Pancreatic Cancer and Precursor Pancreatic Intraepithelial Neoplasia Lesions Are Devoid of Primary Cilia

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Abstract

Primary cilia have been proposed to participate in the modulation of growth factor signaling pathways. In this study, we determined that ciliogenesis is suppressed in both pancreatic cancer cells and pancreatic intraepithelial neoplasia (PanIN) lesions in human pancreatic ductal adenocarcinoma (PDAC). Primary cilia were absent in these cells even when not actively proliferating. Cilia were also absent from mouse PanIN cells in three different mouse models of PDAC driven by an endogenous oncogenic Kras allele. Inhibition of Kras effector pathways restored ciliogenesis in a mouse pancreatic cancer cell line, raising the possibility that ciliogenesis may be actively repressed by oncogenic Kras. By contrast, normal duct, islet, and centroacinar cells retained primary cilia in both human and mouse pancreata. Thus, arrested ciliogenesis is a cardinal feature of PDAC and its precursor PanIN lesions, does not require ongoing proliferation, and could potentially be targeted pharmacologically.

Introduction

Cilia are projections of ciliary axonemes consisting of nine doublet microtubules that are surrounded by ciliary membranes that either have (motile cilia) or do not have (nonmotile primary and motile nodal cilia) a central pair of singlet microtubules. Primary cilia are a ubiquitous feature of epithelial cells, including those of breast, prostate, kidney, liver, and pancreas. These sensory organelles modulate mitogen and morphogen signaling, sequester receptors for growth factors including platelet-derived growth factor and epidermal growth factor (EGF), contain transcription factors, and effect cytosolic calcium fluxes (1–5). Their assembly requires intrflagellar transport (IFT), is templated by mother centrioles, and is associated with interphase and cell cycle arrest (6–8). Conversely, disassembly of primary cilia precedes cell cycle reentry, initiation of DNA synthesis, and mitosis (7, 9).

Centriole ciliation may prevent centrosome duplication and the formation of the mitotic spindle, which are concepts consistent with the timing of primary cilia resorption during the cell cycle. Mutations in genes required for IFT and in other genes required for primary cilia assembly result in visceral epithelial hyperplasia, polycystic kidneys, acinar-to-ductal metaplasia (ADM), and other abnormalities (10–15). Defects in ciliary assembly may also lead to a loss of dependence on exogenous growth factors and an attenuated response to differentiation agents (11, 16), whereas ciliary dysfunction and/or mutation of genes required for ciliogenesis may be associated with cancer development. Thus, the expression of Nek8, a NIMA family kinase that localizes to primary cilia and regulates flagellar assembly and length in Chlamydomonas, is increased in breast cancer (17–19); loss of the von Hippel-Lindau tumor suppressor gene can preclude ciliogenesis in vitro and is associated with renal cyst formation and renal cancers (20, 21); and Aurora A kinase, which is overexpressed in several human epithelial cancers, mediates ciliolysis (22). Intrflagellar transport is required for the assembly of primary cilia, and heterozygous mutations in IFT88 in mice accelerate the rate at which chemical carcinogens induce liver neoplasms (16). However, hepatocellular carcinomas do not exhibit IFT88 mutations (23).

In spite of the histologic, cell biological, and molecular phenotypes associated with mutations interrupting primary cilia assembly, to date it has not been established whether ciliary assembly is interrupted in cancer and/or whether excessive oncogene activation has the potential to alter ciliary function. To address these issues, we examined the abundance and distribution of primary cilia in pancreatic ductal adenocarcinoma (PDAC), a malignancy with a >90% frequency of Kras mutations (24), which has been generally proposed to arise from cell types that assemble primary cilia, such as ductal and centroacinar cells (25–28). Thus, PDAC provides the opportunity to study the relationship between primary cilia and the development of an epithelial malignancy.

Materials and Methods

Human tissue specimens. H&E-stained sections were collected and previewed to confirm pathologic diagnoses and to identify specimens also containing histologically normal pancreatic exocrine tissue. For inclusion in this study, specimens must have contained adjacent regions of histologically normal pancreatic tissue. All studies with human pancreatic tissues were approved by the Human Subjects Committee at Dartmouth Medical School.

