Context-Dependent Transformation of Adult Pancreatic Cells by Oncogenic K-Ras

Sharon Y Gidekel Friedlander[1], **Gerald C. Chu**[4,5,6], **Eric L. Snyder**[1,5], **Nomeda Girnius**[1], **Gregory Dibelius**[1], **Denise Crowley**[1,3], **Eliza Vasile**[1], **Ronald A. DePinho**[4,6,7], and **Tyler Jacks**[1,2,3]

[1]The David H. Koch Institute for Integrative Cancer Research at MIT, Cambridge, Massachusetts 02139, USA

[2]Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

[3]Howard Hughes Medical Institute at Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

[4]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts

[5]Department of Pathology, Brigham and Woman's Hospital, Boston, Massachusetts

[6]Center for Applied Cancer Science, Belfer Foundation Institute for Innovative Cancer Science

[7]Department of Medicine and Genetics, Harvard Medical School, Boston, Massachusetts

Summary

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal human malignancies. To investigate the cellular origin(s) of this cancer, we determined the effect of PDAC-relevant gene mutations in distinct cell types of the adult pancreas. We show that a subpopulation of *Pdx1*-expressing cells is susceptible to oncogenic K-Ras induced transformation without tissue injury, whereas *insulin*-expressing endocrine cells are completely refractory to transformation under these conditions. However, chronic pancreatic injury can alter their endocrine fate and allow them to serve as the cell of origin for exocrine neoplasia. These results suggest that one mechanism by which inflammation and/or tissue damage can promote neoplasia is by altering the fate of differentiated cells that are normally refractory to oncogenic stimulation.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer death in the United States (Li et al., 2004). Identification of the cell(s)-of-origin of PDAC and defining the biochemical and biological changes that accompany their transformation are essential for the development of early detection and treatment tools.

The pancreas is composed of four parenchymal cell types: acinar, ductal, centroacinar (exocrine) and islet (endocrine) cells. The acinar cells synthesize and secrete zymogens into

the ductal lumen. The ducts carry the enzymes to the duodenum and generate bicarbonate. The centroacinar cells (CACs) have been suggested to be progenitor cells in the adult pancreas. The islets of Langerhans are composed of endocrine cell types, which secrete insulin and other proteins that regulate glucose homeostasis (Bardeesy and DePinho, 2002). Rare cells expressing endocrine markers such as insulin can also be found associated with the acini and the ductal epithelium (Mills, 2007).

PDAC arises from *in situ* precursor lesions termed pancreatic intraepithelial neoplasia (PanIN) (Hruban et al., 2001). PanIN lesions include a spectrum of abnormal proliferative ductal structures (termed PanIN 1-3) that are recognized by transformation from a cuboidal to columnar epithelium, mucin production, and nuclear atypia.

Mutations that result in a constitutively active K-RAS are found in over 95% of PDACs and are believed to be an initiating event for this type of cancer (Jones et al., 2008; Rozenblum et al., 1997). *KRAS* encodes a small GTPase that in its active GTP-bound form promotes a wide range of cellular responses including proliferation, survival, migration, and metabolism (Shields et al., 2000). The *CDKN2A* locus encodes the *Ink4A* and *ARF* tumor suppressor genes and is also frequently mutated in PDAC. *INK4A* inactivation mutations are present in virtually all sporadic PDAC, suggesting that INK4A/ARF normally constrains the malignant potential of mutant K-RAS. Other frequent alterations include loss of function mutations of the *SMAD4* and *p53* tumor suppressor genes (Jaffee et al., 2002). Moreover, developmental regulatory pathways, in particular the Notch, Sonic Hedgehog and Wnt pathways, are often activated in PDACs (Berman et al., 2003; Miyamoto et al., 2003; Thayer et al., 2003; Wang et al., 2009).

