse models have provided further insight into the relative importance of PI3K and RAF/MAP/MEK pathways. PI3K-activated models without *Kras* mutation showed no pancreatic abnormalities, whereas *Braf*-mutated models developed PanIn (pancreatic intraepithelial neoplasms; these are discussed later), leading investigators to conclude that this may be the dominant branch of *KRAS*-mediated signaling in PDA.¹⁰ Furthermore, a *Braf* and *Tp53* mutated model showed clear evidence of PDA with extensive metastasis. Treatment with MEK inhibitors suppressed phosphorylation of ERK but led to increased levels of phosphorylated AKT, a marker of PI3K activation, and cell lines treated with both MEK and AKT inhibition led to a synergistic antitumor effect. This antitumor effect, albeit modest, is also seen in dual MEK/PI3K treatment studies in mouse models.^{11,12} Thus, although RAF/MEK/ERK activation may be a dominant effector of *KRAS* activity, its pharmacologic inhibition led to activation of other compensatory downstream pathways driving survival and proliferation, explaining PDA's resistance to single-agent MEK inhibition clinically.¹³

Oncogene addiction has been described as a process in which effector proteins are involved in an intricate network of synergistic positive-feedback and negative-feedback loops to maintain tumor growth, homeostasis, and structural integrity; a process that is not simply a summation of the effects of acquired mutations.¹⁴ The addiction of subsets of PDAs to *KRAS* has been well described, shown by tumor regression with mutant *Kras* extinction.¹⁵ Dependence on mutant *KRAS* can be overcome, however, similar to the mechanisms of resistance described in many targeted therapies, and was recently described via amplification and overexpression of YAP-1.¹⁶ Yap-1 is a transcriptional coactivator involved in cell proliferation, epithelial-to-mesenchymal transition (EMT), and metastasis, previously linked to liver and esophageal tumors.^{17,18} Other routes to *KRAS* independence are also described,¹⁹ suggesting that there may be additional molecular routes by which *KRAS*-independent growth of PDA may be sustained.

INK4a/CDKN2A/P16

Loss of *INK4a* function brought about by mutation, deletion, or promoter hypermethylation occurs in about 80% to 95% of sporadic PDA.^{20,21} The *INK4a* gene encodes the tumor suppressor protein p16, which inhibits CDK4/6-mediated phosphorylation of RB, thereby blocking entry into the S phase (DNA synthesis) of the cell cycle (for a more in-depth review of *INK4a/ARF*, see Sharpless²²). The 9q21 locus that contains *INK4a* also encodes the tumor suppressor gene *ARF*, whose protein product p19 stabilizes p53 by inhibiting MDM2-dependent proteolysis. Pancreatic cancers with mutations at this locus may sustain loss of both *INK4a* and *ARF* tumor suppression pathways, although some mutations have been found to affect loss of p16 alone. The importance of *Ink4a* in restraining PDA has been shown in murine models with engineered mutations in this locus designed to affect both *p16Ink4a* and *p19Arf* genes or *p16Ink4a* alone.^{23,24}

TP53 Tumor Suppressor Pathway

Mutations, predominantly missense, in the *TP53* tumor suppressor gene occur in approximately 75% to 80% of human PDA cases.^{21,25,26} In normal unstressed cells, p53 is bound to MDM2, which targets p53 for proteasomal degradation. In response to cell stress and/or oncogene activation, p53 protein is stabilized and targets activation of genes involved in cell cycle arrest, apoptosis, DNA damage repair, and cellular metabolism.²⁷ The role of *TP53* loss in cancer is well established in that its mutation has been described in most human cancers and germline mutation of *TP53* leads to the early development of sarcomas and carcinomas, also known as the Li-Fraumeni syndrome²⁸ (for in-depth review of p53, see “The p53 Family,” subject collection in *CSH Perspectives in Biology*, 2010). Evidence from PDA precursor lesions (PanINs) suggests that loss or mutation of *TP53* is a late event possibly caused by selective pressure after the collective accumulation of genetic aberrations, reactive oxygen species (ROS), and telomere erosion.^{4,29} The loss of *p19ARF* coexists with *TP53* mutations in only about 40% of tumors, whereas the loss of *TP53* or inactivation of this pathway is a more common feature of PDA, leading many to believe that these tumor suppressors have some overlapping function as well as independent capabilities.^{30,31}

