Cell Intrinsic Role of COX-2 in Pancreatic Cancer Development

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Abstract

COX-2 is upregulated in pancreatic ductal adenocarcinomas (PDAC). However, how COX-2 promotes PDAC development is unclear. While previous studies have evaluated the efficacy of COX-2 inhibition via the use of nonsteroidal anti-inflammatory drugs (NSAID) or the COX-2 inhibitor celecoxib in PDAC models, none have addressed the cell intrinsic versus microenvironment roles of COX-2 in modulating PDAC initiation and progression. We tested the cell intrinsic role of COX-2 in PDAC progression using both loss-of-function and gain-of-function approaches. Cox-2 deletion in Pdx1+ pancreatic progenitor cells significantly delays the development of PDAC in mice with K-ras activation and Pten haploinsufficiency. Conversely, COX-2 overexpression promotes early onset and progression of PDAC in the K-ras mouse model. Loss of PTEN function is a critical factor in determining lethal PDAC onset and overall survival. Mechanistically, COX-2 overexpression increases p-AKT levels in the precursor lesions of Pdx1+/ K-rasG12D/+ ; Pten homozygous mice in the absence of Pten LOH. In contrast, Cox-2 deletion in the same setting diminishes p-AKT levels and delays cancer progression. These data suggest an important cell intrinsic role for COX-2 in tumor initiation and progression through activation of the PI3K/AKT pathway. PDAC that is independent of intrinsic COX-2 expression eventually develops with decreased FBKPS and increased GRP78 expression, two alternate pathways leading to AKT activation. Together, these results support a cell intrinsic role for COX-2 in PDAC development and suggest that while anti-COX-2 therapy may delay the development and progression of PDAC, mechanisms known to increase chemoresistance through AKT activation must also be overcome. Mol Cancer Ther; 11(10); 2127–37. ©2012 AACR.

Introduction

Pancreatic ductal adenocarcinoma (PDAC), the fourth leading cause of cancer-related deaths in the United States, has a 5-year survival rate of 4% (1). The poor outcome of PDAC has been attributed to late detection, the aggressive nature of the disease, and poor response to local and systemic therapies. A better understanding of the underlying mechanisms that lead to PDAC initiation, progression, and chemoresistance would help the development of more effective treatment regimens.

COX, also known as prostaglandin (PG) endoperoxide synthases, are enzymes essential in the conversion of arachidonic acid to prostaglandins. COXs exist as 2 isoforms, COX-1 and COX-2 (2). COX-1 is constitutively expressed in most mammalian tissues and is responsible for mediating various normal physiologic processes. COX-2, on the other hand, is low or undetectable in most normal tissues, but is induced in response to a wide range of stimuli in many cell types including epithelial cells, endothelial cells, and macrophages (3).

COX-2 levels are often elevated in lung, breast, esophageal, bladder, prostate, and pancreatic cancers (4). Substantial data suggest that COX-2 expression is not simply a by-product, but is a causal factor of tumor development. COX-2 overexpression in transgenic mice led to breast cancer formation, a phenotype greatly reduced by the use of the COX-2 inhibitor celecoxib (ref. 5; Fig. 1). Likewise, tumor growth in pancreatic cancers initiated by either COX-2 overexpression (6, 7) or mutant K-ras activation (8), and prostate cancers initiated by the SV40 large T antigen (9), was significantly reduced by celecoxib treatment.