PDAC is commonly believed to arise from transformation of ductal cells, although experimental studies have suggested other differentiated cells or progenitor/stem cells as potential origin(s) for this type of cancer. For instance, mouse models expressing various oncogenes in acinar cells during development, such as the *Elastase-Tgfα* (Wagner et al., 1998) and *Elastase-Kras^{G12D}* (Grippo et al., 2003) transgenic strains and the knock-in *Mist1-Kras4B^{G12D}* animals (Tuveson et al., 2006), have implicated acinar cells as being susceptible to transformation and giving rise to tumors containing ductal elements. Transplantation of mouse islet cell cultures expressing the *polyoma virus middle T (PyMT)* oncogene into histocompatible mice resulted in the induction of pancreatic cancer with ductal histology (Yoshida and Hanahan, 1994), suggesting a potential endocrine origin for PDAC. A role of CACs has been proposed based on studies of mice that harbor a pancreas-specific deletion of the *PTEN* gene; these animals exhibit a proliferative expansion of CACs that can progress to carcinoma (Stanger et al., 2005). Nestin+ exocrine progenitor cells have been shown to be highly responsive to K-Ras oncogenic activation and were suggested to represent a progenitor origin for PDAC (Carriere et al., 2007).

The close developmental relationships of the different pancreatic cell types and the capacity of endodermal lineages to transdifferentiate (Slack, 1995) add complexity to the PDAC cell-of-origin question. PanIN lesions can express markers of gastric and foregut differentiation (Prasad et al., 2005), suggesting that some degree of transdifferentiation occurs routinely during PDAC progression. This developmental plasticity is evident in some human PDACs that show focal expression of non-ductal lineage markers, including endocrine and exocrine factors. More recently, Habbe et al. (2008) and De La O et al. (2008) have shown that activation of oncogenic K-Ras in acinar cells of the adult mouse leads to efficient mPanIN formation. These groups have also demonstrated *in vivo* acinar-to-ductal transdifferentiation by lineage tracing studies in these models (De La O et al., 2008; Habbe et al., 2008). Given this plasticity, it is possible that there is no exclusive cell-of-origin for PDAC and that specific genetic alteration(s) define the resulting malignant phenotype regardless of the cell

in which they occur. Moreover, the physiological context, including tissue damage and inflammation, may alter a given cell's susceptibility to transformation. For example, Guerra et al. (2007) have reported that K-Ras activation in acinar cells (using the *K-Ras$^{+/LSLG12Vgeo}$; Elas-tTA/tetO-Cre* system) resulted in adult onset malignancy only following treatment with caerulein, which causes tissue injury and inflammation (Guerra et al., 2007).

In this study, we investigate whether PDAC arises from an exclusive cell-of-origin and whether a combination of genetic and non-genetic events can collaborate to induce neoplasia in a range of cell types. We describe the use of genetic manipulation of the mouse to systematically determine the effects of oncogenic K-Ras in distinct subsets of pancreatic cells of the adult mouse in a context dependent manner.

Results

Temporally-restricted activation of oncogenic K-Ras in distinct cell populations of the adult pancreas