DPC4/SMAD4/Transforming Growth Factor Beta

The *SMAD4* (*DPC4*) gene is located within chromosome 18q21 and is a key transcriptional regulator of the transforming growth factor beta (TGF- β) signaling cascade, a pathway that mediates proliferation, cell migration, apoptosis, and EMT.^{32–34} Loss of *SMAD4* function has been reported in ~50% to 60% of tumors and is associated with poor outcome in surgical patients.^{26,32} In a rapid-autopsy program examining 76 cases of PDA, *SMAD4* loss was reported in only 22% of locally advanced cases with no metastatic disease at autopsy, but in 78% of those with widespread metastatic disease.²⁶ These clinical findings, along with associated poor outcome in patients with *SMAD4* loss, imply that intact *SMAD4* function may help constrain metastatic spread, and investigators suggest that such patients with functional *SMAD4* may benefit from intensive locoregional therapies.²⁶

Somatic mutation of *Smad4*, as well as the type 2 TGF- β receptor³⁵ in the murine pancreatic epithelium, neither disrupted pancreatic development nor induced malignancy, but hastened

PDA development when coupled with *Kras* mutation.³⁶ Although *SMAD4* is mutated in about 50%, almost all tumors express high levels of both receptor and TGF- β ligands. In both human and murine model systems, activation of this pathway has been found to underlie cellular migration, metastasis, and EMT,³⁷ raising the question of TGF- β inhibition as a therapy. Studies have been mixed depending on models used and underlying tumor genetics.³⁸

Advanced Genetic Sequencing Reveals Novel Mutations

The application of whole-genome sequencing to PDA has revealed additional new mutations in genes and pathways not previously recognized as being important in its pathogenesis as well as insights into its genetic evolution. Exon sequencing of 24 PDAs uncovered 1562 somatic mutations in 1327 genes³⁹ and investigators organized these into a collection of 12 core signaling pathways (Table 1). Although most of the 1327 genes identified were mutated in a small minority of cases, each core pathway was mutated in 67% to 100% of the 24 tumor specimens, suggesting that, although the mutational spectrum may be broad and heterogeneous, the physiologic result is distilled down to effects on conserved pathways.³⁹ Separately, whole-exon sequencing of 99 patients⁴⁰ confirmed these described mutations and identified novel mutations in PDA, including the axon guidance pathway genes *SLIT* and *ROBO*, previously known to be important in embryogenesis and central nervous system development (Table 2).⁴¹ *SLIT2/ROBO2* inactivating mutations were present in 5% of the cohort with copy number losses in *ROBO1* and *SLIT2* in another 15% of the population. The biological impact of *SLIT/ROBO* mutations in PDA is unexplored but, in other contexts, affects both MET and WNT signaling.⁴¹ In addition, novel mutations were discovered in *EPC1* (3%) and *ARID2* (3%), which affect chromatin modification, and in *ATM* (5%), with likely more of the cohort affected because of copy number variation losses in a small percentage of each gene.⁴⁰ *ATM*, which encodes for a serine/threonine kinase DNA damage repair protein, has already been linked to familial cases of PDA along with *BRCA2* and *PALB2*⁴²; this finding supports a role in sporadic PDA as well.

Beyond the identification of genes harboring mutations that may be causal to the disease, these studies have offered more global insights into the genetic landscape and trajectory of the disease. In order to understand the relationship of a primary tumor to its attendant metastatic sites, somatic genetic rearrangements in 13 primary PDA tumors were compared with metastases.⁴³ Although most of the 206 rearrangements were found in the primary tumor and all metastatic sites, supporting a conserved origin, clonal evolution was also identified. Furthermore, some genetic aberrations found in metastatic sites were organ specific, including mutations in *MYC* and *CCNE1* that were found exclusively in lung metastases, suggesting that certain subclones may evolve in an organ-specific manner.⁴³