Mouse colonies and specimens. Mouse colonies of Pdx1-Cre; LSL-KrasG12D, Nestin-Cre;LSL-KrasG12D, and Pdx1-Cre;LSL-KrasG12D;Ink4a/Arflox/ mice were generated as previously described (29–31). Pancreata were collected and processed for analysis as previously described (31). All studies with mice were approved by Dartmouth Medical School Institutional Animal Care and Use Committee.

Establishment of Rink-1 murine pancreatic tumor cells. A mouse pancreatic cancer cell line was generated as described (30). After being passaged in monolayer cultures, cells were assessed visually for homogeneity. Cytokeratin-19 (CK19) positivity was confirmed by Western blot and immunofluorescence microscopy. Rink-1 cells rapidly formed tumors following s.c. injection in nude mice. Cells from passages 8 to 12 were used in the present study, LY294002, U0126, SB203580, and GSK-3β inhibitors I, XII, and XV were from Calbiochem; all-trans retinoic acid (ATRA) was from Sigma-Aldrich. All inhibitors were diluted into DMSO.
Histology and confocal microscopy. Antigen Unmasking Solution (Vector Laboratories) was used according to the manufacturer’s instructions. Images were acquired using a Zeiss LSM 510 confocal microscope and 20× or 63× objectives. For detection of primary cilia, gain and power settings were adjusted to the point of signal saturation while capturing primary cilia in normal regions of exocrine tissue. Identical settings were used for adjacent or paired neoplastic samples. Settings were readjusted for each sample or paired sample. Controls were done for double labeling and triple labeling experiments by treating specimens with secondary antibodies only and by treating specimens with each individual antibody followed by each secondary antibody to determine secondary antibody cross-reactivity.

Results

Primary cilia are not assembled by pancreatic cancer cells or their precursor lesions. We first examined primary cilia in well-preserved surgical resection specimens from 17 patients with PDAC in a tissue array format (Supplementary Fig. S1). A region of histologically normal exocrine tissue was included from each patient to control for any primary cilia loss that may have occurred during sample preparation. Acetylated tubulin was validated for use as a marker of primary cilia by costaining for centrosomes and other ciliary proteins, including IFT88 and detyrosinated α-tubulin (Supplementary Fig. S2). Whereas the abundance of cilia in histologically normal interlobular ducts varied, most intralobular and intercalated ducts were well ciliated in all 17 specimens (Fig. 1, bottom left insets). By contrast, nearly 100% of pancreatic cancer cells were devoid of primary cilia in all 17 cases, irrespective of the tumor grade (Fig. 1). Utilization of tubulin antibodies that recognize the detyrosinated form of α-tubulin or that recognize α-tubulin irrespective of its posttranslational modification status (Fig. 1D and data not shown) did not reveal additional cilia, even after increasing gain and power settings by 150% (Fig. 1D, top inset). These findings indicate that an absence of ciliogenesis is a highly conserved feature of PDAC.

Pancreatic intraepithelial neoplasia (PanIN) lesions represent the most abundant premalignant neoplastic precursor of PDAC. The PanIN-1, PanIN-2, and PanIN-3 designations reflect increasing cytologic atypia and the accumulation of mutations that characterize the progression and eventual malignant transformation of PanIN cells (26, 32). PanIN-1, PanIN-2, and PanIN-3 lesions in the human specimens were completely devoid of primary cilia (Fig. 1A and B, and data not shown), raising the possibility that ciliary assembly arrest may occur very early in PDAC development.

Arrested ciliogenesis in Kras-dependent mouse PanINs and pancreatic cancer cells. To more clearly delineate the role of mutated Kras in the loss of primary cilia in PDAC, we next examined a murine model that readily develops PanIN lesions. In this model, oncogenic Kras (KrasG12D) is knocked in into its own

