HHS Public Access

Author manuscript

Oncogene. Author manuscript; available in PMC 2013 October 11.

Published in final edited form as:

Oncogene. 2013 April 11; 32(15): 1950–1958. doi:10.1038/onc.2012.210.

Maintenance of Acinar Cell Organization is Critical to Preventing Kras-Induced Acinar-Ductal Metaplasia

Guanglu Shi[*], **Daniel DiRenzo**[*], **Chunjing Qu**[*], **Darci Barney**, **David Miley**, and **Stephen F. Konieczny**[1]

Department of Biological Sciences and the Purdue Center for Cancer Research, Purdue University, West Lafayette, IN 47907

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers owing to a number of characteristics including difficulty in establishing early diagnosis and the absence of effective therapeutic regimens. A large number of genetic alterations have been ascribed to PDAC with mutations in the *KRAS2* proto-oncogene thought to be an early event in the progression of disease. Recent lineage-tracing studies have shown that acinar cells expressing mutant $Kras^{G12D}$ are induced to transdifferentiate, generating duct-like cells through a process known as acinar-ductal metaplasia (ADM). ADM lesions then convert to precancerous pancreatic intraepithelial neoplasia (PanIN) that progresses to PDAC over time. Thus, understanding the earliest events involved in ADM/PanIN formation would provide much needed information on the molecular pathways that are instrumental in initiating this disease. Since studying the transition of acinar cells to metaplastic ductal cells *in vivo* is complicated by analysis of the entire organ, an *in vitro* 3D culture system was employed to model ADM outside the animal. $Kras^{G12D}$-expressing acinar cells rapidly underwent ADM in 3D culture, forming ductal cysts that silenced acinar genes and activated duct genes, characteristics associated with *in vivo* ADM/PanIN lesions. Analysis of downstream KRAS signaling events established a critical importance for the Raf/MEK/ERK pathway in ADM induction. Additionally, forced expression of the acinar-restricted transcription factor *Mist1*, which is critical to acinar cell organization, significantly attenuated $Kras^{G12D}$-induced ADM/PanIN formation. These results suggest that maintaining MIST1 activity in $Kras^{G12D}$-expressing acinar cells can partially mitigate the transformation activity of oncogenic KRAS. Future therapeutics that target both the MAPK pathway and *Mist1* transcriptional networks may show promising efficacy in combating this deadly disease.

[1]**Corresponding Author:** Stephen F. Konieczny Department of Biological Sciences and the Purdue Center for Cancer Research Purdue University Hansen Life Sciences Research Building 201 South University Street West Lafayette, IN 47907-2064 Tel: 765-494-7976 Fax: 765-496-2536 sfk@purdue.edu.

[*] these authors contributed equally to this work.

Author Contributions: GS, DD, CQ, DB and DM designed and performed experiments; SFK designed and analyzed experiments and GS and SFK wrote the manuscript.

Conflict of Interest: The authors declare no conflict of interest.

Keywords

Mist1; pancreatic cancer; lineage-tracing; signaling pathways; 3D tissue culture

INTRODUCTION

With a 5-year survival rate of <5% for patients with pancreatic ductal adenocarcinoma (PDAC), the disease remains one of the most lethal malignancies primarily due to late diagnosis and ineffective chemo and radiation therapies (1). Recent identification of three distinct PDAC precursors - pancreatic intraepithelial neoplasia (PanIN), mucinous cystic neoplasm and intraductal papillary mucinous neoplasm - provides new hope for early detection and a much better understanding of the underlying mechanisms that are instrumental in the progression of the disease (2). Among these precursor lesions, PanINs are the best characterized (3). Indeed, a number of genetic mutations associated with PDAC have been detected in PanINs at various stages including mutations in the *KRAS2, TP53* and *SMAD4* genes which also are hallmarks of advanced PDAC, consistent with a PanIN → PDAC step-wise progression model (2). These observations are further supported by genetically engineered mouse models where PanIN/PDAC progression is initiated by single point mutations in the *Kras* gene and modified by additional genetic alterations in other oncogenes or tumor suppressors (4-10). However, the molecular mechanisms that govern each step of PanIN/PDAC progression remain elusive.

More controversial is the cellular origin of PDAC. PDAC has long been considered a disease of pancreatic ducts. Early efforts to model the disease by expressing *Kras* in pancreatic duct cells did not yield discenable pathology (11), although a recent study by Ray *et al.* (12) has shown that activation of endogenous $Kras^{G12D}$ in large pancreatic ducts leads to rare early PanIN lesions. Despite this example, increasing evidence supports the idea that PanIN/PDAC can originate from differentiated acinar cells, which represent the major cellular component of the pancreas parenchyma (10, 13-15). The development of duct-like PanIN lesions from acinar cells necessitates massive remodeling of these cells, both morphologically and with respect to gene expression profiles. The transition from acinar to ductal cell properties has been termed acinar-ductal metaplasia (ADM) and lineage-tracing studies have confirmed that this process results from direct transdifferentiation of adult acinar cells that convert to a duct cell phenotype upon $Kras^{G12D}$ expression (10, 13, 16). The relevance of ADM to PDAC is also supported by the observation that ADM is frequently associated with human PanIN lesions from PDAC patients (17, 18). Additionally, ADM development has been shown to precede PanIN formation in mouse $Kras^{G12D}$ models (17), suggesting that ADM represents the initial stage of PDAC development.

Although it is clear that $Kras^{G12D}$ expression in adult acinar cells can generate ADM lesions that progress to PanINs and PDAC, the molecular signaling pathways that are instrumental in these conversion events remain poorly defined. Indeed, $Kras^{G12D}$ expression alone does not guarantee ADM/PanIN development because in mouse PDAC models where ~95% of acinar cells express $Kras^{G12D}$ only a small cohort of cells generate detectable ADM/PanINs after a significant latent period (months), demonstrating that differentiated acinar cells are

not universally responsive to KRAS signaling. This observation is compounded by the complexity of following ADM/PanIN development within the context of the intact organ. In an effort to develop an *in vitro* system to study the very earliest events of ADM formation, we focused on the initial transition of acinar cells converting to ductal cells upon $Kras^{G12D}$ expression in a 3D culture model. We found that $Kras^{G12D}$-expressing acinar cells in 3D culture rapidly converted to ductal cells that mimicked properties associated with *in vivo* ADM and PanIN lesions, including silencing of acinar genes and expression of duct genes. Analysis of RAS signaling components demonstrated that activation of the Raf/MEK/ERK pathway was essential for $Kras^{G12D}$-induced ADM. Furthermore, the ability of acinar cells to undergo a transdifferentiation process to ductal cells was significantly attenuated by forced expression of the acinar-restricted transcription factor *Mist1*, suggesting that maintaining acinar cell identity mitigates the strong oncogenic potential of $Kras^{G12D}$ expression. These results provide possible new therapeutic approaches to treat early ADM/PanIN cases by simultaneously targeting the MAPK pathway and *Mist1* transcriptional networks.

RESULTS

$Kras^{G12D}$ expression converts pancreatic acini to ductal cysts in vitro

Kras mutations are virtually universal in human PDAC, although identification of the initial adult cell (duct, acinar, centroacinar) that acquires the mutation and initiates the transformation cascade remains unknown. Recent studies examining pancreatic cancer in mouse models have shown that *Kras* oncogene expression in adult acinar cells leads to PDAC that fully mimics the human disease (10, 13, 15, 16). Lineage-tracing of *Elastase-CreER; LSL-*$Kras^{G12D/+}$*; R26R*^{mTmG} mice confirmed that activation of $Kras^{G12D}$ in adult acinar cells induces acinar-ductal metaplasia (ADM), which can progress to pancreatic intraepithelial neoplasia (PanIN) and on to PDAC (Figure 1A).

