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KRAS, Hedgehog, Wnt and the twisted developmental biology of pancreatic ductal adenocarcinoma

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is characterized by near-universal mutations in KRAS and frequent deregulation of crucial embryonic signalling pathways, including the Hedgehog (Hh) and Wnt- β -catenin cascades. The creation of mouse models that closely resemble the human disease has provided a platform to better understand when and in which cell types these pathways are misregulated during PDAC development. Here we examine the central part that KRAS plays in the biology of PDAC, and how the timing and location of Hh and Wnt- β -catenin signalling dictate the specification and oncogenic properties of PDAC.

Tumours frequently display inappropriate activation of signalling pathways that are essential for embryonic development and tissue homeostasis. Not only do such pathways contribute to the ability of tumour cells to proliferate and evade cell death, but they also alter cell plasticity. Pancreatic ductal adenocarcinoma (PDAC) fits this pattern and commonly displays reactivation of embryonic signalling pathways, such as transforming growth factor- β (TGF β), Notch, Hedgehog (Hh), and Wnt- β -catenin signalling (see REF. 1 for a review of the molecular genetics of PDAC).

Unlike human tumours, such as medulloblastoma in which aberrant Hh signalling is sufficient for disease development^{2,3}, or colon cancer⁴ in which deregulated Wnt- β -catenin can represent an initiating event, genetic experiments have revealed that the misregulation of Hh and Wnt- β -catenin signalling alone is not sufficient to drive PDAC development. Instead, analysis of PDAC mouse models driven by targeted pancreatic expression of oncogenic KRAS suggest that both temporal and spatial control of Hh and Wnt- β -catenin activity are involved in specifying a cell lineage that can progress to PDAC. We review the ability of KRAS to alter cell fate in the pancreas and how the timing and location of Hh and Wnt- β -catenin signalling contribute to PDAC development.

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DATABASES Entrez Gene: <http://www.ncbi.nlm.nih.gov/gene/p48> / [Pdx1](http://www.ncbi.nlm.nih.gov/gene/Pdx1)

National Cancer Institute Drug Dictionary: <http://www.cancer.gov/drugdictionarygemcitabine>

Pathway Interaction Database: [http://pid.nci.nih.gov/Wnt- \$\beta\$ -catenin](http://pid.nci.nih.gov/Wnt-beta-catenin)

UniProtKB: <http://www.uniprot.org/DHH> | [GLI1](http://www.uniprot.org/GLI1) | [GLI2](http://www.uniprot.org/GLI2) | [GLI3](http://www.uniprot.org/GLI3) | [IHH](http://www.uniprot.org/IHH) | [KRAS](http://www.uniprot.org/KRAS) | [PTC](http://www.uniprot.org/PTC) | [SHH](http://www.uniprot.org/SHH) | [SMAD4](http://www.uniprot.org/SMAD4)

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Mutant KRAS drives PDAC development

Substantial efforts have been applied to determining the molecular underpinnings of PDAC. Although some (~2–10%) PDACs seem to be associated with hereditary factors^{5,6}, most are associated with high-frequency somatic mutations in a subset of genes, including those that encode the small GTPase protein KRAS⁷, and the tumour suppressors INK4A⁸, p53 (REFS 9,10) and SMAD4 (REF. 11). Of these frequently observed alterations, it is key to note that KRAS mutation is nearly universal (>95%) in human PDAC. The KRAS mutations found in PDAC result in a protein locked in a constitutively active state, unable to hydrolyse GTP, thus promoting persistent signalling to downstream effectors (reviewed in REF. 12). Although large-scale genomic studies are expanding knowledge of the wider landscape of mutations found in PDAC¹³, investigating the function of such ‘classical’ genes listed above in cell culture and animal models has considerably advanced insights into PDAC maintenance and progression. PDAC is associated with non-invasive, preneoplastic lesions that are thought to be precursors to the disease¹⁴ (BOX 1). Pancreatic intraepithelial neoplasias (PanINs) are the most common and most widely studied putative precursors. They are histologically classified into three stages of increasing cellular and nuclear atypia¹⁵ (FIG. 1). Molecular studies have shown that the PanIN stage correlates with increasing mutation frequency and variety¹⁶. For example, PanIN1 lesions frequently possess mutated KRAS (estimates suggest 15–40%¹⁷) but less often harbour mutations in p53 or SMAD4. PanIN3 lesions are more likely to express mutated KRAS, p53 and SMAD4 (REFS 18,19).

Owing to its near universal frequency in PDAC, mutation of *KRAS* was proposed as an initiating genetic lesion in this disease. However, initial efforts to audit the sufficiency of mutant KRAS to initiate PDAC progression were stymied by the limitations of transgenic approaches. Expression of mutant KRAS under acinar and ductal promoters resulted in ductal lesions reminiscent of PanINs and mixed acinar and ductal carcinomas²⁰, or periductal inflammation²¹, respectively. However, neither model resulted in PDAC or a faithful recapitulation of the stepwise progression of precursor lesions. Although it is unclear why these models failed to recapitulate human disease progression, they may have been hampered by hyperphysiological KRAS output, or activation of KRAS in an inappropriate cell type or developmental stage. The ability of mutant KRAS to drive PDAC was not successfully investigated until the development of a Cre-inducible conditional allele (lox-stop-lox *Kras*^{G12D} (*LSL-Kras*^{G12D})) targeted to the endogenous *Kras* locus²², thus allowing expression of constitutively active KRAS under temporal and spatial control. This tool eliminated possible issues of confounding cellular responses to overexpression, as transcription of the mutant *Kras* allele depends on the activity of the endogenous *Kras* promoter. Initially, mice expressing the *LSL-Kras*^{G12D} allele were crossed to mice that expressed Cre recombinase under the control of promoters of the key pancreatic progenitor genes: pancreatic and duodenal homeobox 1 (*Pdx1*) and *p48* (also known as *Ptf1a*), thus targeting mutant KRAS to most cells in the developing pancreas. A small number of *Pdx1-Cre;LSL-Kras*^{G12D} and *p48-Cre;LSL-Kras*^{G12D} mice (TABLE 1) developed PDAC over the course of 1 year²³. Furthermore, early-stage PanINs were universally penetrant, and lesions resembling the entire human PanIN spectrum were observed with increasing age.

Recently, these original models have been modified to begin to determine which pancreatic cell types can develop into PDAC when mutant KRAS is expressed. By using strategies that make use of inducible Cre, which allows the activation of *Kras*^{G12D} in specific populations of adult cells, it has become evident that although PDAC displays ductal characteristics, it may not necessarily emanate from the duct compartment^{24–30} (FIG. 1; TABLE 1). Therefore, mutant KRAS is a crucial determinant of the PanIN–PDAC ‘lineage’ and is capable of driving pancreatic cells from terminal differentiation into a duct-like fate that can ultimately give rise to PDAC.

Additionally, KRAS-driven models have been combined with loss-of-function alleles of the most commonly inactivated tumour suppressors, including *Ink4a*, *Trp53* and various components of the TGF β signalling cascade^{31–38} (the models are summarized in TABLE 1), revealing that these pathways constrain KRAS-directed PDAC development. Interestingly, eliminating different tumour suppressors can dramatically alter the type of precursor lesion that develops and the ultimate differentiation state of the malignant disease. It is important to note that cells in these models are subjected to simultaneous activation of KRAS and loss of tumour suppressor function. This is likely to contrast to human tumour progression in which KRAS mutations seem to occur early in disease development, and cells subsequently undergo selection pressure and accumulate progressive tumour suppressor loss. The observation that enforcing tumour suppressor loss, out of the order that may occur in the spontaneous development of human disease, changes the course of PDAC development suggests that the disease depends on specific and sequential tuning of signalling pathways, in a similar fashion to normal development. Characterization of human tumours and mouse models indicate that this progression depends not just on compromising tumour suppressors, but also on the development of aberrant activity of other signalling pathways, including those that dictate developmental processes. The remainder of this Review focuses on two of these pathways: Hh and Wnt– β -catenin signalling.