![Figure 1](https://cancerres.aacrjournals.org)
locus and transcriptionally silenced by the insertion of a LoxP-Stop-LoxP element (LSL). When LSL-Kras\textsuperscript{G12D} mice are bred with transgenic mice expressing Cre recombinase under control of the Pdx1 promoter, expression of Cre recombinase is directed to early pancreatic progenitor cells, leading to the removal of the floxed transcriptional STOP cassette, activation of the oncogenic Kras allele subsequently in all pancreatic cell types, leading to the formation of mouse PanIN (mPanIN) lesions, and ultimately to pancreatic adenocarcinoma (29). In pancreata from 2-, 4-, and 6-month-old Pdx1-Cre;LSL-KrasG12D mice, cilia were consistently present in the ductal cells, centroacinar cells, and islet cells (Fig. 2A). By contrast, there was a near total absence of primary cilia in all mPanINs examined, including mPanIN-1A lesions and mPanIN-2/3 lesions (Fig. 2). Microscope settings were adjusted to ensure that ciliary acetylated \( \alpha \)-tubulin epifluorescence was saturating at all times and the inclusion of CK19 epifluorescence did not obscure visualization of the primary cilia (Supplementary Fig. S3). Moreover, ciliary staining was absent regardless of whether the antibodies were directed toward acetylated or detyrosinated tubulin.

When LSL-Kras\textsuperscript{G12D} mice are bred with mice expressing Cre recombinase under control of the Nestin regulatory elements, the Kras mutant allele is activated initially only in the cells where the Nestin promoter is activated and Nestin is expressed. Lineage tracing studies have indicated that these are the exocrine progenitor cells that give rise to acinar cells (31, 33). The pancreata of these Nestin-Cre:LSL-KrasG12D mice develop numerous mPanIN-1A/B lesions (31). As observed in PDAC and in the Pdx1-targeted pancreata, all mPanIN lesions in pancreata from Nestin-Cre:LSL-KrasG12D mice were completely devoid of primary cilia, whereas the normal ductal epithelium was unaffected (Fig. 3D). In as much as mPanIN-1A represents the earliest type of PanIN, these observations confirm that ciliogenesis is absent from the earliest stages of pancreatic neoplasia and indicate that both the Pdx1-Cre:LSL-KrasG12D and Nestin-Cre:LSL-KrasG12D models recapitulate the findings in PDAC in humans with respect to absent ciliogenesis at the level of the earliest precursor lesions.

Thin section studies of PanIN lesions reveal that some are contiguous with normal ductal epithelium and seem to arise from this epithelium (26, 34). We reasoned that this transitional architecture would be ideal for comparing ciliogenesis in these cell types. In pancreata from Pdx-Cre:LSL-KrasG12D mice, we were able to identify clear examples of mPanIN-1A completely contained within intralobular and interlobular ductal epithelia (Fig. 3A–C; Supplementary Fig. S4). Thin sectioning also revealed PanIN-1A lesions appearing as projections within normal ducts.
The two most common alterations in PDAC are oncogenic activation of Kras and loss of p16Ink4a, a cell cycle regulating protein (32), and both features are recapitulated in Pdx1-Cre;LSL-KrasG12D;Ink4a/Arflox/lox mice, in which exons 2 and 3 of Kras and Ink4a/Arf alleles are permanently removed (30). These mice develop mPanIN lesions, primary cilia were absent only in the PanIN cells but were retained by the adjacent normal-appearing ductal epithelium (Fig. 3D). These observations mitigate against mechanical stress as a cause for the loss of primary cilia in mPanIN lesions.