Importantly, celecoxib significantly reduced premalignant colon polyp formation in patients at risk for colon cancer (10). However, the addition of celecoxib to gemcitabine therapy, the current standard-of-care for treatment of PDAC did not show significant improvement in the survival of patients with metastatic disease (11), suggesting that the efficacy of celecoxib may be cancer- or cancer stage-dependent.
either overexpressed or deleted. To investigate this hypothesis, we conditionally developed through activation of the PI3K/AKT pathway. We previously showed that K-ras activation and Pten haploinsufficiency cooperate to activate p-AKT in precancerous lesions and accelerate tumor development in our mouse model of PDAC (12), suggesting that PTEN loss-of-function is a critical factor for determining the onset of lethal tumor development. Although PTEN mutation is not commonly found in human PDAC, loss of at least one copy of PTEN or gain/amplification of AKT2 has been reported in 32.8% of the PDAC xenografts derived from primary patient samples (13). Moreover, elevated p-AKT was observed in 68.5% of PDAC tissue microarray samples (13), suggesting that mechanisms other than PTEN deletion or AKT2 gain/amplification contribute to the elevated p-AKT levels seen in PDAC. In addition, our recent integrative, survival-based, molecular profiling of human PDAC provided multiple lines of correlative data showing that dysregulation of the PI3K/AKT pathway is linked to clinical disease progression (14). Taken together, these data provide strong rationale for further studies designed to identify mechanisms through which the PI3K/AKT pathway is regulated in PDAC development in the absence of Pten LOH or mutation. We hypothesized that COX-2 expression, which suppresses PTEN activity in cell culture (15), could play a significant role in tumor development through activation of the PI3K/AKT pathway. To investigate this hypothesis, we conditionally either overexpressed or deleted Cox-2 in the K-ras/Pten mouse model to explore the role of COX-2 in regulating PI3K/AKT pathway activation during tumorigenesis.

While it is clear that COX-2 plays an enhancing and/or causal role in the progression of many types of cancer, the role(s) of COX-2 expression in the initiated epithelial cell versus cells within the tumor microenvironment are unclear. We recently showed that Cox-2 deletion in myeloid and endothelial cells, but not in epithelial cells, exacerbates murine colitis (16). These studies point toward the importance of elucidating the cell intrinsic versus cell extrinsic role of COX-2 in tumorigenesis. To address this issue in pancreatic tumorigenesis, we conditionally deleted or overexpressed Cox-2 in the Pdx1+ pancreatic progenitor cells.

Materials and Methods

Mouse strains

The generation of conditional Cox-2 overexpression (Cox-2 COE) and conditional knockout (Cox-2 KO) mice has been reported previously (17, 18). To conditionally overexpress COX-2 in the pancreas, we crossed the Pdx1-Cre line to the floxed Cox-2 COE transgenic line (17). Primers designed to detect the Cox-2 transgene cassette were used for genotyping (17). The Pdx1-Cre+Cox-2COE line was further crossed to Pdx1-Cre;K-rasG12D;PtenDownloaded from mct.aacrjournals.org on March 31, 2017. © 2012 American Association for Cancer Research.
clustering were done using dChip software. For each gene, its expression in each genotype group was represented by the geometric average of the biologic replicated samples (n = 4). The log ratio between a pair of 2 genotypes was then calculated. The error bar represents ±SEM. Animal survival was determined by the Kaplan–Meier survival method. P < 0.001, log-rank test, for each pairwise combination.

Results

Targeted Cox-2 deletion delays tumor onset in the K-ras/Pten PDAC model

Increased COX-2 expression is observed in chronic pancreatitis, pancreatic intraepithelial neoplasias (PanIN), and PDACs (20). To address the effect of cell intrinsic loss of COX-2 activity on tumor formation, we genetically eliminated Cox-2 by crossing the Pdx1-Creþ;K-rasG12D/+;Ptenlox/lox PDAC model (12) to mice carrying a floxed, conditional Cox-2 deletion allele (Cox-2lox/lox; ref. 18). Pdx1þ pancreatic progenitor cells can give rise to endocrine, exocrine, and ductal tissue, but not to cells in the microenvironment, such as macrophages, or cells associated with blood vessels (21). Because conditional K-ras activation, Pten deletion, and Cox-2 deletions are all dependent on Cre activity driven by the Pdx1 promoter in these mice, Cox-2 will be deleted in the same cells in which mutant K-rasG12D activation and Pten deletion take place.