Hh signalling in PDAC

Hh signalling plays a crucial part in embryonic development and has been implicated in homeostasis (impacting regeneration^{39,40} and stem cell maintenance^{41–43}) and disease development in adult organs (for reviews on the biochemistry and developmental biology dependent on the pathway, see REFS 44,45). Hh signalling is mediated by a family of three, often tissue-specific, secreted ligands (Sonic hedgehog (SHH), Indian hedgehog (IHH) and Desert hedgehog (DHH)). Hh ligands activate signalling in target cells by binding to the 12-pass transmembrane receptor patched (PTC). In the absence of ligand, PTC represses the activity of a 7-pass transmembrane receptor, smoothened (SMO). Ligand binding inactivates PTC, resulting in the activation of SMO. SMO activation, in turn, stimulates downstream intracellular components of Hh signalling that lead to the cytoplasmic accumulation and nuclear localization of active forms of the Gli transcription factors. Three Gli family members are found in mammals, Gli1, Gli2 and Gli3, and each possess unique properties. Gli1 is only present in an active form and Gli2 also functions predominately as an activator, as its repressor form is less stable than its activating form⁴⁶, whereas Gli3 functions mainly as a repressor, as its repressor form is more stable than its transient full-length form⁴⁷. Changing this balance of activating and repressing Gli proteins can lead to the induction of

transcriptional targets that drive Hh-dependent phenotypes, such as proliferation through expression of cyclin D1 (REF. 48) and MYC⁴⁹, evasion of apoptosis by promoting expression of BCL2 (REF. 50) and cell differentiation through the expression of Forkhead family transcription factors^{51,52}. Moreover, the magnitude and duration of Hh signalling can alter owing to changes to positive (such as GII1) and negative regulators (such as PTC) of the pathway.

The role of Hh signalling in tumorigenesis has emerged in parallel with an understanding of Hh biochemistry and its contribution to normal development. Deregulated Hh signalling in tumours can be approximately classified into two types depending on the level in the signalling cascade at which the pathway is activated (reviewed by REF. 53). The first class is defined by cell-autonomous mutations in key regulatory proteins; for example, inactivating mutations in PTC or activating mutations in SMO, such as those observed in basal cell carcinoma^{54,55} and medulloblastoma⁵⁶. The second class of Hh-driven tumours, including cancers of the breast, colon, prostate and PDAC, is characterized by inappropriate ligand expression. Aberrant Hh ligand expression is observed at a high frequency in human PDAC (~75%) and is detectable throughout disease progression, beginning in early PanINs⁵⁷, an expression pattern that is recapitulated in mutant KRAS-driven mouse models³³. Hh signalling in PDAC cells was initially thought to be activated in an autocrine fashion. However, although chemical inhibitors targeting the pathway at the level of SMO (such as cyclopamine) or Hh ligand decrease the tumour burden and metastasis in xenotransplanted primary human tumours and cell lines, and SMO inhibition affects survival and tumour development in mutant KRAS-mutant mouse models⁵⁸⁻⁶¹, recent evidence indicates that epithelial PDAC cells do not respond to Hh ligand and are refractory to ligand inhibition^{62,63}. Instead, Hh signalling in PDAC seems to involve a ligand-dependent component in the tumour microenvironment in which the classical cascade is activated and a ligand-independent module in the tumour epithelium in which Gli activity is deregulated (FIG. 2).

A robust desmoplastic response is one hallmark of PDAC. The desmoplastic stroma of PDAC consists of a complex array of cell types, including cancer-associated fibroblasts (CAFs), inflammatory cells and tumour-associated vasculature. Evidence is mounting that paracrine Hh signalling plays a crucial part in supporting pro-tumorigenic communication between tumour epithelium and stroma, especially with respect to CAFs. CAFs are widely recognized as promoters of tumorigenesis⁶⁴. Reconstitution experiments, in which tumour cells from various cancer types are admixed with CAFs and grown as xenografts, have shown that CAFs can promote transformation of immortalized epithelium (for example, SV40-immortalized prostate cells⁶⁵) and enhance tumour cell growth⁶⁶. PDAC-derived CAFs have been shown to have similar enhancing effects on the growth of xenotransplanted PDAC cells⁶⁷⁻⁶⁹. In support of a paracrine role for Hh ligand in promoting tumour growth, Yauch and colleagues⁶² showed that stromal cells recruited to xenografts of human PDAC cell lines had active Gli signalling, and xenografts treated with a SMO inhibitor displayed a decrease in the expression of mouse Hh target genes, but not in the expression of transplant-derived, human genes. Using mouse embryonic fibroblasts (MEFs) as a proxy for CAFs in reconstitution experiments, Yauch and colleagues⁶² demonstrated that SMO-deficient MEFs were significantly less efficient at promoting tumour growth than SMO-expressing cells.

Furthermore, bailey and colleagues⁷⁰ showed that co-transplantation of human pancreatic fibroblasts with transformed, SHH-overexpressing human duct cells led to increased growth of xenografts. Although paracrine Hh signalling clearly contributes to PDAC maintenance, the exact nature of the paracrine functioning, CAF-derived factors remains unclear. However, profiling experiments suggest that they may include some known pro-tumorigenic factors such as the insulin-like growth factors⁶². Hh ligand signalling may not only drive CAFs to produce pro-tumorigenic factors, but may also support an activated CAF phenotype, characterized by the expression of smooth muscle actin — a marker of the myofibroblast state — and the production of extracellular matrix proteins such as fibronectin and collagen I⁷¹, the latter of which has been shown to enhance PDAC cell proliferation and invasiveness^{72–75}. Treating human pancreatic fibroblasts with SHH increases smooth muscle actin expression, and overexpression of SHH ligand in xenotransplanted cells enhances collagen I and fibronectin expression in recruited host fibroblasts⁷¹. Furthermore, Hh ligand promotes CAF proliferation and stimulates CAF migration⁷¹. Therefore, epithelial-derived Hh ligands may initiate a feed-forward loop that supports CAFs and stimulates the production of factors that act on tumour cells (FIG. 2). As Hh ligand expression increases as the severity of PanINs progresses⁵⁷, this may be a mechanism by which CAFs are expanded and maintained during PDAC progression.

Recent reports indicate that the role of paracrine Hh signalling may not be limited to the tumour cell–CAF axis, and that paracrine Hh ligand signalling may also affect the tumour-associated vasculature. bailey and colleagues⁷⁰ showed that expression of SHH increased angiogenesis in xenografts of transformed human pancreatic ductal cells. This effect depended in part on ligand signalling, as functional inhibition of Hh ligands with a blocking antibody blocked lymphangiogenesis in xenotransplanted tumour cells expressing Hh ligand⁷⁰. Indeed, this effect may be direct owing to the ability of Hh ligands to activate migration and expansion of lymphatic endothelial cells⁷⁰. It may also occur through indirect mechanisms, such as the induction of pro-angiogenic factors; for example, insulin-like growth factor 1 (IGF1) and angiopoietin 1 (REF. 76) produced by bone marrow-derived cells recruited to the tumour microenvironment, and vascular endothelial growth factor A (VEGFA) and matrix metalloproteinase 9 (MMP9)⁷⁰ produced by CAFs.