Despite this fundamental process of initiating a transformation pathway, it has been difficult to study the intricacies of ADM → PanIN → PDAC in the intact animal. In an effort to dissect the initial $Kras^{G12D}$-dependent pathways *in vitro*, we asked if ADM could be modeled in tissue culture. Previous studies showed that wildtype acinar cells treated with TGFα and maintained in a 3D collagen matrix converted to duct-like cysts that activated EGFR downstream signaling pathways, including Ras (4, 19). To determine if constitutive KRAS activity could similarly lead to ductal cyst formation, *Mist1*$^{CreER/+}$*; LSL-*$Kras^{G12D/+}$*; R26R*^{LacZ} or *Elastase-CreER; LSL-*$Kras^{G12D/+}$ mice were treated with corn oil (control) or tamoxifen (TM) to activate acinar-specific $Kras^{G12D}$ expression. Pancreata were then harvested 7 days post-treatment, digested with collagenase and individual acini clusters placed in a 3D collagen matrix. As expected, control and $Kras^{G12D}$-expressing acinar cells initially retained their normal apical-basal polarity with the rER accumulating at the basal aspect and zymogen granules clustered at the apical pole (Figure 1B). However, by 5 days in 3D culture there was a dramatic difference in cell viability between the $Kras^{G12D}$-expressing and non-expressing cell populations. Control acinar cells rapidly became disorganized and underwent massive cell death. In contrast, a high proportion (~70-90%) of $Kras^{G12D}$-expressing acinar cells survived and converted to ductal cyst structures that were comprised

of a single layer of epithelial cells surrounding an empty luminal space, resembling the metaplastic ADM/PanIN lesions observed *in vivo* (Figure 1A,B). Interestingly, these metaplastic cysts were virtually identical to structures formed by primary pancreatic duct cells (20). Lineage tracing using the $R26R^{LacZ}$ reporter allele confirmed that the ductal cysts were derived via transdifferentiation of mature $Kras^{G12D}$-expressing acinar cells (Figure 1B). In agreement with a recent study by Heid *et al.* (21), ductal cyst conversion was dependent on $Kras^{G12D}$ activity as the vast majority of control acinar cells (-TM) failed to convert to ductal cysts over the duration of the experiment (Figure 1C).

The profound morphological changes in the 3D cultures were reminiscent of the *in vivo* changes that take place in the whole organ upon $Kras^{G12D}$ activation. Indeed, a dramatic switch from acinar to ductal gene expression profiles occurred during the 5 day culture period. At day 0, individual acini expressed high levels of amylase, elastase, carboxypeptidase and lipase, with virtually no detectable expression of duct gene products including keratin 19 (K19), cystic fibrosis transmembrane conductance regulator (CFTR) and carbonic anhydrase II (CAII) (Figure 2; 3), confirming the purity of the starting acinar cell preparation. As expected, these cells also expressed high levels of the acinar-specific transcription factors *Ptf1a* and *Mist1* (Figure 2; 3), with *Mist1* being essential to maintaining an adult acinar cell phenotype (22). However, by day 5 $Kras^{G12D}$-induced ductal cysts exhibited a dramatic shift in their gene expression profiles that mirrored the expression pattern of PanIN lesions. Ductal cysts and PanINs uniformly silenced acinar genes and activated expression of duct-specific genes, including CFTR, K19, CAII and the duct-restricted transcription factors Sox9 and HNF6 (Figure 2; 3; S1; S2). These cells also expressed Nestin (Figure 3; S2), a marker of ductal cells in early PanINs (23). As predicted, PanINs in $Mist1^{CreER/+}$; $LSL\text{-}Kras^{G12D/+}$ pancreata were highly proliferative and a similar proliferation index was observed with the $Kras^{G12D}$-induced ductal cysts (Figure 2; S3). The parallel between ductal cyst formation *in vitro* and ADM/PanIN genesis *in vivo* suggests that the 3D acinar culture faithfully mimics the intracellular pathways that regulate acinar-ductal metaplasia.

MAPK activity is elevated in PanINs and is essential for ductal cyst formation

It is well established that $Kras^{G12D}$ expression induces cellular transformation by activating downstream signaling events, including the Raf/MEK/ERK, PI3K/AKT and RalGDS/Ral pathways (Figure 4A). Although these pathways have been the subject of potential therapeutic benefit against pancreatic tumors (24, 25), little is known about the role they have in the earliest transformation events of ADM and PanIN formation. In an effort to dissect the importance of the PI3K/AKT and Raf/MEK/ERK pathways prior to tumor formation, we examined $Mist1^{CreER/+}$; $LSL\text{-}Kras^{G12D/+}$ (HET/Kras) pancreata 3 months post-TM to ascertain if ADM/PanIN regions exhibited elevated KRAS signaling. Interestingly, despite the fact that ≥90% of adult acinar cells express $Kras^{G12D}$, only regions of ADM/PanIN exhibited sufficiently high pAKT and pERK levels that could be detected by immunohistochemistry (Figure 4B). Thus, expression of $Kras^{G12D}$ in acinar cells (for up to 3 months) is not sufficient to uniformly activate the PI3K/AKT and Raf/MEK/ERK arms of the KRAS pathway in all cells.

To examine the importance of the pAKT and pERK pathways in converting acinar cells to ADM, we investigated these KRAS downstream pathways in the 3D culture model. As an initial test, we examined the importance of the upstream EGFR pathway with respect to *Kras*^{G12D}-induced ductal cyst formation. As previously reported (4, 19, 21), wildtype acinar cells placed in 3D culture exhibited only background levels of ductal cysts. However, when cells were provided TGFα, rapid ductal cyst formation occurred (Figure 4C). As expected, inhibition of EGFR by the EGFR tyrosine kinase inhibitor PD153035 completely blocked ductal cyst formation of wildtype acinar cells but had no effect on HET/Kras ductal cyst formation, confirming that KRAS functions downstream of EGFR (Figure 4A,C).

We next examined the importance of the PI3K/AKT and Raf/MEK/ERK pathways for ductal cyst formation in Het/Kras mice. As predicted, elevated pAKT was observed in *Kras*^{G12D}-isolated acinar cells when compared to control cells isolated from corn oil treated littermates (Figure 4D). Treatment of the cultures with the PI3K inhibitor LY294002 led to a ~70% reduction in pAKT levels but had no significant effect on ductal cyst formation in the 3D culture model. Likewise, 90% reduction of pAKT levels by the PI3K inhibitor VIII produced only modest decreases in ADM conversion (Figure S4). Together, these results suggest that the PI3K/AKT pathway is not required for, nor essential to, acinar-ductal metaplasia.

A similar analysis was performed on the Raf/MEK/ERK pathway. Whereas control cells exhibited low levels of pERK, Het/Kras acinar cells had elevated pERK levels (Figure 4E). Treatment of cells with the MEK inhibitor PD98059 efficiently blocked accumulation of pERK at concentrations as low as 20 μM. Interestingly, inhibition of MEK activity perfectly correlated with decreased ductal cyst formation (Figure 4E). We propose that activation of the Raf/MEK/ERK pathway in a subset of *Kras*^{G12D}-expressing acinar cells is required to initiate an ADM response in mouse models of PDAC.