To assess the potential role of oncogenic Kras activation in ciliogenesis, we established a pancreatic cancer cell line from malignant Pdx1-Cre;LSL-KrasG12D;Ink4a/Arflox/lox mouse PDAC cell line. Inhibitors of Kraseffector pathways restore ciliogenesis in a mouse PDAC cell line. To assess the potential role of oncogenic Kras activation in ciliogenesis, Rlnk-1 cells exhibited rapid growth in vitro, formed tumors in nude mice, and were strongly positive for CK19 (data not shown). Following growth for 48 hours in media containing 5% fetal bovine serum (FBS), nearly 100% of these cells were actively proliferating as assessed by Ki67 and proliferating cell nuclear antigen (PCNA) staining (Fig. 4A and B, and data not shown). In contrast, serum starvation for 48 hours arrested nearly 100% of Rlnk-1 cells (Fig. 4A and B). Regardless of the proliferation status, <5% of Rlnk-1 cells were ciliated under these conditions (Fig. 4A and C). When added to Rlnk-1 cultures for 48 hours in serum-free medium, LY294002 (10 μmol/L), an inhibitor of phosphatidylinositol 3-kinase (PI3K), and U0126 (10 μmol/L), a mitogen-activated protein kinase (MAPK)/extracellular signal–regulated kinase kinase (MEK)-1/2 inhibitor, caused the appearance of cilia in 73% and 34% of the cells, respectively (Fig. 4A and C). By contrast, SB203580 (10 μmol/L), an inhibitor of p38 MAPK, and ATRA, a differentiation inducing agent (200 μmol/L), induced the appearance of cilia in only 17% and 9% of the cells, respectively (Fig. 4C). Maximal stimulatory effects on ciliary assembly mediated by LY294002 and U0126 occurred at a concentration of 10 μmol/L, in conjunction with their known ability to inhibit the phosphorylation of their respective downstream targets (data not shown). Moreover, the actions of these inhibitors occurred both in the presence and absence of serum (Supplementary Fig. S5). ATRA, which failed to induce a large increase in ciliogenesis, altered the morphology of Rnk-1 cells, indicating that it was biologically active in these cells (Supplementary Fig. S6). The

Figure 3. Primary cilia are absent in transitional PanIN lesions. A, intralobular mPanIN from Pdx1-Cre;LSL-KrasG12D pancreas reveals normal-appearing ductal epithelium (solid white lines) and neoplastic ductal epithelium (dashed lines). Yellow arrowheads, transition points between normal and neoplastic epithelia. Is, islets. Red, CK19; green, AT; blue, nuclei. Bar, 50 μm. B, magnified region from A, stained as in A, but with anti-Ki67 antibody in white (white arrowhead). Bar, 50 μm. C, interlobular mPanIN from a Pdx1-Kras pancreas. Staining as in A. Bar, 20 μm. Inset, magnified region from C with addition of Ki67 channel in white (white arrowhead). Bar, 50 μm. D, mPanIN from Nestin-Cre;LSL-KrasG12D pancreas stained for CK19 (red), AT (green), and Ki67 (white arrowheads), revealing the terminal cul-de-sac–like architecture (dashed lines) of a mPanIN lesion appearing to arise from a small ductule (solid lines). Inset, magnified view of ciliated normal ductule from within main image. Bar, 20 μm.
actions of GSK-3β, which modulates ciliary elongation and integrity in both Chlamydomonas and mammalian cells, on ciliary assembly in Rink-1 cells were also examined using four different inhibitors of GSK-3β (inhibitors I, XII, and XV and lithium chloride). None of these inhibitors affected ciliogenesis, irrespective of the presence or absence of serum (Supplementary Fig. S5). Taken together, these results indicate that an underlying program of ciliogenesis is actively suppressed in Rink-1 cells; that dominant secondary mutations in genes required for ciliary assembly are unlikely to be responsible for the suppression of ciliary assembly in Kras-dependent pancreatic neoplasia; that ciliogenesis can be restored by specific pharmacologic intervention, especially those that target the PI3K pathway; and that some Kras effector pathways are likely to participate in the interruption of ciliary assembly in the setting of PDAC.

Proliferation and gross centrosomal defects do not account for loss of ciliogenesis. Primary cilia are normally absent in rapidly proliferating cells due to their resorption during cell cycle progression (7, 9). It was important, therefore, to determine whether proliferation-related deciliation was responsible for the absence of cilia in PDAC. Accordingly, the proliferation status of the cancer cells in the human PDAC specimens was assessed by performing Ki67 and PCNA staining. In agreement with previous studies (37, 38), we found that an average of 42.2% of PDAC cells (range, 22.2–55.6%) and no more than one third of PanIN cells exhibited nuclear staining for Ki67 (Fig. 1D). Similar results were obtained by PCNA staining (data not shown). Only rarely were mitotic figures observed in PDAC cells (Fig. 1D, top inset). Inasmuch as over half of the cancer cells in these specimens were not proliferating, as determined by the absence of Ki67/PCNA immunoreactivity, ongoing proliferation does not seem to account for the absence of primary cilia in human PDAC cells.