Activation of oncogenic K-Ras in the common pancreatic embryonic progenitor in the *Pdx1Cre;LSL-Kras^{G12D}* model results in mPanIN formation and spontaneous progression to PDAC (Aguirre et al., 2003; Hingorani et al., 2003). Because human PDAC is an adult-onset malignancy, we sought to engineer PDAC mouse models in which oncogenic K-Ras (Kras^{G12D}) is activated in the adult animal. We crossed the *LSL-Kras^{G12D}* strain to different cell-specific *CreER* lines and compared the phenotypic effect of Kras^{G12D} activation on these distinct cell populations in the adult pancreas (Fig. 1A). The CreER lines used were: (i) the *Pdx1CreER^{TM}* transgenic strain (Gu et al., 2002), which marks the earliest pancreatic progenitor cell during development as well as adult endocrine β cells, some ductal, acinar cells and possibly adult progenitor/stem cells (Koizumi et al., 2003; Stoffers et al., 1999; Swift et al., 1998; Wu et al., 1997); (ii) the *RipCreER^{TM}* transgenic strain (Dor et al., 2004), which is selective for *insulin$^+$* cells; and (iii) the acinar *procarboxypeptidase A1 CreER^{T2} (proCPA1CreER^{T2})* knock-in strain (Zhou et al., 2007). The recombination specificity and efficiency of the different *CreER* strains was determined by crossing them to the *LSL-LacZ* reporter mouse and analyzing the X-gal stained pancreata of their Tamoxifen (TM)-treated compound progeny (Fig. 1B-E and Table S1). As shown in Fig. 1B, in the *Pdx1CreER^{TM}* transgenic strain recombination was achieved in a mosaic fashion in the islets, acinar and ductal cells. The *pro-CPA1CreER^{T2}* strain exhibited recombination mainly in acinar cells and possibly CACs; however, recombination was evident also in a subset of ductal and islet cells. Recombination in this strain was variable, with half of the treated mice showing no evidence of recombination and the other half ranging from 0-49% in acinar cells (Fig. 1C, Table S1 and data not shown). With TM administration, the *RipCreER^{TM}* transgenic strain was very specific and efficient in causing recombination in both insulin-producing β cells located in the islets of Langerhans and in single insulin$^+$ cells found scattered throughout the pancreas parenchyma, as shown by X-gal and co-immunofluorescent (CoIF) staining (Fig. 1D, E respectively and Table S1).

Expression of Kras^{G12D} in different cell types of the adult pancreas was achieved by systemic TM administration in mice at 14-56 days of age. Because newly transformed cells would be expected to resemble their cell-of-origin both genetically and morphologically, we sought to define the earliest time point when transformed cells could be detected after TM administration. Therefore, mice were sacrificed at different times following the last dose, ranging from 4-240 days.

Kras^{G12D} induces transformation in *Pdx1$^+$* cells of the adult pancreas

Pdx1CreER^{TM};LSL-Kras^{G12D} mice were treated with TM at day 14, 21, 24, 27 or 56 after birth and sacrificed after 4-120 days. mPanINs and ductal metaplasia were observed in all age groups (Fig. 2 and Table S2). Low-grade mPanIN 1A lesions were the most prevalent and were observed to a comparable extent across the three main age groups, while mPanIN 1B were less frequent in the 56 day old group. Although mPanIN lesions were predominantly grade 1, grade 2 lesions were also identified, mostly at 120 days after TM administration and only in the 14- and 27-day old treated mice (Table S2, Fig. 2E). These results suggest that the *Pdx1$^+$* target cell for transformation is either less susceptible or less abundant in 56 day old mice. mPanIN 3 was found only in one mouse that was analyzed 188 days after TM administration and it was also the only mouse that developed PDAC. This result is consistent with observations in humans that it is unusual to find PanIN 3 in pancreata lacking invasive carcinoma (Mills, 2007).

TM-treated 14 or 27 day old mice exhibited additional phenotypes (N=23). One of the most notable was the appearance of acinar cells and ductal structures "embedded" in the islets of Langerhans as soon as 10 days following TM treatment (Fig. 3A, B, respectively). The ductal structures grew over time (Fig. 3C), and remained proliferative (data not shown). These lesions became elongated and produced mucin, reminiscent of mPanINs (Fig. 3D). On the other hand, some mPanIN in the exocrine compartment harbored cells that positively stained for endocrine markers such as glucagon, insulin, somatostatin and PYY (Fig. S1A,B and data not shown). Other histologic findings at 120 days after TM treatment included robust atrophy of the pancreas with a hypoplastic exocrine compartment, large cystically dilated areas, and strong stromal reaction (Fig. S1C,D). Importantly, no lesions arose in vehicle-treated mice (N=15) (Table S2). These results exclude the possibility of leaky Cre recombinase activity and TM-independent K-Ras activation during development and/or adulthood. In addition, we observed no evidence of chronic pancreatitis in treated mice. Since TM is dissolved in corn oil, which by itself causes a mild form of peritonitis (Fig. S1E), we treated *Pdx1CreER^{TM};LSL-Kras^{G12D}* mice with TM dissolved in carboxymethyl cellulose (CMC) by oral gavage. Dissolving TM in corn oil or CMC did not affect the specificity and efficiency of Cre-induced recombination as confirmed by X-gal staining in mice harboring a LacZ reporter (data not shown). As shown in Fig. S1F, pancreata of mice treated with TM dissolved in CMC were free of peritonitis yet still developed grade 1 mPanINs (N=5). This result precludes the possibility that the corn oil-induced peritonitis was essential for formation of mPanINs and ductal metaplasia in the *Pdx1CreER^{TM};LSL-Kras^{G12D}* model.