Genetic Pathogenesis

PDA may arise from at least 3 types of precursor lesions: PanIN, intraductal papillary mucinous neoplasm (IPMN), and mucinous cystic neoplasm (MCN). The most prevalent precursor is PanIN, a microscopic lesion graded I to III, encompassing a spectrum of increasing dysplasia and architectural disruption.⁴⁴ High-grade PanIN lesions are commonly found in association with invasive PDA. Furthermore, advanced PanINs harbor many of the

same genetic mutations as PDA, linking these entities molecularly as well as pathologically.⁴⁵ IPMNs are less common precursors and PDA derived from IPMNs are thought by some clinicians to represent a different disease process, especially given its better prognosis and 5-year survival rate of 42% in those surgically resectable patients.⁴⁶ Sixty-six percent of IPMNs harbor mutations in *GNAS*.⁴⁷ Furthermore, investigation of 95 surgical PDA specimens without IPMN yielded no mutations in *GNAS*. These findings were corroborated through sequencing of 48 IPMNs that identified *GNAS* mutations in 79% of patients; only 50% harbored *KRAS* mutations and fewer showed loss of p16 (36%) and *SMAD4* (24%).⁴⁸ The genetics of MCNs are not clearly delineated, but a presentation in young women, mainly in the body and tail of the pancreas, suggests potential differing biology.⁴⁹ Although there are key shared genetic traits between PanINs, PDA, and the lesser common precursors IPMNs and MCNs,⁴ these recent studies show how different genetic events may explain the divergent phenotypes of the malignancies arising from each type of precursor (Box 2).

MICROENVIRONMENT

The pancreatic cancer microenvironment, referred to as stroma, or desmoplasia, is composed of fibroblasts, myofibroblasts, immune cells, vascular components, and a dense extracellular matrix (ECM).⁵⁰ The role of the tumor stroma has been alternatively viewed as either shielding tumor cells from treatment through the creation of a hypoxic and high-tensile environment impenetrable by current available therapies or, more recently, functioning to constrain tumor cells through the facilitation of immune response or impaired vascular access.^{51–53} Several signaling pathways active in PDA can modulate this environment, including TGF- β (discussed earlier), notch, and Sonic Hedgehog (Shh). The Shh pathway, identified as frequently activated in PDA,³⁹ promotes stromal desmoplasia,^{54–57} and is being evaluated as a potential therapeutic target. Administration of IPI-926, an inhibitor of the Shh pathway,⁵⁸ in murine models has been shown to reduce tumor-associated stromal tissue burden, promote angiogenesis, and enhance drug delivery, and combining IPI-926 with gemcitabine resulted in doubling of overall survival compared with those mice treated with gemcitabine alone.⁵⁹

The ECM of the stroma found in PDA contains a large proportion of hyaluronic acid (HA), a large glycosaminoglycan,⁵¹ crucial to normal tissue homeostasis via its influence on cell shape and malleability.⁶⁰ In PDA and other tumors in which hypovascularity, itself a major hindrance to treatment delivery, levels have been associated with tumor metastasis, drug resistance, angiogenesis, and poor prognosis.^{61–64} By administering pegylated hyaluronidase (PEGPH20), tumors showed decreased IFP, increased chemotherapy delivery, and greater patency of the vasculature that previously collapsed amid increased interstitial pressures. Furthermore, improved response rates and overall survival were seen with mouse models when combining PEGPH20 with gemcitabine compared with gemcitabine alone. These findings, combined with improved outcomes found using Shh inhibitors described earlier, strengthened the argument that desmoplasia was a significant culprit in PDA's poor prognosis and drug resistance.^{51,59,65}

Although previous work showed prolonged survival in mice with PDA with pharmacologic inhibition of Shh,⁶⁶ the somatic deletion of Shh in a murine PDA model conversely led to more aggressive pancreatic tumors with an increased metastatic burden.⁵² On histology, Shh knockout PDA is predictably fibroblast deplete, but undifferentiated, with a more prominent vasculature and high expression of Zeb1 and SLUG, both transcription factors that are important for EMT.^{67,68} Further supporting a protective role for the stroma, elimination of myofibroblasts in PDA models led to a decreasing number of T effector cells and an increase in T regulatory cells (T_{reg}), representing weakened immune surveillance (immunomodulation, as discussed later).⁵³

The studies described earlier have created a therapeutic dilemma as to whether the stroma should be targeted and eliminated, to enable drug delivery, or promoted to bolster its antitumoral immune response. Pegylated hyaluronidase is currently being studied as an adjunct to gemcitabine and nab-paclitaxel in a phase II clinical trial ([clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01839487) NCT01839487). It is possible that although some stromal elements function to constrain tumor growth (via enhanced immunity and so forth), others may enhance malignant behavior (ie, conferring chemoresistance; discussed later regarding tumor macrophages) and that a greater understanding of the interplay between the tumor cells and the host will ultimately be necessary to design therapies that target the tumor stroma.