We next examined the proliferation status of mPanIN lesions and cancer cells in the mouse models used in this study. An average of 2.9% and 1.5% of mPanIN-1A cells were positive for nuclear Ki67 staining in Pdx1-Cre;LSL-KrasG12D and Nestin-Cre;LSL-KrasG12D, respectively (Figs. 3B–D, 5C, and 6). Microscope settings were adjusted to ensure that the specific detection of nuclear Ki67 epifluorescence was saturating in all cases (Supplementary Fig. S3). Many mPanIN-1A cells did not exhibit any nuclear Ki67/PCNA labeling, and only a small number of transitional mPanIN-1A cells were positive for either marker (Fig. 3; Supplementary Figs. S3.
and S4). By contrast, advanced mPanIN-2/3 and cancer cells in Pdx1-Cre;LSL-KrasG12D;Ink4a/Arflox/lox pancreata exhibited average Ki67 nuclear labeling indices of 19.9% and 46.7%, respectively (Fig. 6). Similar values were obtained with anti-PCNA antibodies (Fig. 6) and with anti-bromodeoxyuridine (BrdUrd) antibodies following a 24-hour pulse with BrdUrd (data not shown). These results indicate that ongoing proliferation is not responsible for the lack of ciliary assembly in the earliest mPanIN-1A lesions and cannot completely account for the absence of primary cilia in advanced pancreatic neoplasms and invasive PDAC.

Because the assembly of primary cilia is templated from mother centrioles and ciliogenesis is sensitive to centrosomal defects (39, 40), we next examined centrosomal copy number and polarity in mPanIN cells in 1-month-old Pdx1-Cre;LSL-KrasG12D and Nestin-Cre;LSL-KrasG12D pancreata using pericentrin or γ-tubulin antibodies. Some mPanIN lesions exhibited normal centrosomal copy number and polarity whereas others exhibited extensive centrosomal amplification and polarity defects, regardless of whether γ-tubulin or pericentrin antibodies were used (Fig. 2, bottom). The ability to clearly observe centrosomes without associated acetylated-tubulin positive projections further confirms the absence of primary cilia in PanIN lesions and shows that the arrest of ciliogenesis seen in mPanIN can occur in the presence of native centrosome copy number and polarity.

ADM cells assemble primary cilia. PDAC is often associated with foci of ADM, a process whereby acinar cells can, in some cases, transdifferentiate into ductal cells, lose expression of digestive enzymes, and become CK19 positive (41). Histologically, there is loss of acinar cytoplasmic eosinophilia and the appearance of tubular complexes (Fig. 5A, top). ADM is also observed in chronic pancreatitis and in pancreata from either Pdx-Cre;LSL-KrasG12D or Nestin-Cre;LSL-KrasG12D mice (31, 41), and it has been proposed that PanIN lesions may also arise from foci of ADM. It was of interest, therefore, to determine whether cells within ADM lesions were ciliated. Accordingly, ADM lesions were initially studied in surgical resection specimens from four patients with chronic pancreatitis. Foci of CK19-positive cells in these ADM lesions were most often highly ciliated with cilia that were abnormally elongated and that were most frequently associated with cells that were devoid of amylase, but occasionally also present in association with amylase containing cells within these lesions (Fig. 5A). Overall, 52.2% of human ADM cells exhibited a primary cilium, with nearly all foci being ciliated to some extent (Figs. 5A and 6). Moreover, an average of 4.9% of these cells were positive for Ki67 (Fig. 6).

Next, ADM lesions were examined in 4-month-old pancreata from each of the above mouse models. The murine ADM cells were frequently ciliated and were more often Ki67 or PCNA positive than mPanIN-1A cells (Figs. 5B and C and 6). In contrast to the cilia in
the human ADM lesions, the cilia in the mouse ADM lesions were not elongated. Within ADM foci, the loss of amylase content and the gain of CK19 content were accompanied by a loss of Ki67 staining (Fig. 5C). Perhaps due to the fact that primary cilia are resorbed during proliferation, ADM lesions with many Ki67-positive cells had few cilia whereas ADM lesions with few Ki67-positive cells had many cilia (Figs. 5C and 6). However, over half of the Kras-dependent murine ADM cells were neither ciliated nor proliferating (Fig. 6), indicating that the near complete absence of cilia in PDAC is a phenomenon that is restricted to the PanIN cells and pancreatic cancer cells.