Sustained Mist1 expression suppresses ductal cyst formation in vitro and ADM/PanIN formation in vivo

Kras^{G12D}-expressing acinar cells rapidly lose their acinar characteristics as they acquire a duct-like phenotype (13, 17). An early event of this switch is silencing the *Mist1* locus, which encodes a basic helix-loop-helix transcription factor critical to acinar cell differentiation (4, 17). *Mist1* is highly expressed in pancreatic acinar cells and its expression correlates with cellular differentiation and the establishment of the secretory machinery (22, 26). Previous studies have shown that human acinar cells lose *Mist1* expression upon ADM/PanIN formation (4, 17), and in mouse models devoid of the *Mist1* gene, *Kras*^{G12D}-expressing acinar cells rapidly undergo ADM and develop extensive PanIN lesions through a pathway that involves elevated pERK activity (4, 27). Therefore, we hypothesized that MIST1 promotes acinar cell differentiation and suppresses the oncogenic activity of *Kras* by keeping pERK levels low. Given that ADM is dependent on MAPK activity (Figure 4E), we examined if sustained *Mist1* expression could restrict the conversion of *Kras*^{G12D}-expressing acinar cells to ductal cysts.

To test this concept, a transgenic mouse line (*LSL-Mist1*^{myc}) that produces constitutive *Mist1* expression upon Cre-mediated recombination was generated (Figure 5A). Transgene

expression of myc-tagged *Mist1* was driven by a CMV early enhancer/chicken β-actin hybrid promoter, whose activity is independent of the differentiation status of cells (28). When *Mist1*$^{CreER/CreER}$*; LSL-Kras*$^{G12D/+}$*; LSL-Mist1*^{myc} (KO/Kras/Mist1^{myc}) mice were treated with TM, *Mist1*^{myc} expression was observed in >90% acinar cells with no expression detected in duct cells or in corn oil treated (-TM) control mice (Figure 5B). Next, we examined MAPK activity in pancreata +/- *Kras*^{G12D} and *Mist1* expression. As shown in Figure 5C, loss of *Mist1* (KO) led to elevated pERK levels, even in the absence of *Kras*^{G12D} expression. However, sustained *Mist1*^{myc} expression in the KO/Kras/Mist1^{myc} pancreata generated lower pERK levels when compared to KO/Kras littermates (Figure 5C). We then examined how constitutive *Mist1*^{myc} expression influenced ductal cyst conversion in 3D cultures. As expected, *Mist1*$^{+/-}$ (HET) and *Mist1*$^{-/-}$ (KO) acini generated few ductal cysts when placed in collagen matrix for 5 days (Figure 5D). In contrast, TM-treated *Mist1*$^{CreER/+}$*; LSL-Kras*$^{G12D/+}$ (HET/Kras) acini rapidly converted to ductal cysts and the percentage of conversion increased when *Mist1*$^{CreER/CreER}$*; LSL-Kras*^{G12D} (KO/Kras) acinar cells were tested (Figure 5D; S5). Importantly, when KO/Kras/Mist1^{myc} acini were examined, ductal cyst formation was reduced to levels approaching those observed in *Kras*^{G12D}-negative (*Mist1*$^{+/-}$, *Mist1*$^{-/-}$) cells (Figure 5D; S5). Similar results were obtained with the TGFα model where *Mist1*^{myc} expression blocked TGFα-induced ductal cyst formation of *Mist1*$^{-/-}$ acinar cells (Figure S6). These results demonstrate that sustained *Mist1* expression lowers pERK levels and protects acinar cells from TGFα- or *Kras*^{G12D}-induced ADM formation *in vitro*.

Finally, to determine if sustained *Mist1* expression could similarly deter ADM/PanIN development *in vivo*, adult KO/Kras/Mist1^{myc} mice and their littermate controls were treated with TM and pancreata were examined three months post-TM for the presence of ADM and PanIN lesions. HET/Kras mice developed rare focal areas of ADM and PanIN lesions at 3 months and in all cases the *Mist1* locus was transcriptionally silenced in acinar-derived PanINs and in advanced ADM lesions (Figure 6A,B). As expected, KO/Kras pancreata developed organ-wide extensive ADM/PanINs owing to the absence of *Mist1* prior to *Kras*^{G12D} activation (Figure 6A,B). In contrast, sustained *Mist1*^{myc} expression greatly attenuated ADM/PanIN development in the KO/Kras/Mist1^{myc} mice as the majority of the organ consisted of normal appearing acinar tissue with only isolated areas of ADM/PanINs (Figure 6A,B). Indeed, the number of PanINs that developed in KO/Kras/Mist1^{myc} mice and reported in Figure 6B actually represent an upper limit because not all PanINs (~18%) were MIST1^{myc} positive, presumably due to selected Cre-mediated recombination of the *LSL-Kras*^{G12D} locus and not the *LSL-Mist1*^{myc} transgene. Thus, a subset of *Kras*^{G12D}-expressing acinar cells did not have an opportunity to respond to sustained MIST1 protein. However, despite the clear protective effect of *Mist1*^{myc} expression, MIST1^{myc} protein *per se* did not fully inhibit *Kras*^{G12D}-induced PanINs as MIST1^{myc}+ PanINs were still generated, albeit at a greatly reduced level (Figure 6A,B). The MIST1^{myc}-dependent reduction in ADM/PanIN formation was also reflected in the expression profiles of key acinar *vs.* ductal genes where MIST1^{myc} significantly reduced ductal gene activity while maintaining acinar gene expression to levels approaching, or exceeding, those obtained with HET/Kras mice (Figure 6C). These results are consistent with MIST1 exerting an anti-ADM/PanIN activity in the intact pancreas and support the concept that constitutive *Mist1* expression mitigates the

activity of oncogenic *Kras*^{G12D} in acinar cells, leading to a reduction in ADM/PanIN formation.

DISCUSSION

The role of acinar cells in pancreatic tumorigenesis has long been suspected, but definitive evidence had been lacking until lineage tracing studies established that PanINs and PDAC can arise from adult acinar cells (10, 13, 16). These studies also implicated the process of acinar-ductal metaplasia as a bridge between normal acinar cells and duct-like PanINs. Consistent with this hypothesis, ADM occurrence precedes PanIN development in PDAC mouse models (17). Despite the central role for *Kras*^{G12D} in inducing ADM, *Kras* mutations are not a prerequisite. Mutant *Kras* is often detected in ADM and low-grade PanIN lesions in human diseased organs but these mutations are not universally present (2, 29), suggesting that additional underlying events can influence ADM formation. Indeed, exposure to EGFR growth factors leads to metaplastic lesions *in vitro* and *in vivo* in the absence of active *Kras* (30, 31). Similarly, EGFR activity is a hallmark of *Kras*-induced ADM/PanIN lesions (17), implying a central role for this pathway in *Kras*-dependent and *Kras*-independent ADM. ADM is also commonly associated with acute and chronic pancreatitis in both humans and rodents (18, 32, 33), suggesting that inflammation and/or cell damage can simultaneously generate an ADM response without *Kras* activity. Although a number of studies have shown that metaplastic cells in pancreatitis settings eventually resume an acinar cell phenotype (33, 34), the presence of *Kras* mutations efficiently redirects metaplastic cells toward PanIN development (15, 35). These results, together with the observation that chronic pancreatitis increases the risk for PDA (36), underscore the relevance of ADM in pancreatic tumorigenesis and the need to elucidate the molecular mechanisms that regulate the fate of metaplastic cells.