We first examined how Cox-2 deletion affected the survival of Pdx1-Creþ;K-rasG12D/+;Ptenlox/lox mice, which succumb to tumor burden by 3 weeks of age (ref. 12; Fig. 2A; left, green line). Removal of endogenous COX-2 expression in Pdx1-Creþ;K-rasG12D/+;Ptenlox/lox;Cox-2lox/lox mice extended the median survival time to 35 days (Fig. 2A; left, brown line).

![Figure 2](https://example.com/figure2.png)

Figure 2. Targeted Cox-2 deletion causes delayed tumor onset and increased life expectancy in the Pdx1-Cre;K-ras; Pten mouse model. A, Kaplan–Meier analysis comparison of Pdx1-Cre−; K-rasG12D−;Ptenlox/lox and Pdx1-Cre−; K-rasG12D−;Ptenlox/lox Cox-2lox/lox mice. B, histology of Pdx1-Cre−; K-rasG12D−;Ptenlox/lox and Pdx1-Cre−; K-rasG12D−; Ptenlox/lox;Cox-2lox/lox mice. C, histology and COX-2 immunohistochemistry or double immunofluorescence [cytokeratin-19 (CK-19), COX-2], as indicated, on tumors of Pdx1-Cre−; K-rasG12D−; Ptenlox/lox (enlarged version of B) and Pdx1-Cre−; K-rasG12D−; Ptenlox/lox;Cox-2lox/lox mice. Scale bars, 50 μm. DAPI, 4,6-diamidino-2-phenylindole.
At 2 weeks of age, Pdx1-Cre⁺;K-rasG12D/+;Ptenlox/lox mice developed PDAC (Fig. 2B, top). In contrast, removal of intrinsic COX-2 activity in Pdx1-Cre⁺;K-rasG12D/+;Ptenlox/lox, Cox-2lox/lox mice resulted in a predominantly normal pancreas parenchyma (Fig. 2B, bottom). Although lesions in the Pdx1-Cre⁺;K-rasG12D/+;Ptenlox/lox,Cox-2lox/lox mice appeared much later once the tumors developed the phenotypes resembled those of Pdx1-Cre⁺;K-rasG12D/+;Ptenlox/lox mice (Fig. 2C, left).

To confirm that Cox-2 is indeed deleted in tumors from Pdx1-Cre⁺;K-rasG12D/+;Ptenlox/lox,Cox-2lox/lox mice, we evaluated COX-2 expression by immunohistochemistry and double immunofluorescence analyses. Compared with the robust COX-2 staining observed in cytokeratin-19 positive (CK19⁺) neoplastic cells of Pdx1-Cre⁺;K-rasG12D/+;Ptenlox/lox mice (Fig. 2C, red arrow, top), no COX-2 expression can be detected in tumors from Pdx1-Cre⁺;K-rasG12D/+;Ptenlox/lox,Cox-2lox/lox mice (Fig. 2C, red arrow, bottom) except CK19⁺ cells in the microenvironment (Fig. 2C, green arrow, bottom). These observations suggest that, although genetic deletion of Cox-2 in the Pdx1+ pancreatic progenitor cells is not sufficient to fully abrogate tumorigenesis caused by alterations of the RAS/RAF/MAPK and PTEN/P13K pathways, there is an important cell intrinsic role for COX-2 in pancreatic tumorigenesis; without intrinsic COX-2 expression, PDAC development is significantly delayed.

COX-2 overexpression alone is insufficient to promote PDAC development

Having established that loss of cell intrinsic COX-2 expression delays PDAC development, we next sought to determine whether cell intrinsic COX-2 overexpression alone had an effect on PDAC development. To examine this question, we crossed mice carrying a conditional Cox-2 overexpression allele (Cox-2COE; ref. 17) with the Pdx1-Cre⁺ line (22) to generate Pdx1-Cre⁺;Cox-2COE mice.

Examination of H&E-stained sections of pancreata from Pdx1-Cre⁺;Cox-2COE mice revealed structures that have the appearance of ADM, a preneoplastic ductal structure that emerges from acinar cells (Fig. 3A, red arrow) and the appearance of ADM, a preneoplastic ductal structure that emerges from acinar cells (Fig. 3A, red arrow) and may overlap with PI3K/AKT activation in promoting PDAC development.