The effect of SMO inhibition on disease progression in KRAS-driven mouse models further reflects the importance of paracrine Hh ligand signalling in PDAC. Olive and colleagues⁶¹ recently demonstrated that treating *Pdx1-Cre;LSL-Kras^{G12D};Trp53^{R172H/+}* or *Pdx1-Cre;LSL-Kras^{G12D};Trp53^{R270H/+}* mice with a SMO antagonist and gemcitabine, a first-line PDAC chemotherapeutic agent^{77,78}, not only increased survival and decreased metastasis, but also significantly decreased the fibroblastic component of the tumours. Interestingly, SMO inhibitor treatment transiently increased the density of the tumour vasculature, and increased the concentration of gemcitabine reaching the tumour cells. Although this is a surprising finding given the data that indicate a proangiogenic role for stromal Hh signalling, it might reflect the observation that the apparent pro-tumorigenic hypovascularity observed in PDAC depends on a balance between the stromal and epithelial compartments. Nonetheless, these studies suggest that stromal Hh signalling may be a useful therapeutic target that could be explored in parallel with other drugs.

If ligand-mediated, canonical signalling is absent in the PDAC epithelium, does the Hh pathway have a direct role in the evolution of the tumour cells? It probably does, as several studies have shown that active Gli proteins support the proliferation and viability of epithelial PDAC cells^{79–81}. Currently two signalling cascades have been implicated in supporting this non-canonical activation of Gli signalling. Targeting mutant KRAS directly by small interfering RNA (siRNA) or by targeting its downstream RAF–MAPK effector pathway with chemical inhibitors decreases the transcription of Gli-target genes and tumour cell growth^{81,82}. Also, blocking TGF β signalling in PDAC cells inhibits Gli activity and cell growth⁸³. Regulation of Gli signalling by TGF β raises questions regarding how the relative concentrations of the three different forms of Gli proteins contribute to Gli output. Treating cell lines derived from KRAS-driven mouse PDAC with TGF β increases expression of GII1 and GII3, and GII3 is induced to greater levels than GII1 (REF. 81). This is surprising, as GII3 predominantly acts as a repressor of Gli targets. However, sequencing analysis has shown that *GLI3* and the related gene *GLI4* are mutated at a high frequency in PDAC¹³. Therefore, regulation of epithelial Gli signalling might not only occur through non-canonical upstream signals, but also may involve novel interactions and functions of the Gli proteins.

Hh signalling and PDAC development

As Hh signalling seems to be important in PDAC progression and maintenance, a key question is how it contributes to disease initiation. Several genetic approaches have been used to determine how modulating Hh signalling alone, as well as in the context of mutant KRAS, changes the course of PDAC inception (these models are summarized in TABLE 2). A relationship between Hh signalling and PDAC initiation was first implicated in experiments that set out to determine how ligand-mediated Hh signalling directs gastrointestinal development. Several developmental studies illustrated that normal gut development is dependent on the tightly controlled expression of Hh ligands. For example, transgenic mice expressing SHH during early pancreas development under the control of the *Pdx1* promoter (*Pdx1-Shh*)⁸⁴ display near-complete pancreatic agenesis and, in place of a normal pancreas, possess duct structures embedded in an expanded mesenchymal compartment that expresses molecular markers characteristic of tumour-associated stroma⁸⁴. Interestingly, the ductal remnants morphologically resemble early human PanIN1–2 lesions, and several of these transgenic mice also developed spontaneous, pancreas-specific KRAS mutations, suggesting that inappropriate ligand expression may promote changes in tissue architecture and signalling involved in PDAC initiation⁵⁷. However, these animals died shortly after birth (3 weeks), which prevented evaluation of the ability of Hh ligand signalling alone to drive PDAC. To avoid the developmental consequences of aberrant ligand signalling, models aimed at testing if the intracellular modules of the Hh signalling cascade could drive PDAC were developed. First, mice carrying the *CLEG2* transgene, which allows Cre-inducible, conditional expression of a dominantly active GII2 protein lacking the amino-terminus repressor domain, were crossed with mice expressing a *Pdx1-Cre* driver⁸⁵. Approximately one-third of *Pdx1-Cre;CLEG2* mice develop pancreatic tumours⁸⁵ which consist of undifferentiated, spindle-shaped cells that do not molecularly or histologically resemble PDAC. Therefore, although Gli signalling is potentially oncogenic in the pancreas, alone it is inefficient in driving PDAC development.

Given the high frequency of activating KRAS mutations in PDAC and that mutations in KRAS and persistent Gli activity are found in fully transformed PDAC lines, the ability of KRAS and Gli signalling to synergize to drive PDAC initiation and development was determined by intercrossing *Pdx1-Cre; CLEG2* mice with *LSL-Kras^{G12D}* animals. Within 3 to 6 weeks of birth, these animals rapidly developed PanIN lesions, including lesions that possessed characteristics of advanced grade 2 and 3 lesions⁸⁵. PanIN lesions in this model also displayed aberrant Hh ligand expression and were accompanied by an expanded, proliferative stromal compartment, further linking Hh ligand signalling with stromal activation. However, the *Pdx1-Cre; CLEG2; LSL-Kras^{G12D}* mice did not develop PDAC and instead developed an undifferentiated tumour of unclear origin, similar to that observed in *Pdx1-Cre; CLEG2* mice.

The fact that PanINs in the *Pdx1-Cre; CLEG2; LSL-Kras^{G12D}* model invariably displayed aberrant Hh ligand expression further suggested a role for ligand-dependent signalling in PDAC development. To determine if this role depended on ligand-dependent signalling in epithelial cells, Stevaux and colleagues generated *p48-Cre; LSL-Kras^{G12D}; Smo^{flax/flax}* mice⁸¹, therefore rendering pancreatic progenitors insensitive to Hh ligand. Surprisingly, PanIN and PDAC development progressed at an equivalent rate in these mice as in wild-type SMO controls. Similarly, both genotypes developed persistent epithelial ligand overexpression and equivalent levels of Hh-target gene activation. In addition, inhibition of GIII in cell lines derived from *Smo*-deficient and *Smo*-wild-type tumours led to increased apoptosis and decreased cell growth. Therefore, this model suggests that aberrant ligand expression and epithelial Gli signalling contribute to PDAC initiation and progression, but are uncoupled and evolve independently.

Taken together, these mouse models suggest that non-canonical epithelial Gli signalling and stromal ligand-dependent signals synergize with KRAS to drive PanIN initiation and progression to PDAC. However, important questions still remain. Hh ligand is clearly involved in instructing the tumour microenvironment, although which stromal Hh-target genes are key for the maintenance of CAFs and other components of the tumour stroma and which factors exert effects on the epithelium, including impacting differentiation, are mainly unknown. Furthermore, epithelial Gli activity seems to be involved in PDAC progression and develops owing to the activity of pathways other than the canonical Hh ligand–SMO-dependent pathway. A better understanding of how these non-canonical pathways (including KRAS and TGF β signalling) activate Gli signalling and which epithelial Gli targets are crucial for maintenance of tumour biology might provide additional therapeutic targets, in parallel with factors activated by Hh ligand stimulation in stromal cells. Another therapeutic possibility is to determine other developmental signalling pathways that may interact in parallel with Hh signalling. One such candidate pathway that has been shown to have substantial effects on PDAC initiation and progression is Wnt– β -catenin signalling.

Wnt– β -catenin signalling in PDAC

Like Hh signalling, Wnt– β -catenin signalling is an important embryonic signalling pathway that is required for the proliferation, morphogenesis and differentiation of several organs (for a review, see REF. 86). β -catenin signal transduction has been excellently reviewed

elsewhere⁸⁷ (summarized in FIG. 3a). briefly, Wnt ligands bind to receptors of the Frizzled family of proteins and co-receptors. ligand binding results in the inactivation of a complex of cytoplasmic proteins (including adenomatous polyposis coli (APC) and axin) that promote the proteasomal degradation of β -catenin, resulting in its cytoplasmic accumulation and nuclear localization. In the nucleus, β -catenin binds to TCF/LEF factors to activate target genes. Nineteen Wnt ligands have been identified in mammals that are known to activate canonical (β -catenin-dependent) and non-canonical (β -catenin-independent) signal transduction cascades on binding to the Frizzled–LRP co-receptor complex. In this section, we focus on the canonical pathway, as this pathway has been implicated in PDAC development. However, non-canonical Wnts such as WNT5a are deregulated in PDAC and might have a role in PDAC biology^{88,89}.