In this study, we adopted a collagen matrix 3D culture assay to model the conversion of mature acinar cells to ductal metaplastic cells within a compressed timeframe (5 days *vs.* months). As expected, expression of the *Kras*^{G12D} oncogene promoted formation of duct-like cysts, with an efficacy similar to treatment of wildtype acinar cells with exogenous TGFα (4, 19, 21). Further analysis confirmed that the starting cell preparation was highly enriched for amylase-expressing acinar cells, which converted to K19-expressing ductal cells in the presence of *Kras*^{G12D}. Using inhibitors that specifically blocked individual signaling components, we also demonstrated that Raf/MEK/ERK represent a critical downstream effector pathway through which KRAS operates to induce ADM. Interestingly, *Braf*^{V599E} and *Kras*^{G12D}, the predominant *raf* and *ras* gene mutations, respectively, in pancreatic cancer operate in a mutually exclusive pattern in pancreatic tumors, suggesting that upstream (*Kras*^{G12D}) or downstream (*Braf*^{V599E}) effectors of the Raf/MEK/ERK pathway exhibit interchangeable functions in converting acinar cells to ADM lesions (37). Recent lineage-tracing studies of primary human acinar cells also revealed that surviving acinar cells undergo an ADM response that is dependent on MAPK activity (38). Likewise, we have found that TGFα-induced ADM is also dependent on the Raf/MEK/ERK pathway (unpublished results), revealing that these downstream effectors represent a common regulator of ADM in mouse and human Kras-dependent and Kras-independent settings.

Future studies will test if individual dominant-negative mutants of the MAPK pathway similarly block ADM in models of tumorigenesis and pancreatitis.

While these studies were in progress, Heid *et al.* (21) reported that acinar cells isolated from *Ptf1a*$^{Cre/+}$*; LSL-Kras*^{G12D} mice were also capable of producing ductal cysts in 3D culture. Additionally, conditional deletion of *Rac1* significantly blocked ductal cyst formation *in vitro* and PanIN development *in vivo*. This was a surprising finding as Rac1 is often thought to be activated by the PI3K pathway which we showed by selective inhibition to be nonessential for ADM formation. However, a number of studies have shown that Rac can also be activated in a PI3K-independent fashion via the Raf-specific guanine exchange factor Tiam1 (39, 40). Importantly, *Tiam1* null mice are resistant to Ras-induced tumor formation (41), confirming the importance of the Tiam1-Rac1 axis for the Ras signaling pathway. Thus, our study and the work by Heid *et al.* (21) strongly suggest that there are two key pathways (MAPK and Rac1) that are instrumental in *Kras*-induced ADM and that both pathways are essential to converting normal acinar cells into ADM and PanIN lesions. Future PDAC therapeutic strategies that target both pathways are likely to have higher efficacy than approaches targeting only a single effector.

Are there other ways to block ADM? We can find some leads in studies of pancreatitis, where most ADM spontaneously heals and re-differentiates in the absence of *Kras*^{G12D} signaling. For example, β-catenin activation following acute pancreatitis is required for acinar cell regeneration, while stabilized β-catenin protects acinar cells against *Kras*--induced PanIN formation (16). Similarly, *Mist1* exhibits a protective role not only in *Kras*^{G12D}-induced ADM but also in ADM associated with acute pancreatitis, where *Mist1* null pancreata endure more severe damage following caerulein treatment (42). At this time, it is unclear how *Mist1* influences ADM decisions. One possibility is that MIST1 protects acinar cells from metabolic stress associated with pancreatitis or *Kras*^{G12D} expression by regulating aspects of the MAPK pathway. Indeed, *Mist1* is silenced in pancreatitis-associated acinar cells (unpublished results) and *Mist1*^{KO} acinar cells exhibit increased MEK and ERK phosphorylation (4). Given the central role of the MAPK pathway in ADM and the observation that pancreatitis enhances *Kras*^{G12D}-induced tumorigenesis (15, 35, 43), it is likely that *Mist1* inhibits ADM by maintaining basal levels of these MAPK effectors. Since *Mist1* is influential in converting embryonic stem cells to an acinar cell fate (44), sustained *Mist1* expression also likely keeps key acinar characteristics intact in *Kras*^{G12D} cells. Further investigation into the mechanisms of this regulatory network and identification of MIST1 target genes should lead to better therapeutic interventions and improved clinical outcomes for PDAC patients.

Materials and Methods

Mouse strains and genotyping

Mist1$^{CreER/+}$, LSL-Kras$^{G12D/+}$, R26R^{LacZ} and R26R^{mTmG} reporter mouse lines have been described previously (4, 13, 45). *Elastase*$_{pr}$*-CreER* mice were generated according to standard protocols using a previously reported Elastase$_{pr}$-Mist1^{MB} construct where the Mist1^{MB} coding region was replaced by a CreER^{T2} coding region to drive acinar-specific expression of CreER^{T2} (46). *LSL-Mist1*^{myc} transgenic mice were generated using the *pCAG-*

LoxP-CAT-LoxP-LacZ construct in which the *LacZ* gene was replaced by a myc-tagged *Mist1* coding sequence (47). Induction of $CreER^{T2}$ activity was accomplished by providing adult mice (6-9 wk) tamoxifen (TM, 4 mg/mouse/day) for 2-3 consecutive days. Genotyping primer sets are listed in Supplemental Table I. All animal studies were conducted in compliance with NIH and the Purdue University IACUC guidelines.

3D acinar cell culture

Mice were treated with TM 7 days prior to harvesting the pancreas. Immediately after sacrificing, pancreata were rinsed in cold 1X HBSS (Invitrogen, Carlsbad, CA) and cut into small pieces with scissors. Primary acini were released by collagenase P (Roche Applied Science, Mannheim, Germany) digestion (200 μg/ml in 1x HBSS) for 10-20 minutes at 37°C. Isolated acini were washed 3 times in cold 5% FBS, 1x HBSS and then filtered sequentially through 500 μm and 105 μm nylon meshes (Spectrum Laboratories, Rancho Dominguez, CA). Cell suspensions were carefully layered on top of 30% FBS, 1x HBSS and acini were collected by centrifugation (1000 rpm, 2 min at 4°C) and then resuspended in 8 ml of 3D culture base medium (RPMI, 10% fetal bovine serum, 0.1 mg/ml soybean trypsin inhibitor, 1 μg/ml dexamethasone and antibiotics). 24-well tissue culture plates were coated with a 250 μl/well collagen layer (100 μl 10x RPMI, 900 μl 3mg/ml collagen, neutralized with either 4.2% $NaHCO_3$ or 0.34N NaOH) at least one hr prior to acini isolation. The cell suspension was then mixed with collagen 1:1 and plated (0.5 ml per well). The cell-collagen mix was allowed to solidify for 1 hr at 37°C before adding 1 ml 3D culture media. Media was changed on days 1 and 3. On day 5 the percentage of acini that converted to ductal cysts was calculated by counting individual clusters in all wells. Collagen matrix was then digested for 10-15 min at 37°C with 200 μg/ml collagenase P in 1x HBSS to release cells. RNA was prepared using the E.Z.N.A. total RNA kit (Omega Bio-Tek, Norcross, GA). Alternatively, collagen discs were fixed in 4% formaldehyde, embedded in paraffin and 5 μm sections were prepared for standard immunohistochemistry as described below.

Histology and immunohistochemistry

Mouse pancreata were fixed in 10% neutral buffered formalin, paraffin embedded, sectioned to 5 μm and stained using conventional hematoxylin and eosin. Sections were deparaffinized and retrieved using the 2100-Retriever (PickCell Laboratories, Amsterdam, The Netherlands) and antigen unmasking solution (Vector Laboratories, Burlingame, CA). Samples were blocked using the MOM blocking reagent (Vector Laboratories) and incubation of primary antibodies was conducted for 1 hr at room temperature or overnight at 4°C. Biotinylated secondary antibodies were applied for 10 min at 25°C. Visualization was accomplished via 3,3'-Diaminobenzidine peroxidase staining or tertiary avidin-conjugated fluorescent antibodies. Primary antibodies and conditions are provided in Supplemental Table II.

Protein immunoblots

Twenty μg of whole cell protein extracts were separated on 12% acrylamide gels, transferred to PVDF membranes and incubated with primary antibodies (antibody conditions are

provided in Supplemental Table III). Immunoblots were developed using an ECL kit (Thermo Scientific, Waltham, MA).