COX-2 overexpression promotes earlier onset of PDAC development and reduces survival of Pdx1-Cre⁺;K-rasG12D/+;Ptenlox/lox mice

Consistent with our finding that COX-2 overexpression alone is not sufficient to promote PDAC development, Guerra and colleagues (23) showed that injury-induced pancreatitis, in which Cox-2 overexpression plays an essential role, cannot cause PDAC unless a K-ras activation mutation is present. Given this precedent, we tested whether intrinsic COX-2 overexpression would accelerate tumorigenesis in a mouse model capable of developing PDACs.

Mice expressing K-rasG12D from its endogenous locus generate PDACs only after a prolonged latency (22), unless coupled with other genetic alterations commonly found in human PDAC, including loss of Inktm1/Arf (24), TGF-β receptor type 2 (Tgfrb2; ref. 25), Smad4 (26), mutation in p53 (27), or haploinsufficiency for Pten (12). COX-2 overexpression leads to significant reduction of median survival time in the Pdx1-Cre⁺;K-rasG12D/+;Ptenlox/lox mice from 525 to 410 days (Fig. 4A, compare black and green lines).

When one allele of Pten was removed from the Pdx1-Cre⁺;K-rasG12D/+;Ptenlox/lox mice, we observed a substantial reduction in the overall survival in the Pdx1-Cre⁺;K-rasG12D/+;Ptenlox/lox mice (Fig. 4A, blue line). Of note, the Pdx1-Cre⁺;K-rasG12D/+;Ptenlox/lox,Cox-2COE mice have a similar median survival times (Fig. 4A, blue and red line, respectively). This genetic evidence suggests that although COX-2 overexpression can collaborate with RAS/RAF/MAPK pathway activation, the biologic effects of COX-2 overexpression may overlap with PI3K/AKT activation in promoting PDAC development.

To further investigate the effects of COX-2 overexpression and RAS/RAF/MAPK and PI3K pathway activations, we examined the pancreata of each model between the age of 1 and 3 months (n = 12 for each cohort), and scored the presence of ADMs, mPanINs, or PDACs. As summarized in Fig. 4B, Pdx1-Cre⁺;K-rasG12D/+;Ptenlox/lox and Pdx1-Cre⁺;K-rasG12D/+;Ptenlox/lox,Cox-2COE mice developed a similar spectrum of lesions. Although Cox-2 overexpression had no significant impact on the incidence of early ADM and mPanIN1A lesions, it promoted the progression of these early lesions as evidenced by the larger and higher grade lesions found in Pdx1-Cre⁺;K-rasG12D/+;Ptenlox/lox,Cox-2COE mice when compared with age- and genetically background-matched Pdx1-Cre⁺;K-rasG12D/+;Ptenlox/lox mice (Figs. 4B and C). Only 3 of 12 Pdx1-Cre⁺;K-rasG12D/+;Ptenlox/lox,Cox-2COE mice developed mPanIN2/3s during this time frame. Pdx1-Cre⁺;K-rasG12D/+;Ptenlox/lox,Cox-2COE mice also displayed an increased incidence of invasive PDACs as quantified in Fig. 4B and shown in the lower panel of Fig. 4C. The fact that the early progression observed histopathologically does not seem to have a major impact on the overall survival of the 2 cohorts suggests that the kinetics of disease progression from the early lesions to lethal disease may not be linear because of the timing and nature of secondary mutations acquired by localized lesions in each cohort. Because metastatic incidences are rare in both cohorts (<5%), it is difficult to quantitatively compare the rates and onset of these lesions.
AKT activation is associated with COX-2-mediated tumor progression