Mutations in genes encoding regulatory proteins of the canonical signalling cascade are associated with many tumour types and in some cases, such as colon cancer, can act as initiating genetic lesions. loss-of-function mutations in negative regulators, such as APC⁴, and gain-of-function mutations in β -catenin itself⁹⁰ activate constitutive, ligand-independent β -catenin signalling in a similar fashion to PTC and SMO mutations. like these Hh pathway mutations, genetic lesions in classical β -catenin regulatory modules are rare in PDAC^{91–93}. Therefore, the role of β -catenin in PDAC has been controversial.

However, recent studies have begun to cement an important role for Wnt– β -catenin signalling in PDAC. Although β -catenin accumulation is not a universal characteristic of this disease, both nuclear (10–60%) and cytoplasmic accumulation (25–65%) of β -catenin are observed in PanIN lesions and PDAC^{94–96}. Functional evidence is also accumulating that implicates a supporting role for β -catenin in PDAC maintenance and progression. Inhibiting β -catenin with siRNA substantially compromises PDAC proliferation and increases apoptosis⁹⁶. Furthermore, increased levels of cytoplasmic and nuclear β -catenin correlate with PanIN grade and the development of invasive PDAC^{94,97}. Recently, ataxia telangiectasia group D-associated protein (ATDC, also known as TRIM29) has been shown to contribute to the progressive accumulation of β -catenin and persistent activation of β -catenin-target genes in PDAC⁹⁷. Inhibiting ATDC function in PDAC cell lines decreases β -catenin activity, tumour growth and metastasis. Therefore, genes such as *ATDC* might comprise a set of non-classical players that influence oncogenic β -catenin activity in PDAC in the absence of mutations normally observed in other β -catenin-driven tumours. Interestingly, accumulation of β -catenin mediated by ATDC⁹⁷ leads to increased expression of MYC, a key node in β -catenin-driven transformation in other tissues. There is also emerging evidence that β -catenin accumulation and signalling could be increased through paracrine signalling which occurs in the PDAC micro-environment. Recent studies have shown that PDAC cells admixed with CAFs grown in three-dimensional extracellular matrix plugs display increased cytoplasmic β -catenin accumulation⁹⁸, and growth on collagen I promotes nuclear β -catenin accumulation and activation of known β -catenin-target genes⁷⁵.

With the exception of the specific examples discussed above, the general mechanism by which β -catenin accumulation occurs in PDAC is poorly understood (summarized in FIG. 3b). For example, although β -catenin accumulation correlates with disease severity, it is unclear whether accumulation depends on an autocrine response to Wnt ligands

overexpressed by tumour cells. In support of this, canonical Wnt ligands (including WNT3 and WNT8b) are strongly expressed in PDAC samples⁹⁶, and enforced expression of secreted frizzled receptor protein (SFRP, an inhibitor of Wnt ligands) decreases β -catenin transcriptional activity in a subset of human PDAC lines⁹⁹. However, overexpression of DKK1, another inhibitor of secreted Wnt ligands, is associated with more advanced PanINs and PDAC, and increases PDAC cell growth and motility¹⁰⁰. Furthermore, which of the vast number of β -catenin target genes are crucial for PDAC maintenance and progression are currently unknown. Therefore, although classical β -catenin oncogenic targets might be important in PDAC, more work is required to determine whether and at what level β -catenin signalling should be targeted for potential therapeutic effects.

β -catenin is insufficient to initiate PDAC

Increasing evidence indicates that β -catenin signalling contributes to PDAC maintenance. by contrast, the role of β -catenin function during PDAC initiation has only recently been analysed. Three studies have investigated the ability of uncontrolled β -catenin signalling to transform cells in the pancreas. Our studies have used a β -catenin allele in which the third exon (encoding phosphorylation sites important for protein degradation) is floxed and can be eliminated through Cre-mediated recombination¹⁰¹, resulting in enforced, conditional β -catenin stabilization and signalling. When crossed to a Cre driver line that expresses Cre in all pancreatic progenitor cells — *Pdx1-Cre^{Early}* — stabilized β -catenin impaired pancreatic development, resulting in severe exocrine agenesis and the formation of large cysts¹⁰². Interestingly, this effect on pancreatic development was stage- and cell type-dependent. Crossing the stabilized β -catenin allele with another Cre driver line, *Pdx1-Cre^{Late}*, in which Cre expression is delayed and restricted to acini and endocrine cells, resulted in normal pancreatic development. Surprisingly, postnatal expansion of the exocrine pancreas was observed in ageing mice that correlated with increased acinar proliferation and accumulation of β -catenin. Interestingly, although β -catenin accumulation persisted in older mice, no tumours were observed. Strom and colleagues¹⁰³ generated *Pdx1-Cre* mice expressing floxed alleles of the crucial destruction complex component APC (*Pdx1-Cre;Apc^{flox/flox}*), thereby enforcing canonical Wnt signalling in cells of the developing pancreas. These mice also had increased postnatal pancreatic mass that correlated with enhanced exocrine proliferation, age-dependent accumulation of nuclear β -catenin, and increased expression of β -catenin-target genes; yet, these mice also failed to develop tumours by 1 year of age. Therefore, this work supported the ability of β -catenin to potently activate proliferation in the exocrine pancreas and again demonstrated that the transforming capacity of β -catenin is tightly regulated in pancreatic cells.

Additional studies have shown that enforced β -catenin signalling can induce pancreatic transformation when activated at an appropriate developmental stage. Activation of stabilized β -catenin using a *p48-Cre* driver (*p48-Cre;Ctmb1^{exon3/+}*) resulted not only in acinar proliferation and activation of some β -catenin-target genes, but also the development of large benign tumours that resemble human solid pseudopapillary tumours (SPTs)¹⁰⁴. These SPT-like tumours, which in humans have been shown to carry activating mutations in β -catenin¹⁰⁵, are morphologically and molecularly distinct from PDAC. Taken together, these models suggest that β -catenin signalling is a crucial mediator of exocrine proliferation

and, although it can induce pancreatic tumorigenesis in a temporal and cell-type-dependent manner, it does not seem to be sufficient to initiate PDAC.

Given its predominant role in initiating PDAC, an important question is whether KRAS can synergize with β -catenin signalling to drive PDAC development, as observed in other tumour types such as colon cancer^{106–108}. Surprisingly, *p48-Cre; Ctnnb1^{exon3/+}; LSL-Kras^{G12D}* mice did not develop SPT-like tumours, or PanINs or PDAC. Instead, they developed a distinct ductal tumour similar to rare intraductal tubular tumours (ITTs) observed in humans¹⁰⁴. Therefore, although PDAC shows concomitant KRAS activity and β -catenin accumulation, simultaneous activation of these two pathways in the developing pancreas seems incompatible with specification of the PanIN–PDAC ductal lineage.

The unexpected lack of synergy between β -catenin and KRAS in PDAC initiation suggests that developmental signalling pathways must be tuned to appropriate levels at key time points during transformation to specify the PanIN–PDAC lineage. Recently, the discovery that pancreatitis, a potent risk factor for PDAC in humans^{109–111}, accelerates PanIN and PDAC development in mice expressing mutant KRAS in the exocrine compartment has led to insights into the role of β -catenin in this process. This discovery has also prompted interesting findings regarding which cells in the pancreas have the capacity to be reprogrammed into PanIN–PDAC lesions.

Acinar plasticity and the PanIN–PDAC lineage

Unlike other gastrointestinal organs, such as the intestine and colon, turnover of the pancreatic parenchyma occurs at a very slow rate (for example, as shown by rare incorporation of bromodeoxyuridine into the DNA of healthy, adult mouse pancreas cells¹¹²). However, the pancreas has potent regenerative capacity. Investigations of pancreatic regeneration following various types of pancreatic damage (such as chemically induced pancreatitis¹¹³ and partial pancreatectomy¹¹⁴) have yielded extensive knowledge regarding the source of cells that repopulate damaged pancreatic compartments and the inherent plasticity of adult pancreatic lineages.