RT-qPCR gene expression analysis

Pancreas RNA or RNA from 3D cultures was isolated using the E.Z.N.A. total RNA kit and reverse transcribed using the iScript™ cDNA synthesis kit (Bio-Rad, Hercules, CA). Equal amounts of cDNA reactions were amplified with FastStart Universal SYBR Green Master (Roche Applied Science, Indianapolis, IN) using the primer sets listed in Supplemental Table IV. Target sequences were amplified with 95°C/30 sec, 59°C/60 sec, 72°C/30 sec conditions. Fold changes in gene expression between TM-treated and control animals or between day 0 and day 5 3D cultures were calculated using the comparative $2^{-\Delta\Delta Ct}$ method. Error bars represent the S.E.M.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Judy Hallett of the Purdue Center for Cancer Research Transgenic Mouse Core Facility for generating the transgenic mouse strains used in this study and David Hess and Anju Karki for critical reading of the manuscript. This work was supported by grants to SFK (NIH CA124586, NIH DK55489 and the Phi Beta Psi Sorority for Cancer Research) and to DD (CTSI Predoctoral Fellowship).

Abbreviations

ADM	acinar-ductal metaplasia
AMY	amylase
β-gal	β-galactosidase
CAII	carbonic anhydrase II
CFTR	cystic fibrosis transmembrane conductance regulator
CPA	carboxypeptidase
Cre-ER	Cre recombinase-estrogen receptor
Elas	elastase
Het	Mist1 heterozygous
K19	keratin 19
KO	Mist1 homozygous null
MAPK	mitogen-activated protein kinase
PanIN	pancreatic intraepithelial neoplasia
PDAC	pancreatic ductal adenocarcinoma
PI3K	phosphatidylinositol 3-OH kinase

RT-qPCR	reverse transcriptase-quantitative polymerase chain reaction
TM	tamoxifen

References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. Sep-Oct;2010 60(5): 277–300. [PubMed: 20610543]
2. Maitra A, Fukushima N, Takaori K, Hruban RH. Precursors to invasive pancreatic cancer. Adv Anat Pathol. Mar; 2005 12(2):81–91. [PubMed: 15731576]
3. Hruban RH, Adsay NV, Albores-Saavedra J, Compton C, Garrett ES, Goodman SN, et al. Pancreatic intraepithelial neoplasia - A new nomenclature and classification system for pancreatic duct lesions. Am J Surg Pathol. May; 2001 25(5):579–86. [PubMed: 11342768]
4. Shi G, Zhu L, Sun Y, Bettencourt R, Damsz B, Hruban RH, et al. Loss of the acinar-restricted transcription factor Mist1 accelerates Kras-induced pancreatic intraepithelial neoplasia. Gastroenterology. Apr; 2009 136(4):1368–78. [PubMed: 19249398]
5. Guerra C, Mijimolle N, Dhawahir A, Dubus P, Barradas M, Serrano M, et al. Tumor induction by an endogenous K-ras oncogene is highly dependent on cellular context. Cancer Cell. 2003; 4(2):111–20. [PubMed: 12957286]
6. Hingorani SR, Wang L, Multani AS, Combs C, Deramaudt TB, Hruban RH, et al. Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. Cancer Cell. May; 2005 7(5):469–83. [PubMed: 15894267]
7. Bardeesy N, Aguirre AJ, Chu GC, Cheng K-h, Lopez LV, Hezel AF, et al. Both p16Ink4a and the p19Arf-p53 pathway constrain progression of pancreatic adenocarcinoma in the mouse. Proceedings of the National Academy of Sciences. Apr 11; 2006 103(15):5947–52. 2006.
8. Bardeesy N, Cheng K-h, Berger JH, Chu GC, Pahler J, Olson P, et al. Smad4 is dispensable for normal pancreas development yet critical in progression and tumor biology of pancreas cancer. Genes & Development. Nov 15; 2006 20(22):3130–46. 2006. [PubMed: 17114584]
9. Siveke JT, Einwachter H, Sipos B, Lubeseder-Martellato C, Kloppel G, Schmid RM. Concomitant Pancreatic Activation of Kras(G12D) and Tgfa Results in Cystic Papillary Neoplasms Reminiscent of Human IPMN. Cancer Cell. Sep; 2007 12(3):266–79. [PubMed: 17785207]
10. De La OJ-P, Emerson LL, Goodman JL, Froebe SC, Illum BE, Curtis AB, et al. Notch and Kras reprogram pancreatic acinar cells to ductal intraepithelial neoplasia. Proceedings of the National Academy of Sciences. Dec 2; 2008 105(48):18907–12. 2008.
11. Brembeck FH, Schreiber FS, Deramaudt TB, Craig L, Rhoades B, Swain G, et al. The mutant K-ras oncogene causes pancreatic periductal lymphocytic infiltration and gastric mucous neck cell hyperplasia in transgenic mice. Cancer Res. May 1; 2003 63(9):2005–9. [PubMed: 12727809]
12. Ray KC, Bell KM, Yan J, Gu G, Chung CH, Washington MK, et al. Epithelial tissues have varying degrees of susceptibility to Kras(G12D)-initiated tumorigenesis in a mouse model. PLoS One. 2011; 6(2):e16786. [PubMed: 21311774]
13. Habbe N, Shi G, Meguid RA, Fendrich V, Esni F, Chen H, et al. Spontaneous induction of murine pancreatic intraepithelial neoplasia (mPanIN) by acinar cell targeting of oncogenic Kras in adult mice. Proc Natl Acad Sci U S A. Dec 2; 2008 105(48):18913–8. [PubMed: 19028870]
14. Friedlander SYG, Chu GC, Snyder EL, Girnius N, Dibelius G, Crowley D, et al. Context-Dependent Transformation of Adult Pancreatic Cells by Oncogenic K-Ras. Cancer Cell. 2009; 16(5):379–89. [PubMed: 19878870]
15. Guerra C, Schuhmacher AJ, Canamero M, Grippo PJ, Verdaguer L, Perez-Gallego L, et al. Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. Cancer Cell. Mar; 2007 11(3):291–302. [PubMed: 17349585]
16. Morris JP, Cano DA, Sekine S, Wang SC, Hebrok M. [beta]-catenin blocks Kras-dependent reprogramming of acini into pancreatic cancer precursor lesions in mice. The Journal of Clinical Investigation. 2010; 120(2):508–20. [PubMed: 20071774]