Discussion

In the present study, we determined that ciliogenesis is arrested in the cancer cells in human PDAC and in the precursor PanIN-1, PanIN-2, and PanIN-3 lesions that occur in this malignancy, indicating that ciliary assembly arrest occurs very early in PDAC development. This conclusion is supported by the observation that cilia were also absent in mPanIN lesions in pancreata from Pdx1-Cre;LSL-KrasG12D and Nestin-Cre;LSL-KrasG12D mice, including all mPanIN-1A lesions, which represent the earliest type of PanIN. Cilia were also absent in the mPanIN lesions and cancer cells in pancreata from Pdx1-Cre;LSL-KrasG12D;Ink4a/Arflox/lox mice,
indicating that an arrest or absence of ciliogenesis is a highly conserved feature of PDAC at all stages of this malignancy.

In contrast to the absence of cilia in the PanINs, the ADM lesions in the human chronic pancreatitis samples exhibited abundant and unusually long cilia, whereas the cilia in the ADM lesions in the mouse models used in this study, irrespective of the genotype, were less abundant and were of normal length. Whereas ciliary elongation has also been reported in experimental chronic pancreatitis (42), the mechanisms that allow for ciliary elongation to occur are not fully characterized, but seem to be dependent on several factors including IFT itself (8). Taken together, these observations indicate that PanINs and ADMs are distinct lesions.

To compare the proliferation status of cancer cells, PanINs and ADMs, we used the proliferation markers Ki67 and PCNA. Ki67 is present in all phases of the cell cycle but is absent in arrested (G0) cells, and PCNA recruits DNA polymerase to the replication fork and is therefore a useful marker of S-phase cells. In agreement with previous studies (37, 38), we found that an average of 42.2% of human and 46.7% of Pdx1-Cre;LSL-KrasG12D;Ink4a/Arflox/lox cancer cells exhibited nuclear staining for these proteins, indicating that ongoing proliferation does not account for the absence of primary cilia in the majority of the cancer cells in PDAC. Moreover, less than one third of PanIN cells in human samples, less than 3% of mPanIN-1A cells, and less than 20% of mPanIN-2/3 cancer cells were proliferating as assessed by nuclear Ki67 staining, indicating that increased cell cycle activity could not account for the nearly total absence of ciliation in these lesions. By contrast, up to 20% of the cells in the murine ADM lesions were Ki67 positive, an observation that supports the concept that PanINs and ADMs are distinct lesions.

The cell type that gives rise to PanINs and PDAC in humans has not been definitively identified. Histologic analyses suggest that PanIN can arise from within native ductal epithelia, hence the terminology pancreatic intraepithelial neoplasia (26, 34). By contrast, evidence from murine models of PDAC development suggests that, in addition to ductal cells, centroacinar cells and/or acinar cells give rise to mPanINs (27, 31, 43). Because centroacinar and ductal cells are normally ciliated, our data suggest that PanIN formation via either cell type would involve suppression of ciliogenesis. Whereas acinar cells do not assemble primary cilia, it has been proposed that acinar-derived PanIN arise from ADM lesions (41) and that acinar cells can differentiate into ductal cells and into mucinous metaplastic lesions that are equivalent to mPanINs but that do not arise from native pancreatic ducts (44). Our current data show that ADM cells form cilia, even in Nestin-Cre;LSL-KrasG12D mice, in which oncogenic Kras expression is restricted to the acinar compartment (31), indicating that if acinar cell–derived ADMs were to progress into PanINs, such a transformation would be associated with suppression of ciliogenesis. Thus, irrespective of its derivation, PanIN formation is associated with the absence of ciliogenesis.