Because Pdx1-Cre\textsuperscript{+};K-ras\textsuperscript{G12D/+};\textit{Pten}\textsuperscript{lox/+} mice and Pdx1-Cre\textsuperscript{+};K-ras\textsuperscript{G12D/+};\textit{Pten}\textsuperscript{lox/+};\textit{Cox-2}\textsuperscript{COE} mice have a similar median survival time, we reasoned that COX-2 overexpression may functionally mimic \textit{Pten} LOH, an event critical for PDAC progression in the Pdx1-Cre\textsuperscript{+};K-ras\textsuperscript{G12D/+};\textit{Pten}\textsuperscript{lox/+} model (12). To test this hypothesis, we assessed both the \textit{Pten} genomic and functional status in ADM and PDAC lesions in the Pdx1-Cre\textsuperscript{+};K-ras\textsuperscript{G12D/+};\textit{Pten}\textsuperscript{lox/+};\textit{Cox-2}\textsuperscript{COE} and Pdx1-Cre\textsuperscript{+};K-ras\textsuperscript{G12D/+};\textit{Pten}\textsuperscript{lox/+};\textit{Cox-2}\textsuperscript{lox/lox} mice. PTEN functional status was determined by p-AKT immunohistochemical analysis, and \textit{Pten} genomic status was investigated by genomic PCR, using laser-captured lesion tissues. AKT is significantly activated in the ADM lesions of Pdx1-Cre\textsuperscript{+};K-ras\textsuperscript{G12D/+};\textit{Pten}\textsuperscript{lox/+};\textit{Cox-2}\textsuperscript{COE} mice, but not in similar lesions of either Pdx1-Cre\textsuperscript{+};K-ras\textsuperscript{G12D/+};\textit{Pten}\textsuperscript{lox/+} (Hill and colleagues 2010; and data not shown) or Pdx1-Cre\textsuperscript{+};K-ras\textsuperscript{G12D/+};\textit{Pten}\textsuperscript{lox/+};\textit{Cox-2}\textsuperscript{lox/lox} mice (Fig. 5A; comparing left and right). In contrast, PDAC lesions from COX-2 overexpressing and Cox-2 deleted mice have comparably enhanced p-AKT staining, further supporting our hypothesis that AKT activation is a limiting step in ADM to PDAC progression (12).

We then extracted genomic DNA from laser-captured ADM and PDAC lesions from cohorts of Pdx1-Cre\textsuperscript{+};K-ras\textsuperscript{G12D/+};\textit{Pten}\textsuperscript{lox/+};\textit{Cox-2}\textsuperscript{COE} and Pdx1-Cre\textsuperscript{+};K-ras\textsuperscript{G12D/+};\textit{Pten}\textsuperscript{lox/+};\textit{Cox-2}\textsuperscript{lox/lox} mice (\(n = 4\)) and conducted PCR analysis. The WT \textit{Pten} allele (Fig. 5B; in red) is maintained at near-equal molar ratio to the \textit{Pten}\textsuperscript{-deleted} allele (Fig. 5B; in blue) in both precancer (ADM) and cancer (PDAC) lesions, indicating that majority of cells in these lesions did not acquire \textit{Pten} LOH. This result suggests that COX-2 overexpression, together with \textit{Pten} haploinsufficiency,
promotes AKT activation—a necessary step for the progression of precursor lesions to PDAC (12). The presence of elevated COX-2 activity, therefore, removes the selective pressure for Pten LOH that is observed in the Pdx1-Cre+/K-rasG12D+/Ptenlox/þ model (15).

Upregulated GRP78 expression correlates with p-AKT activation in the precursor lesions