In humans, chronic pancreatitis is a potent risk factor for PDAC^{109–111}. Recently, several studies have used different versions of KRAS-driven mouse PDAC models to evaluate whether pancreatitis is functionally relevant to PDAC initiation and progression. Not only have these studies shown that pancreatitis accelerates PanIN and PDAC development, suggesting that tissue damage and inflammation cooperate with KRAS signalling to drive the disease, they have also indicated that KRAS substantially alters pancreatic regeneration and plasticity. The first study to directly investigate the effect of pancreatitis on PDAC development used a mouse model that allowed doxycycline-induced, temporal expression of constitutively active, mutant KRAS (*Kras^{G12V}*) exclusively in the acinar and centroacinar compartment²⁴. like models in which KRAS is induced by Cre under control of the *Pdx1* or *p48* promoters, activation of KRAS in these mice during embryogenesis or soon after birth resulted in the development of frequent PanIN lesions and some PDACs. Although the presence of acinar markers in human and mouse PanINs had been noted¹¹⁵, this result provided direct evidence for a non-ductal source for the PanIN–PDAC sequence of events.

Interestingly, these mice were refractory to PanIN–PDAC development when mutant KRAS was activated 60 days after birth. Therefore, the authors investigated whether persistent damage in the form of chronic pancreatitis could provide a permissive environment for the development of PanIN–PDAC. They induced pancreatitis through long-term treatment with caerulein, a cholecystokinin analogue that stimulates precocious activation of acinar cell digestive enzymes, resulting in pancreatic auto-digestion and cellular damage that is associated with inflammation. In response to chronic pancreatitis, mice in which KRAS was activated in adulthood developed PanINs and PDAC at a high frequency. Thus, this study functionally linked pancreatitis and, therefore, tissue damage to the initiation and progression of PDAC.

Since this study, other groups have verified its key findings, namely that KRAS can reprogram acinar cells into the ductal PanIN–PDAC lineage, and that pancreatitis potently accelerates the initiation and progression of KRAS-driven PanIN–PDAC. Using other inducible Cre lines, adult acinar cells have been shown to be sensitive to spontaneous ductal reprogramming into PanINs by KRAS^{G12D} in the absence of pancreatitis^{25,26}. Although the mechanisms remain unclear, these results suggest that different KRAS mutations (in this case KRAS^{G12D} versus KRAS^{G12V}) that both render the protein constitutively active may exert distinct biological and biochemical effects on exocrine cells, potentially due to differences in the levels of Ras signalling. Indeed, there is emerging evidence that acinar to ductal reprogramming into the PanIN–PDAC lineage depends on breaching a crucial KRAS activity threshold (FIG. 4). Ji and colleagues²⁸ showed that levels of active, GTP-bound KRAS increase between cells derived from non-transformed pancreas expressing KRAS^{G12D} and cells derived from KRAS^{G12D}-driven PDAC. Enforcing KRAS activity in acini at levels which mirror those observed in PDAC resulted in acinar to ductal metaplasia that was reminiscent of chronic pancreatitis in young mice. This was followed by the development of PanINs and PDAC as animals aged²⁸. In support of the need for a crucial KRAS activity threshold to initiate ductal reprogramming, Siveke and colleagues¹¹⁶ showed that combining overexpression of TGF α , which can activate KRAS downstream of the epidermal growth factor receptor, with mutant KRAS dramatically accelerates the elimination of normal acini and the development of PanINs and intraductal papillary mucinous neoplasm (IPMN). Currently, which KRAS effectors are important for reprogramming acini into the PanIN–PDAC lineage is unclear; however, MAPK activity²⁹ and Akt signalling¹¹⁷ (downstream of Ras-activated PI3K) have been associated with acinar to ductal plasticity.

Other studies have verified the ability of pancreatitis to provide a permissive environment for specifying PDAC precursors, demonstrating that acute pancreatitis potently accelerates PanIN development, and in some cases PDAC, in mice in which mutant KRAS is expressed in the exocrine compartment^{29,118}. These studies, alongside that of Guerra and colleagues²⁴, suggest that tissue damage is a permissive environment for the development of PDAC precursors and altering pancreatic regeneration may play a parallel part with KRAS activity in initiating ductal reprogramming into the PanIN–PDAC lineage. In wild-type mice, acinar cells rapidly regenerate following severe caerulein-induced pancreatitis^{112,113,119}. However, acinar cells undergo substantial morphological and molecular changes immediately following injury. During this regenerative phase, acinar cells transiently reactivate elements

of embryonic pancreatic development¹¹³, frequently assume duct-like morphology¹¹² and express cytokeratin 19, a marker of ductal differentiation²⁹. However, it is important to note that in wild-type animals this dedifferentiated, ductal state does not become fixed and cells resume acinar differentiation. This limited acinar to acinar plasticity has been noted in response to other insults that activate acinar regeneration such as partial pancreatectomy¹¹⁴. Therefore, in the absence of aberrant activation of some signalling pathways, fixed acinar to ductal reprogramming (or acinar to ductal metaplasia (ADM)) is a tightly restricted differentiation fate. It can occur in wild-type acini *in vivo*, but seems to require persistent damage. Strobel and colleagues¹²⁰ noted that mice with 7 to 10 weeks of chronic caerulein-induced pancreatitis developed mucinous metaplastic lesions (MMLs) that had some characteristics of early PanINs (and newly defined pancreatic duct glands¹²¹). Although most MMLs were derived from ductal or centroacinar cells, only a small percentage (~5%) were the result of ADM. Desai and colleagues¹¹⁴ also noted ADM in mice following pancreatic ductal ligation, a process characterized by severe acinar apoptosis and prolonged acinar loss. However, ADM can readily be induced through activation of other signalling pathways, such as epidermal growth factor (EGF)¹²² and Notch signalling^{123,124}, by exposing acini to matrix metalloproteinase 7 (MMP7)¹²⁴ and, as recently appreciated, activation of mutant KRAS²⁵. Furthermore, enforced expression of PDX1 in exocrine cells during development induces ADM¹²⁵. Therefore, some factors associated with acinar regeneration (such as Notch signalling and PDX1 expression) can drive acinar to ductal reprogramming when inappropriately activated, suggesting that developmental signalling pathways must be tightly regulated to control acinar plasticity.

Taken together, these data raise the possibility that developmental signalling pathways must be specifically tuned for KRAS to reprogram acini into ductal PanINs. Our recent work has compared the regenerative response to acute pancreatitis of normal acini versus acini expressing mutant KRAS and found that this is the case with β -catenin signalling²⁹. Although acini expressing mutant KRAS assume a de-differentiated, ductal state similar to wild-type acini in response to acute pancreatitis, their ability to regenerate the acinar state is blocked. Instead, acini expressing mutant KRAS persistently express duct markers and reactivated elements of embryonic development, and rapidly give rise to PanIN lesions. As such persistently active, regeneration-associated elements of pancreatic development are characteristics of PanINs, these data suggest that assuming a de-differentiated state may be a rate-limiting step in KRAS-driven PDAC development. Other mouse models that combine mutant KRAS with mutations in genes involved in maintaining acinar differentiation support this potentially obligate role for de-differentiation. For example, inhibiting muscle, intestine and stomach 1 (MIST1) function, a transcription factor expressed in acinar cells, results in ADM that expresses markers characteristic of the de-differentiated acini found following acute pancreatitis and during KRAS-driven ductal reprogramming and PanIN formation¹²⁶. Indeed, combining *Mist1*-knockout mice with mutant KRAS significantly accelerates the development of acinar-derived PanINs²⁷, supporting a role for de-differentiation as a component of KRAS-driven PDAC initiation.