Activation of Kras^{G12D} in combination with mutations of *Ink4A/Arf* (Aguirre et al., 2003) or *Trp53* (Jonkers et al., 2001) in *Pdx1$^+$* cells in the adult mouse resulted in formation of moderately to poorly differentiated PDAC in 10/13 mice tested (Fig. 4 and Table S2). Many of the tumors contained undifferentiated areas with sarcomatoid or anaplastic features, and extension beyond the pancreas was common. We observed direct invasion into the stomach and small intestine as well as metastasis to mediastinal lymph nodes, diaphragm and peritoneal adipose tissue (Fig. 4 and data not shown). We also identified a full range of mPanIN lesions in these mice, including grade 3 (Table S2, Fig. 4A, B). Thus, *Pdx1$^+$* cells, or at least a subpopulation of *Pdx1$^+$* cells, represent an excellent candidate to be the/a cell-of-origin for PDAC in the mouse and in humans.

Targeted expression of Kras^{G12D} in *proCPA1$^+$* cells

Targeting the expression of Kras^{G12D} to pancreatic acinar cells and other cell types by treating *proCPA1CreER^{T2};LSL-Kras^{G12D}* mice with TM resulted in low grade mPanIN-1A formation in only 2/20 mice (Table S3 and Fig. S2A). All mice were tested and confirmed

for *LSL-Kras^G12D* recombination by PCR analysis of DNA extracted from tissue sections (Table S3). These results suggest that *proCPA1^+* cells are not efficiently transformed by Kras^G12D into mPanIN lesions in the adult mouse. It has been recently shown that Kras^G12D activation in acinar cells of the adult mouse leads to efficient mPanIN formation in the absence of tissue injury in the *Ela-CreERT2^Tg/+*, *Mist1^CreERT2/+* and *ElaCreERT* strains (De La O et al., 2008; Habbe et al., 2008). The phenotypic diversity observed between these models is discussed below (see also Table S7).

To determine whether loss of p53 or Ink4A/Arf could affect the neoplastic potential of *proCPA1^+* cells, we combined the *LSL-Kras^G12D* allele with the *Trp53^flox* or *Ink4A/Arf^flox* alleles on the background of the *proCPA1CreER^T2* strain. The pancreata derived from TM-treated *proCPA1CreER^T2;LSL-Kras^G12D;Ink4A/Arf^flox/flox* mice (N=7) showed no overt cancer phenotype when examined at 50, 83, 113, 158, 188 and 309 days after TM administration. However, one *proCPA1CreER^T2;LSL-Kras^G12D;Trp53^flox/flox* mouse (N=5) developed a moderately to poorly differentiated PDAC 81 days after TM injection at 53 days of age. The tumor contained undifferentiated areas, was locally invasive, and metastasized to the liver and diaphragm (Table S3; Fig. S2B). As described earlier, all mice in this study were analyzed for DNA recombination by PCR (Table S3). We conclude that highly aggressive PDAC can be initiated in *proCPA1^+* cells in adult mice without chronic pancreatitis, albeit with low penetrance in this system.