Upregulation of GRP78, a member of the Hsp70 protein family, is associated with poor prognosis of a number of human cancers (28, 29). While GRP78 is known to be expressed in the cytoplasm, the recent discovery of GRP78 on the cell surface and its role in activating the AKT (28, 29) prompted us to investigate whether GRP78 expression is involved in initiation and progression in human PDAC. While no GRP78 expression was observed in the ducts of wild-type murine samples (Supplementary Fig. S1A, green arrow in left), GRP78 expression is significantly upregulated in the ductal structures of human ADM and PDAC lesions (Fig. 6A, top, high power magnification; Supplementary Fig. S1A, right, low power magnification). Importantly, GRP78 is strongly expressed in both the cytoplasm and the membranes of these human lesions (Fig. 6A, top).
We then analyzed GRP78 expression in our murine models. Similar to the human disease, both ADM and PDAC lesions in the Pdx1-Cre+; K-rasG12D/++; Pten+/++; Cox-2COE mice have strongly upregulated GRP78 expression, especially its membrane-associated form (Fig. 6A, middle), corresponding to the AKT activation (Fig. 5A, I and II panel from left). The specificity of this staining was confirmed by showing that excess GRP78 peptide can block labeling with this antibody (Supplemental Fig. S1B). In contrast, only low cytosolic GRP78 expression (Fig. 6A, bottom left) and nearly undetectable AKT activation (Fig. 5A, 3rd panel from left) can be observed in the ADM lesions from mice with the targeted Pten allele (WT allele), the floxed Pten allele (lox allele), and the deleted Pten allele (del band).

These data suggest that COX-2 overexpression may lead to upregulated expression of membrane-associated GRP78 and to subsequent AKT activation. However, even in the absence of epithelial COX-2 expression, PDAC lesions in the ADM show substantial upregulated GRP78 expression (Fig. 6A, mid-range of Fig. 6A; Supplementary Fig. S1B), which colocalizes with enhanced p-AKT in the membrane (Fig. 6B), indicating that GRP78 and AKT can be upregulated and activated in a COX-2–independent manner as the disease progresses.

**Decreased FK506-binding protein 5 (FKBP5) negative feedback leads to enhanced AKT activation in PDAC of Pdx1-Cre+; K-rasG12D/++; Pten+/++; Cox-2lox/lox mice**

Although Cox-2 conditional deletion led to increased median survival of Pdx1-Cre+; K-rasG12D/++; Pten+/++; Cox-2lox/lox mice (65 days; data not shown), all Cox-2–deleted animals ultimately succumbed to aggressive PDAC without Pten LOH (Fig. 5A), suggesting that resistance to COX-2–directed therapy is likely to occur in patients. Decreased expression of scaffold protein FKBP5 has been identified as one of the mechanisms that enhances AKT activity and causes PDAC chemoresistance (30). FKBP5, by acting as a scaffolding protein for AKT and PH domain and leucine-rich repeat protein phosphatase 1 (PHLPP), promotes PHLPP dephosphorylation of AKT at amino acid S473 (31), thereby suppressing AKT activity (30, 32). We examined the expression level of FKBP5 mRNA and found that FKBP5 is downregulated significantly in PDACs of Pdx1-Cre+; K-rasG12D/++; Pten+/++; Cox-2lox/lox mice (Fig. 7A; P = 0.01; 2-tailed t test, determined as the Log2 ratio of the FKBP5 expression of knockout [Pdx1-Cre+; K-rasG12D/++; Pten+/++; Cox-2lox/lox] over Pdx1-Cre+; K-rasG12D/++; Pten+/+, and overexpressed [Pdx1-Cre+; K-rasG12D/++; Pten+/++; Cox-2COE over Pdx1-Cre+; K-rasG12D/++; Pten+/+, respectively].

Immunohistochemical analysis revealed strong FKBP5 staining in PDACs of Pdx1-Cre+; K-rasG12D/++; Pten+/++; Cox-2COE and Pdx1-Cre+; K-rasG12D/++; Pten+/++; Cox-2lox/lox mice, whereas FKBP5 expression was reduced to almost undetectable levels in Pdx1-Cre+; K-rasG12D/++; Pten+/++; Cox-2lox/lox mice (Fig. 7B). Furthermore, enhanced membrane-associated p-AKT correlates with reduced FKBP5 staining in PDACs of Pdx1-Cre+; K-rasG12D/++; Pten+/++; Cox-2lox/lox mice (Fig. 7C, region indicated by green arrow, Supplementary Fig. S2). These results suggest that, in the absence of Pten LOH, downregulation of FKBP5 could...
activate AKT, a gate-keeping event in PDAC progression, leading to accelerated tumor development. Mechanistically, AKT activation may occur as a result of increased membrane-associated GRP78 expression, induced as a consequence of enhanced COX-2 signaling (Fig. 7D, left). Alternatively, AKT can be activated by decreased FKBP5-PHLPp-AKT negative feedback circuitry associated with Cox-2 abrogation (Fig. 7D, right) or, perhaps, in response to NSAID or celecoxib treatment. Our findings suggest that, while anti-COX-2 therapies may delay PDAC onset and progression, tumors undergoing NSAID or celecoxib treatment may acquire changes that lead to AKT activation and therapeutic resistance. These alternate mechanisms that lead to AKT-mediated resistance must also be overcome to achieve therapeutic efficacy (33–35).