IMMUNE RESPONSE

Immunosuppression in cancer, specifically in PDA, that is promoted by cancer-associated inflammation is now recognized to foster tumor development and growth.⁶⁹ The microenvironment of PDA is crucial to this process because it harbors a range of immunosuppressive cells, including tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and T_{reg} (see review by Vonder-heide and Bayne⁶⁹). Current understanding is that these leukocytes help to deplete T effector cells, which are an integral part of antitumoral immunity,⁷⁰ via several underlying mechanisms, including oncogenic activation of KRAS and granulocyte-macrophage colony-stimulating factor (GM-CSF)^{71,72} and the CCL2/CCR chemokine axis.⁷³ Recent developments in understanding these systems are described later, as well as how targeting the immune response, or lack thereof, in PDA may provide clinical benefit.

In a *Kras*-mutant mouse model, an intense desmoplastic reaction with a prominent leukocytic infiltration was observed that was predominantly made up of T_{reg} cells, which can abrogate the immune response,⁷⁴ MDSCs, and TAMs and lacked effector T cells.⁷⁰ After an inverse relationship was discovered between MDSCs and T effector cells, it was found *in vitro* that MDSCs are capable of driving down T-cell proliferation.⁷⁰ In addition, investigators found that these immunosuppressive cells are present in PanINs and throughout progression to PDA, indicating that immunomodulation and suppression are possibly early events of PDA development.

GR-1+ CD11b+ immature myeloid cells are MDSCs that are precursors to macrophages, dendritic cells, and granulocytes and have been found to infiltrate PanIN and PDA lesions in mouse models.⁶⁹ Upregulation of GM-CSF, which has been found to be *Kras* dependent in

mice, drives the accumulation of these immunosuppressive cells in PDA.^{71,72} When GM-CSF is knocked down in Kras-mutated grafts, GR-1+ CD11b+ cells are scarce, there is an increase in CD8+ T cells, and orthotopic engrafted pancreatic tumor cells are subsequently eliminated.⁷² CD8+ T-cell immunity was proved to be a dominant force in this response as tumor growth was restored when CD8+ T cells were ultimately eradicated by anti-CD8 antibody injection into the host.

Depleted numbers of CD81 T cells in PDA was also described in a study examining the CCL2/CCR chemokine axis and its ability to regulate antitumor immunity.⁷³ The CCL2/CCR chemokine axis is an important modulator of inflammatory monocyte (IM) recruitment from the bone marrow to the peripheral blood and tumor sites where they are destined to become immunosuppressive TAMs.^{75,76} CCR+ TAMs, which made up 28% of tumor-infiltrating leukocytes,⁷³ were suppressed in tumor-bearing mice with a CCR2 antagonist leading to an increase in the number of T effector cells, and diminished T_{reg} cell numbers and associated decreased tumor growth.⁷³ Furthermore, combining the CCR2 antagonist with gemcitabine had a synergistic effect in reducing tumor growth. In addition, they observed that treating these mice with gemcitabine led to persistent levels of IMs and an increase in TAMs, which could provide some insight into why PDA becomes resistant to chemotherapy. These findings provide the basis for an open-phase Ib/II trial combining chemotherapy with a CCR2 antagonist (NCT01413022; clinicaltrials.gov).

METABOLIC REQUIREMENTS

The recognition that PDA has unique metabolic requirements, compared with untransformed epithelial cells, has emerged from the basic study of mutations associated with the disease (eg, *KRAS*, *TP53*) as well as direct attention paid to metabolites that promote the tumor's growth.^{77–79} Beyond metabolic pathways identified downstream of *KRAS*, PDA uses evolutionary-conserved cellular survival mechanisms (specifically, autophagy and macropinocytosis) to meet its heightened metabolic demands.^{79,80} Furthermore, the hypoxic and metabolically active environment of PDA requires an increased capacity to contend with sequelae such as increased ROS stress.