In a previous study, Guerra et al. (2007) have reported that selective expression of an endogenous K-Ras^(G12V) oncogene in adult cells of the acinar/centroacinar lineage using the *K-Ras^+/LSLG12Vgeo; Elas-tTA/tetO-Cre* mouse model resulted in no phenotype unless the mice were pre-treated with caerulein (Guerra et al., 2007). Caerulein induces chronic pancreatitis that results in regeneration of normal tissue and formation of metaplastic lesions of a ductal phenotype in the exocrine compartment (Strobel et al., 2007). The authors concluded that chronic pancreatitis can be a co-factor in the induction of PDAC by the K-Ras^(G12V) oncogene in adult mice (Guerra et al., 2007). Because the *proCPA1CreER^T2;LSL-Kras^G12D* mice described above were largely refractory to Kras^G12D induced transformation, we set out to test whether chronic pancreatitis could facilitate PDAC formation in these mice.

We treated 34-60-day-old mice (N=9) with caerulein for a total of 44-193 days. One month after caerulein treatment initiation, the mice were treated with TM to activate Cre. In addition to caerulein-induced inflammation and ductal metaplasia, 3/9 *proCPA1CreER^T2;LSL-Kras^G12D* and *proCPA1CreER^T2;LSL-Kras^G12D;LSL-LacZ* mice developed grade 1 mPanINs (Fig. 5A and Table S4). We confirmed *LSL-Kras^G12D* recombination by PCR analysis of DNA extracted from laser-captured microdissected (LCM) lesions (Fig. 5C and Table S4). Hence, the mPanINs arose from *ProCPA^+* cells. Importantly, 3 of 6 *proCPA1CreER^T2;LSL-Kras^G12D;p53^flox/flox* developed PDAC when treated first with caerulein and then with TM (Fig. S3B and Table S4). mPanIN grades 1 and 2 were observed, and the neoplastic lesions and advanced tumors were similar to those arising in *Pdx1CreER^TM* compound mutant mice with respect to histologic appearance, local invasion and distant metastasis. In addition, a single *proCPACreER^tT2;LSL-Kras^G12D;Ink4A/Arf^f/f;* mouse that was treated first with TM and then with caerulein developed PDAC (Fig. S3C and Table S4). To rule out false-positive results due to non-specific Cre recombinase activity upon caerulein-induced injury, *proCPACreER^T2;LSL-Kras^G12D;LSL-LacZ* mice (N=3) were treated with caerulein without prior TM administration for 8, 15 and 22 days. LacZ^+ cells were not observed in these mice (data not shown). Thus, the *proCPACreER^T2* allele remains tightly controlled under chronic pancreatitis conditions. Immunofluorescence for CPA revealed that most mPanIN cells in the *proCPA1CreER^T2;LSL-Kras^G12D* mouse model failed to express this marker (Fig. 6D).

This result supports the hypothesis that *proCPA⁺* cells can undergo injury-induced transdifferentiation and give rise to mPanIN formation in the context of Kras^{G12D} activation. In addition, our results provide evidence that pancreatic injury synergizes with Kras^{G12D} in transforming *proCPA⁺* cells and initiating PDAC.

Targeted expression of Kras^{G12D} in *insulin⁺* cells

Targeting the expression of Kras^{G12D} to *insulin⁺* cells by treating *RipCreER^{TM};LSL-Kras^{G12D}* mice with TM did not result in mPanIN formation in any of the different age groups (N=30), even after 8 months (Table S3). Although these results suggest that *insulin⁺* cells are not targeted for transformation by Kras^{G12D}, we nonetheless combined the *Kras^{G12D}* allele with the conditional *Trp53* or *Ink4A/Arf* alleles on the background of the *RipCreER^{TM}* strain. None of the *RipCreER^{TM};LSL-Kras^{G12D}; Trp53$^{flox/flox}$* mice or the *RipCreER^{TM};LSL-Kras^{G12D}; Ink4A/Arf$^{flox/flox}$* mice developed mPanINs or PDAC (N=14). As described above, recombination of *LSL-Kras^{G12D}* was confirmed by PCR analysis of DNA extracted from tissue sections of all TM-treated mice in this study (Table S3). These results strongly suggest that *insulin⁺* cells are highly refractory to transformation by multiple oncogenic mutations.