17. Zhu L, Shi G, Schmidt CM, Hruban RH, Konieczny SF. Acinar cells contribute to the molecular heterogeneity of pancreatic intraepithelial neoplasia. Am J Pathol. Jul; 2007 171(1):263–73. [PubMed: 17591971]
18. Brune K, Abe T, Canto M, O'Malley L, Klein AP, Maitra A, et al. Multifocal neoplastic precursor lesions associated with lobular atrophy of the pancreas in patients having a strong family history of pancreatic cancer. The American journal of surgical pathology. Sep; 2006 30(9):1067–76. [PubMed: 16931950]
19. Means AL, Meszoely IM, Suzuki K, Miyamoto Y, Rustgi AK, Coffey RJ Jr. et al. Pancreatic epithelial plasticity mediated by acinar cell transdifferentiation and generation of nestin-positive intermediates. Development. Aug 15; 2005 132(16):3767–76. 2005. [PubMed: 16020518]
20. Schreiber FS, Deramaudt TB, Brunner TB, Boretti MI, Gooch KJ, Stoffers DA, et al. Successful growth and characterization of mouse pancreatic ductal cells: functional properties of the Ki-RAS(G12V) oncogene. Gastroenterology. Jul; 2004 127(1):250–60. [PubMed: 15236190]
21. Heid I, Lubeseder-Martellato C, Sipos B, Mazur PK, Lesina M, Schmid RM, et al. Early requirement of rac1 in a mouse model of pancreatic cancer. Gastroenterology. Aug; 2011 141(2): 719–30. e7. [PubMed: 21684285]
22. Pin CL, Rukstalis JM, Johnson C, Konieczny SF. The bHLH transcription factor Mist1 is required to maintain exocrine pancreas cell organization and acinar cell identity. J Cell Biol. Nov 12; 2001 155(4):519–30. [PubMed: 11696558]
23. Carriere C, Seeley ES, Goetze T, Longnecker DS, Korc M. The Nestin progenitor lineage is the compartment of origin for pancreatic intraepithelial neoplasia. Proc Natl Acad Sci U S A. Mar 13; 2007 104(11):4437–42. [PubMed: 17360542]
24. Hezel AF, Kimmelman AC, Stanger BZ, Bardeesy N, DePinho RA. Genetics and biology of pancreatic ductal adenocarcinoma. Genes & Development. May 15; 2006 20(10):1218–49. 2006. [PubMed: 16702400]
25. Downward J. Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer. Jan; 2003 3(1):11–22. [PubMed: 12509763]
26. Ramsey VG, Doherty JM, Chen CC, Stappenbeck TS, Konieczny SF, Mills JC. The maturation of mucus-secreting gastric epithelial progenitors into digestive-enzyme secreting zymogenic cells requires Mist1. Development. Jan; 2007 134(1):211–22. [PubMed: 17164426]
27. Tuveson DA, Zhu L, Gopinathan A, Willis NA, Kachatrian L, Grochow R, et al. Mist1-KrasG12D knock-in mice develop mixed differentiation metastatic exocrine pancreatic carcinoma and hepatocellular carcinoma. Cancer Res. Jan 1; 2006 66(1):242–7. [PubMed: 16397237]
28. Sakai K, Miyazaki J. A transgenic mouse line that retains Cre recombinase activity in mature oocytes irrespective of the cre transgene transmission. Biochemical and biophysical research communications. Aug 18; 1997 237(2):318–24. [PubMed: 9268708]
29. Shi C, Hong SM, Lim P, Kamiyama H, Khan M, Anders RA, et al. KRAS2 mutations in human pancreatic acinar-ductal metaplastic lesions are limited to those with PanIN: implications for the human pancreatic cancer cell of origin. Mol Cancer Res. Feb; 2009 7(2):230–6. [PubMed: 19208745]
30. Sandgren EP, Luetteke NC, Palmiter RD, Brinster RL, Lee DC. Overexpression of TGF alpha in transgenic mice: induction of epithelial hyperplasia, pancreatic metaplasia, and carcinoma of the breast. Cell. Jun 15; 1990 61(6):1121–35. [PubMed: 1693546]
31. Wagner M, Luhrs H, Kloppel G, Adler G, Schmid RM. Malignant transformation of duct-like cells originating from acini in transforming growth factor transgenic mice. Gastroenterology. Nov; 1998 115(5):1254–62. [PubMed: 9797382]
32. Detlefsen S, Sipos B, Feyerabend B, Kloppel G. Pancreatic fibrosis associated with age and ductal papillary hyperplasia. Virchows Archiv : an international journal of pathology. Nov; 2005 447(5): 800–5. [PubMed: 16021508]
33. Strobel O, Dor Y, Alsina J, Stirman A, Lauwers G, Trainor A, et al. In vivo lineage tracing defines the role of acinar-to-ductal transdifferentiation in inflammatory ductal metaplasia. Gastroenterology. Dec; 2007 133(6):1999–2009. [PubMed: 18054571]

34. Jensen JN, Cameron E, Garay MV, Starkey TW, Gianani R, Jensen J. Recapitulation of elements of embryonic development in adult mouse pancreatic regeneration. Gastroenterology. Mar; 2005 128(3):728–41. [PubMed: 15765408]

35. Carriere C, Young AL, Gunn JR, Longnecker DS, Korc M. Acute pancreatitis markedly accelerates pancreatic cancer progression in mice expressing oncogenic Kras. Biochemical and biophysical research communications. Mar 16.2009

36. Malka D, Hammel P, Maire F, Rufat P, Madeira I, Pessione F, et al. Risk of pancreatic adenocarcinoma in chronic pancreatitis. Gut. Dec; 2002 51(6):849–52. [PubMed: 12427788]

37. Calhoun ES, Jones JB, Ashfaq R, Adsay V, Baker SJ, Valentine V, et al. BRAF and FBXW7 (CDC4, FBW7, AGO, SEL10) Mutations in Distinct Subsets of Pancreatic Cancer: Potential Therapeutic Targets. The American Journal of Pathology. 2003; 163(4):1255–60. [PubMed: 14507635]

38. Houbracken I, de Waele E, Lardon J, Ling Z, Heimberg H, Rooman I, et al. Lineage tracing evidence for transdifferentiation of acinar to duct cells and plasticity of human pancreas. Gastroenterology. Aug; 2011 141(2):731–41. e4. [PubMed: 21703267]

39. Lambert JM, Lambert QT, Reuther GW, Malliri A, Siderovski DP, Sondek J, et al. Tiam1 mediates Ras activation of Rac by a PI(3)K-independent mechanism. Nat Cell Biol. Aug; 2002 4(8):621–5. [PubMed: 12134164]

40. De Corte V, Bruyneel E, Boucherie C, Mareel M, Vandekerckhove J, Gettemans J. Gelsolin-induced epithelial cell invasion is dependent on Ras-Rac signaling. EMBO J. Dec 16; 2002 21(24): 6781–90. [PubMed: 12485999]

41. Malliri A, van der Kammen RA, Clark K, van der Valk M, Michiels F, Collard JG. Mice deficient in the Rac activator Tiam1 are resistant to Ras-induced skin tumours. Nature. Jun 20; 2002 417(6891):867–71. [PubMed: 12075356]

42. Kowalik AS, Johnson CL, Chadi SA, Weston JY, Fazio EN, Pin CL. Mice lacking the transcription factor Mist1 exhibit an altered stress response and increased sensitivity to caerulein-induced pancreatitis. Am J Physiol Gastrointest Liver Physiol. Apr; 2007 292(4):G1123–32. [PubMed: 17170023]

43. Carriere C, Young AL, Gunn JR, Longnecker DS, Korc M. Acute pancreatitis accelerates initiation and progression to pancreatic cancer in mice expressing oncogenic kras in the nestin cell lineage. PLoS One. 2011; 6(11):e27725. [PubMed: 22140463]

44. Rovira M, Delaspre F, Massumi M, Serra SA, Valverde MA, Lloreta J, et al. Murine embryonic stem cell-derived pancreatic acinar cells recapitulate features of early pancreatic differentiation. Gastroenterology. Oct; 2008 135(4):1301–10. 10, e1–5. [PubMed: 18725222]

45. Hingorani SR, Petricoin EF, Maitra A, Rajapakse V, King C, Jacobetz MA, et al. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell. Dec; 2003 4(6):437–50. [PubMed: 14706336]

46. Zhu L, Tran T, Rukstalis JM, Sun P, Damsz B, Konieczny SF. Inhibition of Mist1 homodimer formation induces pancreatic acinar-to-ductal metaplasia. Mol Cell Biol. Apr; 2004 24(7):2673–81. [PubMed: 15024058]

47. Araki K, Araki M, Miyazaki J, Vassalli P. Site-specific recombination of a transgene in fertilized eggs by transient expression of Cre recombinase. Proc Natl Acad Sci U S A. Jan 3; 1995 92(1): 160–4. [PubMed: 7816809]