Autophagy is a catabolic pathway that recycles damaged organelles and proteins for use as an alternative energy source in response to starvation.⁸¹ Although autophagy has been described previously as a tumor suppressor involved in cell death in other contexts,^{82,83} levels of autophagic markers are increased in PDA.⁸⁴ Autophagic flux was found to be upregulated in PDA cell lines at baseline, nutrient-rich conditions, and not only when under stress,⁸⁰ suggesting that autophagy is an essential metabolic process for PDA cell sustainability. Autophagy in pancreatic cancers seems in part to be driven by a constitutively active RAS.⁸¹ When autophagy genes *Atg5* or *Atg7* were knocked down, RAS-expressing cells died in nutrient-deplete conditions; cells were found lacking substrates for tricarboxylic acid (TCA) cycle metabolism normally produced in the mitochondria. Furthermore, *Ras*-mutated mice had suppressed tumor growth with these same genes knocked down. Inhibition of autophagy in PDA cell lines using chloroquine, which increases lysosomal pH and blocks fusion with the phagosome,^{85,86} led to diminished oxidative phosphorylation and decreased cellular proliferation.⁸⁰ Further supporting a role for autophagy in PDA tumor progression,

Plac8, a protein upregulated by oncogenic mutations, is important to lysosomal/autophagosomal fusion and its absence hampers tumor growth. The role of p53 in mediating autophagy dependence is a matter of some debate with a dichotomous effect depending on the context studied.^{86–88} Taken together, these studies support the critical role of autophagy in PDA cell nutrition, growth, and survival (possibly related to attenuation of ROS stress) and have prompted further investigation of autophagy as a therapeutic target.

RAS mutation has also been found to be pivotal in other metabolic pathways responsible for sustaining growth, including broadly reprogramming glucose use and glutamine metabolism to maintain optimal reduction and oxidation (REDOX) balance as well as via direct transcriptional upregulation of NRF2 pathways.^{77,89,90} Glutamine consumption is increased in cancer as well as other highly proliferative, nonmalignant cells.^{78,91} In healthy cells, glutamine can be converted to α -ketoglutarate to refuel the TCA cycle⁹² through 2 distinct enzymatic processes: glutamate de-hydrogenase 1 or transaminases including glutamic-oxaloacetic transaminase 1 (GOT1), an enzyme critical to glutamine metabolism in PDA and upregulated transcriptionally with *Kras* mutation. Loss of GOT1 function in PDA led to an increase in ROS and decreased ratio of NADPH/NADP⁺. PDA cells deprived of glutamine fail to thrive, likely because it plays a part in preventing accumulation of ROS and ensuring REDOX homeostasis. Macropinocytosis, a conserved signal-dependent endocytic process by which extracellular solutes and nutrients are transmitted into the cell,⁹³ is active in PDA and downstream of RAS, contributing to intracellular glutamine replenishment.⁷⁹

KRAS mutations lead to a coordinated acquisition of macromolecules, via both internal scavenging, through autophagy, and from the external environment, via macropinocytosis, and then use of pathways that optimize cellular REDOX balance can be appreciated. Because *KRAS* has proved to be an elusive therapeutic target, attacking these adaptive metabolic pathways may be efficacious with minimal toxicity because they are likely to be less crucial in benign functional tissues (Box 3).

SUMMARY

PDA remains a clinical challenge and although treatments options have changed over the past 5 years, most of these advances have been through the novel combinations of previously established and known chemotherapeutics.^{94,95} Thus far, enlightenment on the downstream activities of *Kras*, the tumor's unique metabolic needs, and how the stroma and immune system affect it has remained untranslated to clinical practice (Fig. 1). Given the numbers of diverse therapies in development and a growing knowledge about how to evaluate these systems preclinically and clinically, this is expected to change significantly and for the better over the next 5 years.

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KEY POINTS

- Pancreatic ductal adenocarcinoma remains a clinical challenge.
- Thus far, enlightenment on the downstream activities of Kras, the tumor's unique metabolic needs, and how the stroma and immune system affect it have remained untranslated to the clinical practice.
- Given the numbers of diverse therapies in development and a growing knowledge about how to evaluate these systems preclinically and clinically, this is expected to change significantly and for the better over the next 5 years.