Furthermore, β -catenin signalling seems to be a crucial difference between transiently de-differentiated, regenerating acini and acini undergoing persistently de-differentiated ductal

reprogramming and PanIN formation²⁹. In wild-type mice, β -catenin signalling is activated during acinar regeneration following caerulein-induced pancreatitis. However, β -catenin signalling is blocked at an equivalent time point in mice expressing mutant KRAS, representing an early stage of ductal reprogramming. Challenging adult acinar cells expressing both mutant KRAS and stabilized β -catenin with caerulein inhibits PanIN formation, and instead results in abnormal duct structures that frequently display nuclear accumulation of β -catenin²⁶. Taken together, it seems that the ability of mutant KRAS to exploit the de-differentiated acinar state to drive the usually restricted, ductal PanIN lineage is sensitive to the molecular activity of developmental signalling pathways and specifically involves a low threshold of β -catenin signalling (FIG. 4).

Therefore, β -catenin seems to have opposing roles during PanIN initiation and progression to PDAC. These studies suggest that mutations in classical β -catenin pathway modulators are mainly absent in PDAC because they may block the ability of KRAS to initiate cells into a progenitor-like lineage capable of being driven into PDAC. However, as discussed above, β -catenin accumulation and signalling occur in PanIN lesions and support PDAC maintenance. These data suggest that β -catenin signalling is tuned, similar to the temporal regulation of developmental signalling pathways required during normal organ specification, in an ordered fashion to allow KRAS to specify the PanIN–PDAC lineage. The exact mechanisms that block β -catenin signalling during the reprogramming of acini into the PanIN lineage and enable its reactivation during PanIN progression are currently being investigated. Insights from recent studies suggest that differing β -catenin levels may be achieved because of the reactivation of other developmental signalling pathways. Siveke and colleagues¹¹⁹ have shown that Notch receptor activation inhibits β -catenin activity in acinar cells in culture and during regeneration following acute pancreatitis. Also, De la O and colleagues²⁶ showed that transgenic NOTCH1 activation significantly accelerated ADM–PanIN. This may indicate that Notch signalling (which is activated in response to acute pancreatitis) may help to maintain a permissive β -catenin signalling threshold during KRAS-driven ductal reprogramming. It is likely that the roles of Notch in PanIN initiation, PDA progression and control of β -catenin signalling will prove to be complex. For example, Hanlon and colleagues have recently demonstrated that elimination of Notch1 in *Pdx1-Cre;LSL-Kras^{G12D}* mice accelerates PanIN and PDAC development¹²⁷. However, these mice showed neither gross changes in the activation of Notch signalling targets nor an increase in β -catenin accumulation. Therefore, the interactions between Notch and β -catenin in PanIN development and PDAC progression may depend on input from other signalling cascades. For example, Gli signalling activated non-canonically in PDAC epithelium⁹⁶ and TGF- β signalling have been shown to support β -catenin signalling in PDAC cells¹³¹. These pathways, along with other novel regulators such as ATDC, may help drive β -catenin signalling in PDAC after the PanIN–PDAC lineage has been established.

Conclusion

The advent of KRAS-driven models has generated much knowledge about how PDAC is initiated and how the disease progresses. both Hh and β -catenin signalling clearly affect PDAC development and maintenance. The next challenge is to understand how to exploit these pathways for therapeutic success. Crucial to this effort will be determining the

molecular basis of how each pathway is activated, through both known mechanisms and discovering new regulators. Also, it is important to understand how the pathways interact and to compare and contrast these interactions in the tumour epithelium and the microenvironment. Finally, efforts must be made to determine which Hh and β -catenin targets are ‘mission critical’ for maintaining proliferation, viability and differentiation, and to develop efficient methods (pharmacological and otherwise) to block these crucial signalling nodes. As these important goals are achieved, they open a window of opportunity to twist the developmental biology of PDAC away from malignancy.

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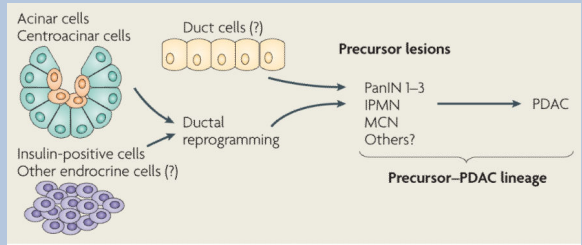
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Box 1

KRAS necessity and sufficiency in the stepwise progression to pancreatic ductal adenocarcinoma

Mucinous cystic neoplasm (MCN), intraductal papillary mucinous neoplasm (IPMN) and pancreatic intraepithelial neoplasia (PanIN)¹ are thought to represent precursor stages for pancreatic ductal adenocarcinoma (PDAC). This view is supported by KRAS-driven mouse models in which such preneoplastic lesions precede the development of PDAC (TABLE 1). Indeed, in models that develop PanINs (the most commonly observed putative precursor lesion in humans) and PDAC, PanIN lesions increase in severity and PDAC becomes more prevalent as mice age. Therefore, in this Review, we refer to this cell fate as the PanIN–PDAC lineage (indicated as precursor–PDAC lineage in the figure below). However, other mouse models suggest that IPMNs and MCNs could be parallel routes to PDAC development. All three lesions share expression of ductal markers and therefore would require reprogramming into a ductal lineage if they emerge from non-ductal compartments, such as acini, centroacinar cells or endocrine cells.

Even though mutant KRAS is sufficient to initiate the PanIN–PDAC lineage in mice, there is some evidence that it may not be necessary. Although frequently observed, KRAS mutation is not universal in early human PanINs. Furthermore, in mice, lesions resembling early-stage PanINs can develop owing to Hedgehog ligand overexpression during pancreatic development⁵⁷ and chronic inflammation¹²⁰. KRAS mutation becomes increasingly frequent in advanced PanINs and PDAC, leading to an important but unresolved question as to when deregulated KRAS activity becomes necessary for disease progression.



At a glance

- Mutations in KRAS are nearly universal in human pancreatic ductal adenocarcinoma (PDAC). Mouse models in which mutant KRAS is targeted to the pancreas reveal that KRAS signalling is sufficient to reprogram pancreatic cells into duct-like lineages capable of progressing through preneoplastic lesions and, ultimately, PDAC in stages that are reminiscent of human disease.
- The latency, differentiation and type of preneoplastic lesion observed in KRAS-driven PDAC mouse models is sensitive to tumour suppressor loss, suggesting that PDAC evolution is dependent on sequential tuning of signalling pathways.
- PDAC is characterized by frequent deregulation of embryonic signalling pathways, including Hedgehog (Hh) and Wnt- β -catenin signalling. Recent evidence points to temporal and spatial control of these pathways in PDAC development and maintenance.
- PDAC cells frequently display aberrant Hh ligand expression. Recent studies suggest that classical ligand-dependent signalling is activated in cells in the tumour microenvironment, supporting tumour maintenance in a paracrine fashion, but not in the tumour epithelium. However, Hh signalling at the level of Gli transcriptional factors is active in the tumour epithelium, dictated by non-canonical regulators of the pathway. Both paracrine ligand activity and epithelial Gli signalling seem to independently support KRAS-driven PDAC evolution in mouse models.
- Wnt- β -catenin signalling is frequently activated in PDAC and contributes to tumour cell proliferation and biology. Genetic models that allow Wnt- β -catenin deregulation reveal that this pathway can transform pancreatic cells but is insufficient to drive PDAC initiation.
- Mouse models have revealed that the ability of KRAS to reprogram cells into a duct-like fate that can give rise to PDAC is sensitive to cell differentiation and levels of KRAS signalling. Temporal regulation of embryonic signalling pathways seems to play a part in preneoplastic reprogramming, as shown by a requirement for control of Wnt- β -catenin signalling during KRAS-driven de-differentiation of acinar cells into PDAC precursor lesions.