Oncogenic Kras activates multiple signaling pathways and exerts pleiotropic effects on proliferation and morphology depending on dosage and context (45). The most common Kras mutation in PDAC in humans is on codon 12 (KrasG12D), which is associated with loss of both intrinsic and extrinsic GTPase activities. In all three mouse models used in the present study, KrasG12D expression remained under the control of its own endogenous promoter, thereby allowing for oncogenic Kras to be expressed at levels observed in human PDAC. Because cell lines derived from PanIN lesions are not readily available, we used Rlnk-1 mouse pancreatic cancer cells that we established from Pdx1-Cre;LSL-KrasG12D;Ink4a/Arflox/lox mice to determine whether Kras-induced arrest of ciliogenesis was reversible. The proliferation-independent defect in ciliary assembly remained stable in vitro, as evidenced by the failure of the cells to ciliate even when grown arrested by 48-h serum starvation. However, LY294002, a PI3K inhibitor, and U0126, a MEK1/2 inhibitor, caused the reappearance of cilia in these cells, with LY294002 being twice as effective as U0126, whereas serum starvation induced cell cycle arrest but failed to induce ciliation. These findings indicate that the absence of primary cilia is independent of ongoing proliferation, that arrest of ciliogenesis is reversible, and that excessive activation of Kras pathways participates in the interruption of ciliary assembly in PDAC. Our findings also suggest that additional dominant mutations in genes participating directly in the assembly of primary cilia are most likely not required for arrest of ciliary assembly in PanIN and PDAC cells.

Inasmuch as oncogenic Kras is expressed throughout the pancreas in Pdx1-Cre;LSL-L-KrasG12D mice and Pdx1 expression is maintained in adult islet cells, it is appropriate to ask how Kras blocks ciliary assembly only in the PanINs and cancer cells. It would seem, however, that the mature islet cells which express Pdx1 are relatively resistant to Kras oncogenic stimuli, especially in models where the KrasG12D allele remains subject to physiologic regulation via its endogenous promoter and therefore do not undergo malignant transformation in the Pdx1-Cre;LSL-L-KrasG12D mouse model. Moreover, because oncogenic Kras in this model should continue to be expressed in all downstream progeny, even after Pdx1 silencing, it is intriguing that normal duct cells continue to exhibit cilia, raising the possibility that the ductal cell may not readily give rise to PDAC and may be resistant to Kras-induced suppression of ciliogenesis. In this regard, the observation that the Nestin-Cre;LSL-KrasG12D model recapitulates the findings with the Pdx1-Cre;LSL-L-KrasG12D, with respect to both PanIN formation and loss of cilia, suggests that the acinar cell lineage that arises from Nestin-positive progenitor cells may indeed be the cell type that is exquisitely sensitive to oncogenic Kras and arrested ciliogenesis. This interpretation is consistent with a recent report that PANC-1 and CFPAC-1 human pancreatic cancer cell lines are relatively resistant to growth arrest–induced ciliogenesis (46).

PDAC is the fourth leading cause of cancer deaths in the United States, and ~85% of patients present with advanced disease that precludes them from undergoing resection (47, 48). Nonsurgical therapies have had limited success because of the intrinsic resistance of pancreatic cancer cells to cytotoxic agents (48). The use of gemcitabine alone or in combination with erlotinib has slightly prolonged survival (49), whereas targeting of Kras with farnesyl transferase inhibitors has been completely ineffective (50). Although primary cilia are essential for hedgehog (Hh) signaling in normal mammalian cells (51), pancreatic cancer cells are highly abnormal. Thus, in spite of absence of cilia, it is now appreciated that PDAC is associated with excessive Hh signaling, which contributes to PDAC growth and metastasis (52). Moreover, it has been recently shown that oncogenic Kras activates Hh signaling in pancreatic cancer cells (53). Taken together, these observations raise the possibility that oncogenic Kras may induce loss of tumor suppressor functions that are embedded in primary cilia and may lead to perturbations in Hh signaling pathways that result in their aberrant activation in the absence of cilia. Moreover, the EGF receptor, MAPK, Wnt, Hh, and MAPK pathways are all dysregulated in ciliary assembly mutants (14, 15), just as they are in
PDAC (54–56). Thus, perturbations in ciliary function occurring in conjunction with oncogenic Kras may contribute to the early activation of multiple aberrant signaling cascades in PDAC, raising the possibility that it may be necessary to restore ciliogenesis by pharmacologic means to improve the effectiveness of other therapeutic options.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

References
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