Box 1**Genetics of PDA**

- KRAS is the most common mutational hallmark of PDA and can activate the RAF/MEK/ERK and PI3K pathways.
- Mouse models with K-RAS mutations typically require subsequent genetic events, such as loss of tumor suppressor genes INK4A, P53, or SMAD4, to develop tumors similar to human PDA.
- Finding that loss of SMAD4 has a greater propensity for metastatic spread is an example of how genetic sequencing may help tailor future therapy to the individual.

Box 2**Advanced genetic sequencing efforts in PDA**

- More than 1500 mutations have been identified to occur in PDA; focusing on the core signaling cascades these affect may show greater promise than focusing on individual mutations.
- Next-generation sequencing efforts have identified many novel genetic aberrations in PDA, such as those in axon guidance pathways and chromatin modification, which require further investigation.
- PanIns are precursor lesions that have tight genetic linkage with PDA; IPMNs are less common PDA precursor lesions found to have frequent mutation of GNAS and slightly better outcomes, suggesting a potential divergent phenotype.

Box 3**Adaptive mechanisms for invasion and growth**

- The PDA microenvironment is shifted to favor immunosuppressive leukocytes like TAMs, MDSCs, and T_{reg} rather than cells like T effector cells, which stimulate an immune response.
- Autophagy is a KRAS-driven process upregulated in PDA that provides alternate means for nutrition and growth that may be a therapeutic target and is under investigation.
- Glutamine metabolism is essential in PDA cells for protection against ROS; glutamine stores are replenished by macropinocytosis.

Fig. 1. Evolution of PanIN (pancreatic intraepithelial neoplasms) to PDA and the many mechanisms by which RAS influences tumor growth.

Table 1

Core signaling pathways in PDA as described by Jones and colleagues³⁹ and highlighted mutations of each pathway

Core Pathway	Key Mutated Genes
KRAS signaling	KRAS, MAP2K4, RASGRP3
Regulation of G1/S phase	CDKN2A, FBXW7, CHD1, APC2
TGF- β signaling	SMAD4, SMAD3, TGFBR2, BMPR2
DNA damage control	TP53, ERCC4, ERCC6, RANBP2
Hedgehog signaling	TBX5, SOX3, LPR2, GL1, BOC, CREBBP
WNT/Notch signaling	MYC, GATA6, WNT9A, TCF4, MAP2, TSC2
Apoptosis	CASP10, VCP, CAD, HIP1
C-Jun N-terminal kinase signaling	MAP4K3, TNF, ATF2, NFATC3
Regulation of invasion	ADAM11, DPP6, MEP1A, PCSK6, APG4A, PRSS23
Homophilic cell adhesion	CDH1, CDH10, PCDH15, PCDH17, FAT
Small GTPase-dependent signaling (non-KRAS)	AGHGEF7, CDC42BPA, DEPDC2, PLCB3
Integrin signaling	ITGA4, ITGA9, LAM1, FN1, ILK

Data from Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 2008;321:1801–6.

Table 2

Significant novel mutations identified by genetic sequencing of human PDA samples in studies in by Jones and colleagues³⁹ and Biankin and colleagues⁴⁰

Jones et al,³⁹ 2008	Biankin et al,⁴⁰ 2012
MLL3 (transcriptional activator)	ARID2 (chromatin modification)
CDH10, PCDH15, PCDH18 (cadherin homologs)	ATM (DNA damage repair)
CTNNA2 (alpha-catenin)	ZIM2 (transcriptional regulator)
DPP6 (dipeptidyl-peptidase)	NALCN (Na-channel activity)
BAI3 (angiogenesis inhibitor)	MAGEA6 (protein binding)
GPR133 (G protein–coupled receptor)	MAP2K4 (toll-like receptor signaling pathway)
GUCY1A2 (guanylate cyclase)	SLC16A4 (monocarboxylate transporter)
PRKCG (protein kinase)	SLIT2, ROBO1, ROBO2 (axon guidance pathway genes)
Q9H5F0 (unknown function)	SEMA3A, SEMA3E (semaphorins, axon guidance)