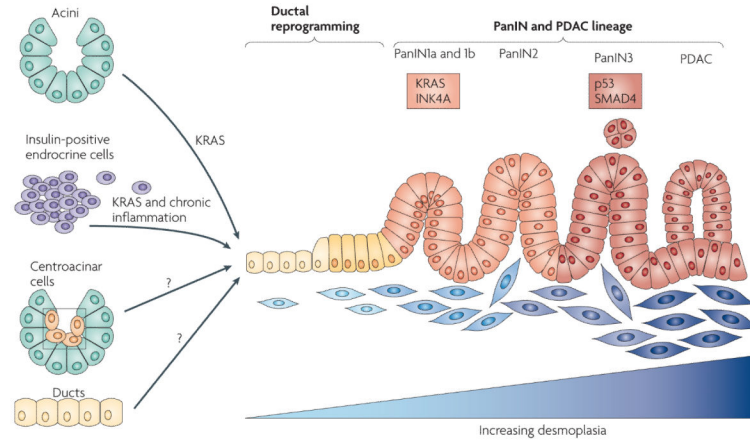


Figure 1. KRAS is a master regulator of pancreatic ductal adenocarcinoma initiation and progression

Constitutively active KRAS (caused by *Kras*^{G12D} or *Kras*^{G12V} mutations) is sufficient to initiate the development of pancreatic intraepithelial neoplasia (PanIN) and pancreatic ductal adenocarcinoma (PDAC). PanINs are classified into three stages of increasing cellular atypia and, in humans, have been found to possess increasing numbers of mutations (common mutations are indicated in boxes). Changes in the epithelium are matched by desmoplastic changes in the stroma. In mouse models, the human PanIN spectrum followed by progression to PDAC has been recapitulated by activating mutant KRAS in embryonic pancreatic progenitors. Eliminating tumour suppressors commonly inactivated in the human disease dramatically decreases PDAC latency (a limited set of examples is indicated). Mouse models in which KRAS is activated specifically in some adult cell types have shown that both acini and insulin-positive cells can give rise to PanINs and, in some cases, PDAC depending on tissue damage and tumour suppressor inactivation. For these cell types, reprogramming into a ‘ductal’ cell type is required to assume the PanIN–PDAC lineage. Question marks are shown for centroacinar and duct cells as they have not been specifically assessed for their ability to be reprogrammed into a lineage capable of becoming PDAC under the control of KRAS. However, until specific targeting has been achieved, they cannot be ruled out as sources of the precursor–PDAC lineage. Figure is modified, with permission, from REF. 128 © (2000) American Association of Cancer Research.

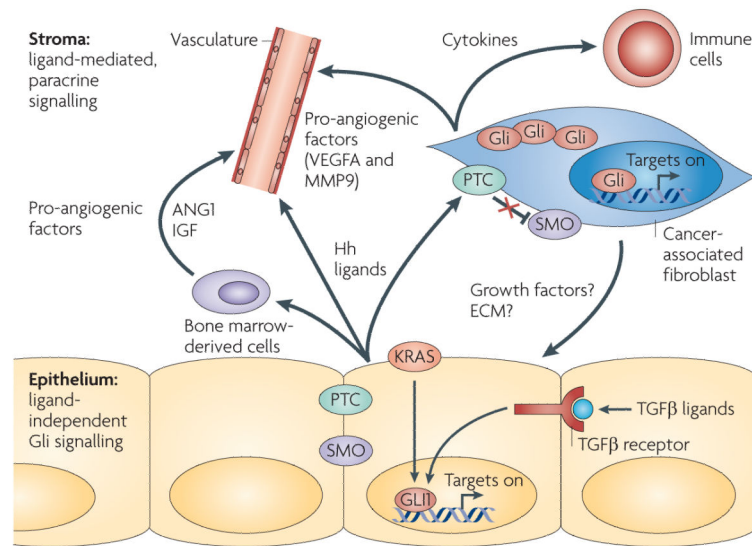


Figure 2. Hedgehog signalling in pancreatic ductal adenocarcinoma

Although the pancreatic ductal adenocarcinoma (PDAC) epithelium overexpresses Hedgehog (Hh), ligand-dependent canonical signalling is activated in stromal cells, including cancer-associated fibroblasts, infiltrating bone marrow-derived cells and subsets of endothelial cells, through the patched (PTC)–smoothened (SMO) axis. In turn, these cells directly proliferate or produce factors that might enhance tumour cell growth (potentially through secreted growth factors or by changing extracellular matrix (ECM) composition) and angiogenesis in a paracrine fashion (the factors that have been identified are indicated). Furthermore, cancer-associated fibroblasts and other Hh-responsive cells might produce cytokines and other molecules that communicate with infiltrating immune cells. Conversely, autocrine activation through this canonical pathway does not seem to occur in the tumour epithelium. Gli activity is maintained in part by activation of GLI1 through alternative signalling pathways, such as mutant KRAS expression and transforming growth factor- β (TGF β) signalling. ANGPT1, angiopoietin 1; IGF, insulin-like growth factor; MMP9, matrix metalloproteinase 9; VEGFA, vascular endothelial growth factor A.

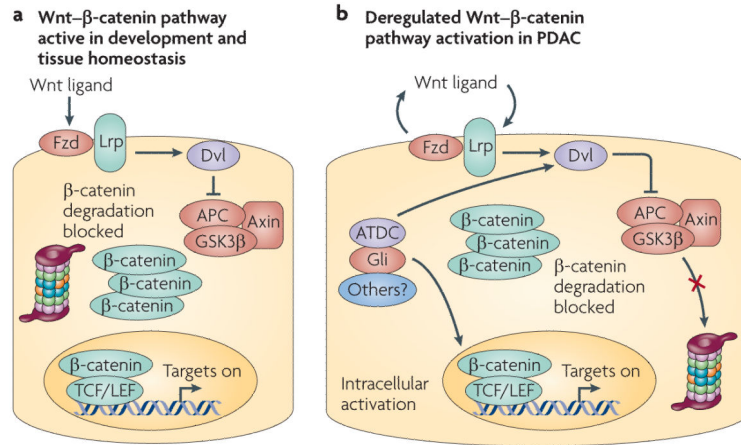


Figure 3. Canonical Wnt-β-catenin signalling in pancreatic ductal adenocarcinoma

a | In normal development, canonical Wnt-β-catenin signalling depends on secreted ligands (Wnt ligands) that activate receptors (Frizzled (Fzd)-Lrp complex) that block the proteosomal degradation of β-catenin promoted by the destruction complex (comprising adenomatous polyposis coli (APC), axin, glycogen synthase kinase 3β (GSK3β) and other proteins) through activation of Dishevelled (Dvl). **b** | β-catenin accumulation is frequently observed in pancreatic ductal adenocarcinoma (PDAC). Accumulated β-catenin can translocate into the nucleus and activate target genes in concert with TCF/LEF co-factors. Presently, the dominant mechanism of persistent β-catenin accumulation and activity in PDAC is unclear. There is evidence for both autocrine (owing to epithelial-derived Wnt ligands) and cell-autonomous activation (through Gli signalling and genes such as ataxia telangiectasia group D-associated (ATDC), which activates Dvl). There may also be contributions from the stromal cells and extracellular matrix that may promote β-catenin accumulation.