We next set out to test the effect of chronic pancreatitis on PDAC formation in the *RipCreER^{TM};LSL-Kras^{G12D}* mice. Two *RipCreER^{TM};LSL-Kras^{G12D};LSL-LacZ* mice were treated with caerulein for a total of 84 and 89 days. One month after caerulein treatment initiation, the mice were treated with TM to activate Cre. Interestingly, grade 1 mPanINs were detected in one of these mice (Fig. 5B and Table S4). We confirmed *LSL-Kras^{G12D}* recombination in the pancreas of the mouse that did not show mPanIN formation as well as in mPanINs derived from the second mouse by PCR analysis of DNA extracted from tissue section and of DNA extracted from LCM lesions, respectively (Fig. 5C and Table S4). Hence, the mPanINs observed arose from cells in which the transgenic Rat insulin promoter was active. Importantly, all three *RipCreER^{TM};LSL-Kras^{G12D}; p53$^{flox/flox}$;LSL-LacZ* mice developed poorly- and undifferentiated PDAC (N=2 and N=1, respectively) when treated first with caerulein and then with TM (Fig. S3D and Table S4). As in the *Pdx1CreER^{TM};LSL-Kras^{G12D};p53$^{flox/flox}$* mutant mice, these were highly aggressive tumors that exhibited a capacity for local invasion and distant metastasis.

One interpretation of these results (as well as those of Guerra et al. (2007)) is that caerulein treatment or inflammation caused a change in differentiated cells that made them susceptible to K-Ras activation. To investigate this possibility, we crossed the *RipCreER^{TM};LSL-Kras^{G12D}* mice to the *LacZ* reporter strain and pulsed the triple compound progeny of these mice with TM 7-14 days prior to caerulein treatment initiation (N=6) (Table S4). In this way, differentiated insulin⁺ cells and their progeny would be marked by the LacZ expression and their fate could be identified after caerulein treatment by X-gal staining (Fig. 5D). Strikingly, LacZ⁺ mPanINs were found in 4/6 pancreata of these mice (Fig. 6A, and Table S4). In the remaining 2 mice mPanINs were not observed, which might be explained by the small pancreatic area analyzed (less than 2 mm², Table S6). In addition, 2 mice developed LacZ⁺ poorly to undifferentiated tumors with local invasion to adjacent tissues. These results suggest that Kras^{G12D} expressing insulin⁺ cells can be transformed and give rise to mPanINs and PDAC specifically in the context of chronic pancreatitis.

To explore the possibility of non-specific Cre recombinase activity upon caerulein-induced injury, *RipCreER^{TM};LSL-Kras^{G12D};LSL-LacZ* mice (N=5) were treated with caerulein without prior TM administration. Surprisingly, despite the absence of TM, LacZ⁺ cells were observed in the islets of Langerhans and in scattered cells throughout the parenchyma as early as 2 days after caerulein treatment initiation (data not shown). Thus, although the *RipCreER^{TM}* strain is tightly controlled and does not normally show leaky expression of the