Cell intrinsic role for COX-2 in pancreatic tumor development

Previous studies have evaluated the efficacy of COX-2 inhibition, via the use of NSAIDs or celecoxib treatment, in PDAC models (6–8). However, there are no previous studies that have been able to discriminate between the effect of COX-2 inhibition in both the tumor cells and the microenvironment, and COX-2 inhibition in only the tumor cells. The murine models we have generated provided an opportunity to elucidate the cell intrinsic function of COX-2 in PDAC development.

Unlike previous studies, which showed that cytotkeratin-5-driven expression of COX-2 led to the development of PDAC, (6), we observed that COX-2 expression in Pdx1+ progenitor cells alone is not sufficient for the development of PDAC in Pdx1-Cre+/+;Cox-2COE mice. This result raises the question of what is preventing tumor development in Pdx1-Cre+/+;Cox-2COE mice or in mice with injury-induced pancreatitis, where COX-2 plays a critical role in subsequent tumor development (23). Recent studies indicate K-ras mutation is essential for the progression of premalignant lesions to PDAC in mice with injury-induced pancreatitis (23, 36). Consistent with these observations, our genetic study suggests that the cross-talk between COX-2 and RAS/MAPK signaling pathway is essential in promoting PDAC development. Furthermore, the median survival of Pdx1-Cre+/+;K-rasG12D+/+;Cox-2COE mice was significantly longer than that of Pdx1-Cre+/+;K-rasG12D+/+;Ptenlox/+;Cox-2COE mice, establishing the importance of PTEN pathway function in determining both PDAC progression and overall survival. The similar survival trends of Pdx1-Cre+/+;K-rasG12D+/+;Ptenlox/+;Cox-2COE and Pdx1-Cre+/+;K-rasG12D+/+;Ptenlox/+;Cox-2lox/+;Cox-2COE mice also support the hypothesis that the effects of COX-2 expression overlap with the PI3K/AKT pathway and suggest that COX-2 is a key modulator of PI3K/AKT activation in pancreatic tumorigenesis, without the need for Pten loss of heterozygosity.

The PTEN pathway and PDAC progression

Tumors in the K-ras/Pten mice recapitulate the pathologic features of human PDACs. Moreover, these mice
shows that this PI3K/AKT expression are, should aid the development (14). The identification of the driver(s) for PI3K/AKT patient subgroups with more aggressive disease progression significantly associated with distinct pancreatic cancer has shown that dysregulated PI3K/AKT signaling is mechanisms is of critical importance, as our recent work elevated p-AKT levels seen in PDAC. Elucidating these mechanisms other than PTEN deletion contribute to the PDAC tissue microarray samples (13), suggesting that PDAC, elevated p-AKT was frequently observed in the AKT-activating mechanisms, both related to COX-2 regulation. AKT activation is associated with decreased COX-2 expression, which is reduced in the absence of COX-2 expression. model the loss of PTEN function, which is now recognized as a frequent, major contributor in human PDAC progression. While the loss of both alleles of PTEN is infrequent in PDAC, elevated p-AKT was frequently observed in the PDAC tissue microarray samples (13), suggesting that mechanisms other than PTEN deletion contribute to the elevated p-AKT levels seen in PDAC. Elucidating these mechanisms is of critical importance, as our recent work has shown that dysregulated PI3K/AKT signaling is significantly associated with distinct pancreatic cancer patient subgroups with more aggressive disease progression (14). The identification of the driver(s) for PI3K/AKT pathway expression, and what the downstream effects of this PI3K/AKT expression are, should aid the development of more effective therapies. Our previous work shows that Pten heterozygosity leads to earlier precursor lesion development, which is associated with increased cell proliferation (12). Moreover, our findings suggest 2 AKT-activating mechanisms, both related to COX-2 expression, may occur during the development of PDAC. p-AKT activation in Pdx1-Cre;K-rasG12D;Pten−/−;Cox-2COE mice is associated with an increase in membrane-bound GRP78, which was diminished upon Cox-2 deletion, in precursor lesions. Increased GRP78 expression has recently been identified as a critical factor in protecting cells from cell death in response to cellular insults, including inflammation and pancreatitis (37). It seems likely that increased COX-2 expression regulates GRP78 activity. The GRP78 promoter contains a cAMP responsive element (38). COX-2 signaling via epithelial cell EP receptors may activate GRP78 via the cAMP/PKA pathway (39).