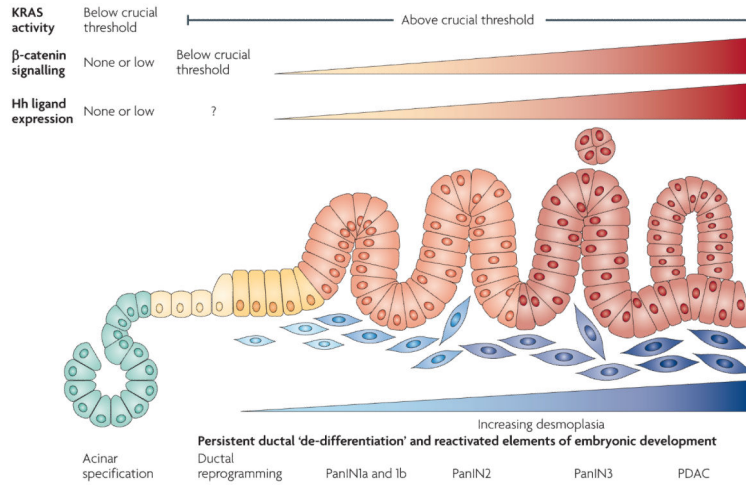


Figure 4. Crucial temporal thresholds of developmental signalling pathways and KRAS activity allow pancreatic epithelial neoplasia — pancreatic ductal adenocarcinoma initiation and progression

KRAS activity above a crucial threshold can drive differentiated pancreatic cells (acinar cells, for example) into a de-differentiated, ductal state that persists in pancreatic epithelial neoplasia (PanIN) and pancreatic ductal adenocarcinoma (PDAC). For these de-differentiated ductal cells to become PanINs, β-catenin signalling must be maintained below a crucial low level. However, once the PanIN state is established, β-catenin signalling is reactivated in parallel with increasing expression of Hedgehog (Hh) ligand that activates target genes in stromal cells of the developing desmoplastic response. Gli activity in the developing tumour epithelium emerges independently of autocrine stimulation. Although Gli activity is probably active in PanINs, its role in the progression from PanIN to PDAC is unknown. Finally, Gli activity is probably important for PDAC maintenance. Figure is modified, with permission, from REF. 128 © (2000) American Association of Cancer Research.

Table 1

Mouse models of *Kras*-driven pancreatic ductal adenocarcinoma

| Genetic model | Phenotype | Refs |
|---|---|------|
| <i>Pdx1-Cre;LSL-Kras^{G12D}</i> <i>p48Cre;LSL-Kras^{G12D}</i> | Universally penetrant PanIN development. Age-dependent increase in lesion severity and occasional PDAC with long latency | 23 |
| <i>Pdx1-Cre;LSL-Kras^{G12D};Cdkn2a^{fllox/fllox}</i> | Accelerated PanIN and PDAC development | 31 |
| <i>Pdx1-Cre;LSL-Kras^{G12D};Trp53^{R172H/+}</i> | Accelerated development of PanIN and metastatic PDAC. PDAC was routinely focal, well differentiated, with extensive genomic instability | 33 |
| <i>Pdx1-Cre;LSL-Kras^{G12D};Smad4^{fllox/fllox}</i> <i>p48-Cre;LSL-Kras^{G12D};Smad4^{fllox/fllox}</i> | Development of IPMN and PDAC | 34 |
| <i>p48-Cre;LSL-Kras^{G12D};Tgfb2^{fllox/fllox}</i> | Accelerated PanIN and PDAC development. Latency further decreased with nullizygosity compared with heterozygosity | 35 |
| <i>Pdx1-Cre;LSL-Kras^{G12D};Trp53^{fllox/fllox}</i> | Accelerated PanIN and well-differentiated PDAC development | 32 |
| <i>Pdx1-Cre;LSL-Kras^{G12D};Ink4a^{fllox/fllox}</i> | Accelerated development of PanIN and poorly differentiated PDAC | 32 |
| <i>p48-Cre;LSL-Kras^{G12D};Smad4^{fllox/fllox}</i> | Development of MCN and PDAC. Latency shortened with nullizygosity | 36 |
| <i>Pdx1-Cre;LSL-Kras^{G12D};Smad4^{fllox/fllox}</i> | Accelerated PanIN, IPMN and PDAC development | 37 |
| <i>Nestin-Cre;LSL-Kras^{G12D}</i> | Development of exocrine-derived PanINs | 129 |
| <i>p48-Cre;LSL-Kras^{G12D};Ela-Tgfa</i> <i>Ela-Cre^{ERT};LSL-Kras^{G12D};Ela-Tgfa</i> | Accelerated PanIN, IPMN and PDAC development | 116 |
| <i>Ela-Tta;tetO-Cre;LSL-Kras^{G12V}</i> | Development of acinar- and centroacinar-derived PanIN and PDAC when <i>Kras^{G12V}</i> activated during development or postnatally. Chronic pancreatitis required for PanIN and PDAC development when <i>Kras^{G12V}</i> activated in adult cells | 24 |
| <i>Ela-Cre^{ERT2};LSL-Kras^{G12D}</i> <i>Mist1-Cre^{ERT2};LSL-Kras^{G12D}</i> | Development of acinar-derived PanIN | 25 |
| <i>Pdx1-Cre^{ERT};LSL-Kras^{G12D};R26-Notch^{NIC}</i> <i>Ela-Cre^{ERT};LSL-Kras^{G12D};R26-Notch^{NIC}</i> | Accelerated development of acinar-derived PanIN | 26 |
| <i>Mist1-Cre^{ERT2};LSL-Kras^{G12D};Mist1^{-/-}</i> | Accelerated development of acinar-derived PanIN | 27 |
| <i>Pdx1-Cre;LSL-Kras^{G12D};Tif1^{fllox/fllox}</i> | Rapid development of IPMN | 38 |
| <i>Ela-Cre^{ERT};CAG-lox-GFP-lox-Kras^{G12V}</i> | Rapid development of chronic pancreatitis, PanIN, cystic papillary carcinoma and PDAC | 28 |

Cdkn2a, cyclin-dependent kinase inhibitor 2A (encodes both INK4A and ARF); *Ela*, elastase; IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; *Mist1*, muscle, intestine and stomach 1; *Notch^{NIC}*, constitutively active form of Notch; PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma; *Pdx*, pancreatic and duodenal homeobox; *Tgfb2*, transforming growth factor- β receptor type II.

Table 2Mouse models of Hedgehog and β -catenin deregulation in the pancreas

| Genetic model | Phenotype | Refs |
|---|--|------|
| Hedgehog | | |
| <i>Pdx1-Shh</i> | Atrophic pancreas displaying lesions reminiscent of PanIN1 and 2 embedded in intestinal-like stroma | 57 |
| <i>Pdx1-Cre;CLEG2</i> | Development of large, undifferentiated pancreatic tumours | 85 |
| <i>Pdx1-Cre;CLEG2;LSL-Kras^{G12D}</i> | Accelerated PanIN development and undifferentiated 'CLEG2-like' tumours | 85 |
| <i>CAGGS-CreER;R26-SmoM2</i> | Development of MCN-like lesions | 130 |
| <i>p48-Cre;LSL-Kras^{G12D}; Trp53^{fllox/+};Smo^{fllox/fllox}</i> | No difference in PanIN and PDAC development compared to <i>p48-Cre; LSL-Kras^{G12D};Trp53^{fllox/+}</i> mice | 81 |
| <i>p48-Cre;LSL-Kras^{G12D}; SmoM2</i> | No acceleration of PanIN development compared to <i>p48-Cre;LSL-Kras^{G12D}</i> mice | 63 |
| β-catenin | | |
| <i>Pdx1-Cre^{Late}; Ctnnb1^{exon3/+}</i> | Acinar proliferation and postnatal pancreatomegaly. No tumour development | 102 |
| <i>Pdx1-Cre;Apc^{fllox/fllox}</i> | Acinar proliferation and postnatal pancreatomegaly. No tumour development | 103 |
| <i>p48-Cre;Ctnnb1^{exon3/+}</i> | Development of tumours resembling human solid pseudopapillary tumour | 104 |
| <i>p48-Cre;Ctnnb1^{exon3/+}; LSL-Kras^{G12D}</i> | Development of tumours resembling human intraductal tubular tumour | 104 |

Apc, adenomatous polyposis coli; *Ctnnb1*, β -catenin; MCN, mucinous cystic neoplasm; PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma; *Shh*, sonic hedgehog; *Smo*, smoothened.