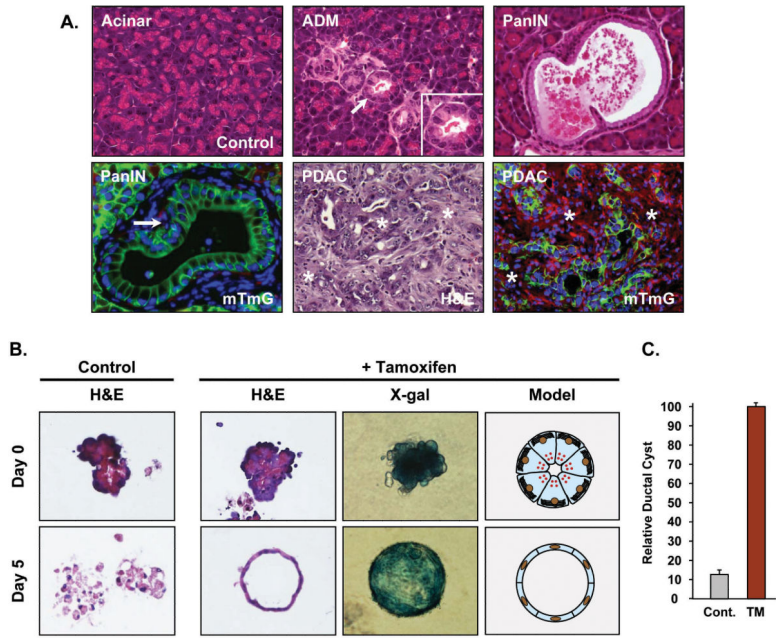

Figure 1.

Acinar-specific Kras^{G12D} expression leads to ADM → PanIN → PDAC. **(A)** Adult *Elastase-CreER; LSL-Kras$^{G12D/+}$; R26R^{mTmG}* and Elastase-CreER; LSL-Kras$^{G12D/+}$; LSL-Trp53^{R172H}; R26R^{mTmG} mice were given corn oil (control) or TM and pancreas sections were processed for standard histology after 1-5 months. The earliest lesions to appear are ADM (inset) followed by PanINs and then PDAC. *R26^{mTmG}* lineage tracing confirms that ADM, PanINs and PDAC are derived from *Kras^{G12D}*-expressing acinar cells (green). Red signal represents unrecombined *R26^{mTmG}* stromal cells. arrows - ADM and PanIN lesions; asterisks - adjacent stromal tissue. **(B)** *Mist1$^{CreER/+}$; LSL-Kras$^{G12D/+}$; R26R^{LacZ}* acinar cells were isolated from 7 day post-treated mice and individual acini were cultured for 5 days in a 3D collagen matrix. The majority of control acinar cells undergo extensive cell death by 5 days, whereas *Kras^{G12D}*-expressing acinar cells rapidly transdifferentiate into ductal cysts (H&E sections) that retain β-gal expression (whole mount X-gal staining). Identical results were obtained with *Elastase-CreER; LSL-Kras$^{G12D/+}$; R26R^{mTmG}* mice. **(C)** Whereas corn oil treated control *Mist1$^{CreER/+}$; LSL-Kras$^{G12D/+}$; R26R^{LacZ}* acinar cells generate very few ductal cysts, TM-treated acinar cells generate a large number of ductal cysts in 3D collagen matrix.

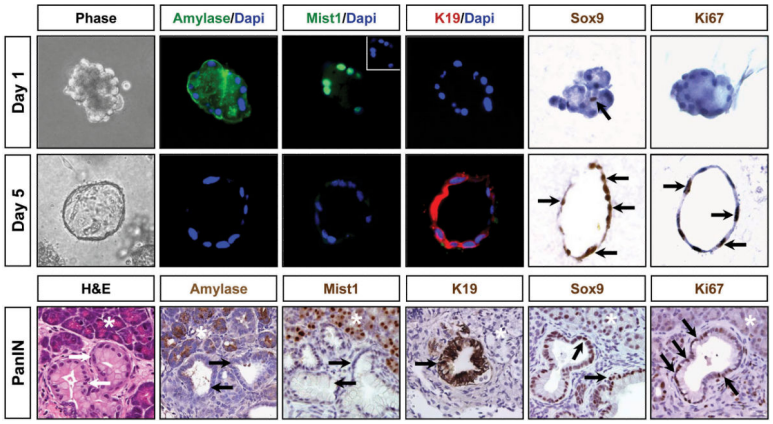

Figure 2. Kras^{G12D}-expressing acinar cells undergo conversion to a ductal phenotype when maintained in 3D collagen matrix

Fluorescence and light microscopy reveals that *Kras^{G12D}* acinar cells express acinar gene products at day 1 but rapidly convert to a ductal cell phenotype with activation of K19 and Sox9 expression. Inset for day 1 Mist1 panel shows the Dapi stained nuclei for this acinus. Note that a single centroacinar cell (arrow) in an isolated acinus is SOX9 positive. Pancreas serial sections from experimental littermates confirm the absence of AMYLASE and MIST1 and the expression of SOX9 and K19 in acinar-derived PanINs (arrows). Both PanINs and ductal cysts are also Ki67 positive (arrows). asterisks - adjacent acinar tissue.

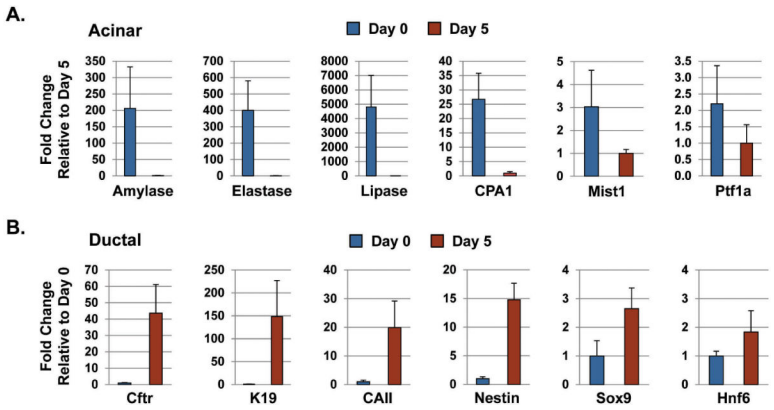

Figure 3. Kras^{G12D}-induced ductal cyst conversion leads to repression of the acinar gene program and activation of the ductal gene program

(A,B) RT-qPCR of day 0 and day 5 cultures confirms that *Kras^{G12D}* acinar cells express high levels of acinar gene products at day 0 but rapidly down-regulate acinar genes and up-regulate duct genes by day 5, a time that corresponds to maximum ductal cyst formation.