The high level of GRP78 we observed in tumors in our in vivo models raises the question of whether GRP78 is essential for pancreatic tumor progression. Targeted knockout of Grp78 in the prostate epithelium and hematopoietic stem cells inhibited Pten null prostate tumorigenesis, leukemia development, and AKT activation (40, 41), suggesting GRP78 as a potential therapeutic target for cancers that result from PTEN loss or inactivation. PDACs invariably developed in all Pdx1-Cre;K-rasG12D;Pten−/−;Cox-2−/− mice, suggesting that tumors can compensate for COX-2 loss through other mechanisms. AKT activation is associated with decreased expression of the FKBP5 scaffold protein in Pdx1-Cre;K-rasG12D;Pten−/−;Cox-2−/− mice (Fig. 7C). Decreased FKBP5 expression has been identified as one of the mechanisms that enhances AKT activity and results in PDAC chemoresistance (30, 42). These data suggest that FKBP5 may function as a tumor suppressor in PDAC development. On the basis of our data showing a correlation between loss of COX-2 expression and decreased
FKBP5 expression, we suggest that celecoxib treatment could result in loss of FKBP5-mediated suppression of AKT activation.

Therapeutic implications
Our results and those of others (8) show that therapeutic regimens designed to target COX-2 expression may be most useful for delaying both precursor lesions and PDAC initiation in patients at high risk for pancreatic cancer. Recent studies have shown that dissemination of pancreatic epithelial tumor cells can occur much earlier than previously thought and that these cells can seed distant metastasis (43). Given the fact that PDAC can grow at an exponential rate with micrometastases developing from small primary tumors before surgical resection (44), it is possible that therapeutics, which delay precursor lesion formation, like COX-2 inhibitors, could be administered in this previously unappreciated therapeutic window to significantly delay/prevent seeding of metastases in patients at high-risk for PDAC development.

Moreover, our results emphasize the importance of AKT activity in the effectiveness of anti-COX-2 therapies. Recent studies have shown that COX-2 inhibition in lung and colon cancer cells was ineffective at eliciting cell death unless COX-2 inhibitors were used in combination with PPARY ligand ciglitazone (45) and atorvastatin (46). Induction of cell death, in both studies, was associated with decreased p-AKT activation. We identified increased GRP78 and decreased FKBP5 as 2 mechanisms through which pancreatic tumors could activate AKT. The lack of increased therapeutic benefit from combination celecoxib + gemcitabine treatment of metastatic PDACs (11) could be the result of increased therapeutic resistance resulting from AKT activation. We suggest, therefore, that pathways that cause AKT activation will likely have to be targeted to enhance therapeutic efficacy. Cotargeting the COX-2, RAS/RAF/MAPK, and PI3K/AKT pathways may be necessary to slow progression of late-stage PDACs.

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

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Conception and design: R. Hill, A.J. Garcia, T.R. Donahue, H.R. Herschman, H. Wu
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