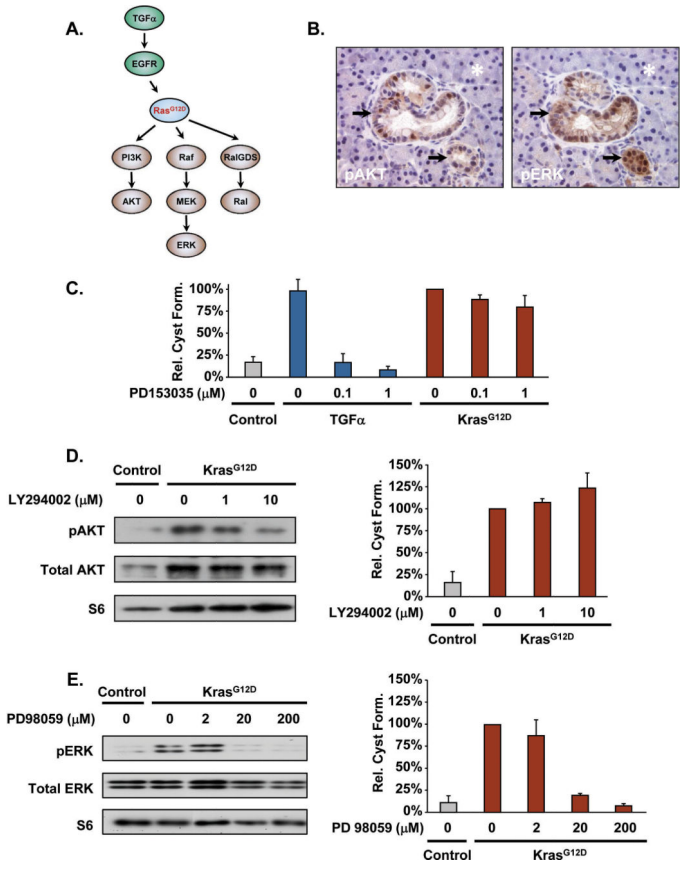

Figure 4. The Raf/MEK/ERK pathway is essential to early ADM/PanIN formation in vitro and in vivo

(A) Canonical upstream and downstream pathways associated with *Kras*. **(B)** ADM/PanIN lesions (arrows) in the HET/Kras pancreas exhibit elevated levels of pAKT and pERK. Note that the majority of acinar cells (asterisks) in HET/Kras pancreata are $Kras^{G12D}$ positive but pAKT/pERK negative. **(C)** TGFα treatment of control wildtype acinar cells leads to ductal cyst formation in 3D culture. As expected, inhibition of EGFR activity (PD153035 treatment) blocks TGFα-dependent ductal cyst formation but has no effect on the downstream $Kras^{G12D}$ pathway of HET/Kras acinar cells. **(D)** Inhibition of PI3K by LY294002 blocks pAKT activity but does not inhibit ductal cyst formation. **(E)** The MEK inhibitor PD98059 efficiently blocks pERK activity in HET/Kras acinar 3D cultures. Inhibition of MEK also leads to complete inhibition of $Kras^{G12D}$-induced ductal cyst formation.

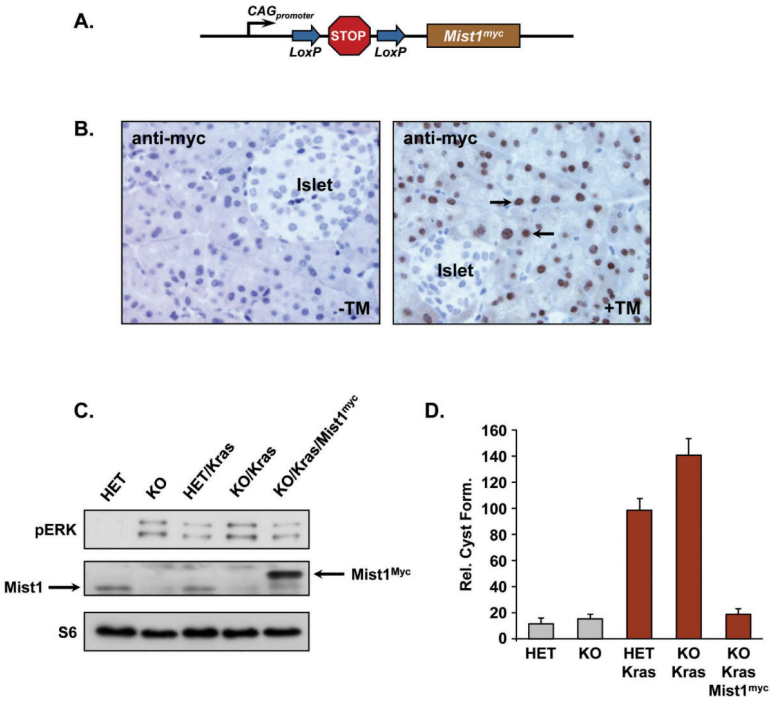

Figure 5. Sustained Mist1 expression suppresses Kras^{G12D}-induced ductal cysts

(A) Schematic diagram of the LSL-Mist1^{myc} transgene. **(B)** Anti-myc immunohistochemistry of KO/Kras/Mist1^{myc} pancreata reveals extensive acinar-specific expression of LSL-Mist1^{myc} in tamoxifen-treated mice (arrows). **(C)** Immunoblot of pERK activity in HET *vs.* KO and KO/Kras *vs*. KO/Kras/Mist1^{myc} pancreata. The absence of *Mist1* leads to increased pERK activity while sustained *Mist1^{myc}* expression reduces pERK levels in KO/Kras/Mist1^{myc} pancreata. **(D)** Quantification of ductal cyst formation in control and *Kras^{G12D}*-expressing cells. Mice were treated with TM and acinar cells placed in 3D culture as described in Materials and Methods. Sustained *Mist1^{myc}* expression dramatically inhibits *Kras^{G12D}*-induced ductal cyst formation.

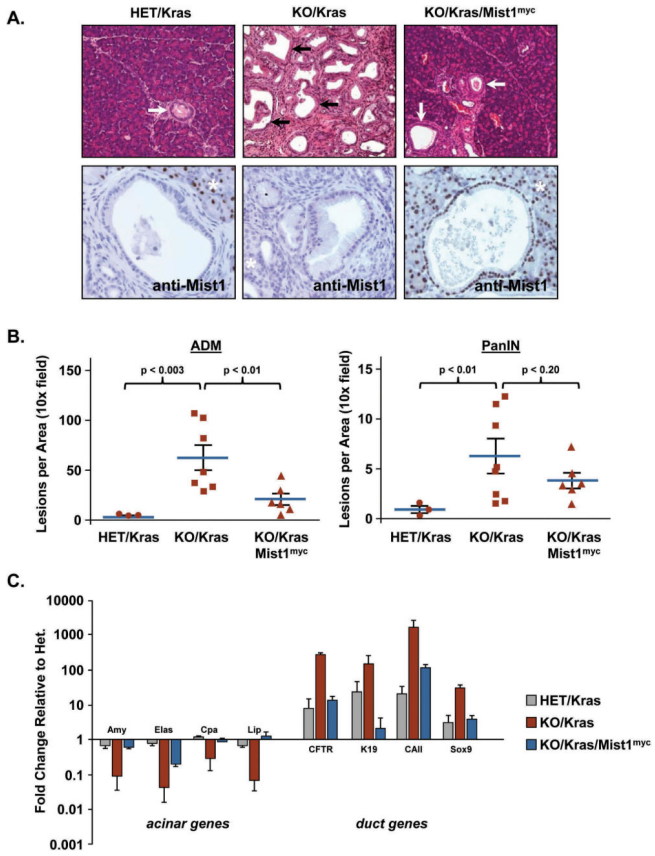

Figure 6. Sustained Mist1^{myc} expression inhibits ADM and PanIN formation in vivo

(A) PanIN formation (arrows) is greatly accelerated in KO/Kras pancreata but suppressed by sustained *Mist1^{myc}* expression in KO/Kras/Mist1^{myc} mice. Acinar-derived PanINs are Mist1 negative in HET/Kras mice. Interestingly, induced *Mist1^{myc}* expression alone is not sufficient to inhibit all PanIN formation since some MIST1^{myc}+ PanINs still develop in KO/Kras/Mist1^{myc} pancreata. asterisks - adjacent acinar tissue. **(B)** Quantification of ADM and PanIN lesions in HET/Kras, KO/Kras and KO/Kras/Mist1^{myc} samples. Note that constitutive *Mist1^{myc}* leads to significant reductions in ADM/PanIN formation. **(C)** Analysis of gene expression profiles by RT-qPCR confirms the reduced ADM/PanIN formation in KO/Kras/Mist1^{myc} samples when compared to KO/Kras littermates. All values were normalized to the control HET samples, which were set to 1.0.