Rat insulin promoter in insulin⁻ cells or TM-independent activation of Cre recombinase (Table S1), Cre activity was more promiscuous under chronic pancreatitis conditions. This result raised the possibility that the mPanIN lesions observed in the experiment described above arose in non-insulin expressing cells following inappropriate Cre activation during the caerulein treatment. To address this possibility directly, *RipCreER^TM;LSL-LacZ* mice (N=5) were treated with TM for 5-7 days followed by caerulein treatment for 65 days and then analyzed for coexpression of LacZ and insulin by CoIF (Table S5). In all cases, LacZ⁺insulin⁺ cells were observed within the islet of Langerhans. In addition, we scored a total of 83 LacZ⁺insulin⁺ and 6 LacZ⁺insulin⁻ single cells scattered throughout the pancreata. We then calculated the frequency of mPanIN lesions per mm² in the TM/ caerulein treated *RipCreER^TM;LSL-Kras^G12D;LSL-LacZ* and *RipCreER^TM;LSL-Kras^G12D* mice. Importantly, the frequency of mPanIN lesions in these mice was 6-fold higher than the frequency of LacZ⁺insulin⁻ cells in TM/caerulein treated *RipCreER^TM;LSL-LacZ* mice (P=0.04 by Wilcoxon Rank Sum Test) (Tables S5 and S6). Thus, a minority of the mPanINs observed in the TM and caerulein treated *RipCreER^TM;LSL-Kras^G12D;LSL-LacZ* and *RipCreER^TM;LSL-Kras^G12D* mice might have arisen from insulin⁻ cells. However, based on this statistical analysis, it is likely that the vast majority of the mPanINs can be attributed to transformation of insulin⁺ cells. Thus, our results provide evidence that insulin⁺ cells can be made susceptible to Kras^G12D and give rise to mPanIN and PDAC formation following tissue injury and inflammation. Interestingly, immunohistochemistry (IHC) revealed that most mPanIN cells in this model failed to express insulin (Fig. 6B and C). Although the mechanism through which mature insulin-producing cells undergo this fate change is unclear, it is notable that these mPanINs harbored Pdx1⁺ cells and cells that positively stain for the Notch intracellular domain (NotchIC) (Fig. 6E and data not shown).

To further study transformation of insulin⁺ cells, four *RipCreER^TM;LSL-Kras^G12D;p53^flox/flox;LSL-LacZ* mice were treated with TM and 14-40 days later with caerulein for additional 42-52 days. These mice developed high-grade mPanIN and poorly differentiated to undifferentiated carcinoma capable of local invasion and distant metastasis (Fig. 7A, B and Table S4). The undifferentiated areas were morphologically identical to those observed in *Pdx1CreER^TM;LSL-Kras^G12D; Trp53^flox/flox* mice and also resembled human undifferentiated pancreatic carcinoma (Hoorens et al., 1998). Tumors in these mice were negative for insulin as well as the neuroendocrine marker synaptophysin by IHC (Fig. 7C, D). Furthermore, no preneoplastic lesions were observed in the islets of these mice (data not shown). In conclusion, our results provide evidence that in combination with pancreatic injury, Kras^G12D-expressing insulin⁺ cells of the endocrine lineage, which are refractory to transformation under normal conditions, can also serve as a cell-of-origin of PDAC, a malignancy with an exocrine phenotype.

Discussion

We have studied the susceptibility of adult pancreatic cells to transformation by Kras^G12D. We show that *Pdx1⁺* cells can efficiently give rise to mPanIN and ductal metaplasia and, in combination with *p53* or *Ink4A/Arf* deletion, can develop into PDAC. In contrast, in the absence of tissue injury and inflammation, *insulin⁺* cells (which also express *Pdx1*) did not show any overt phenotype, even in the context of tumor suppressor gene mutation. Likewise, *proCPA1⁺* cells were inefficiently transformed. Thus, we conclude that under non-inflammatory conditions, a subpopulation of *Pdx1⁺* cells of the adult pancreas have heightened sensitivity to the Kras^G12D oncogenic effects and represent a cell-of-origin of PDAC. Of note, Habbe et al. (2008) have reported that *Pdx1⁺* cells are relatively resistant to Kras^G12D-induced transformation in 42 day-old mice. Our data suggest that younger mice are more susceptible to transformation of this cell type. It is also possible that the different

observations are attributable to background differences between the mice used in both studies.

Four Cre lines have been used to target the expression of oncogenic K-Ras to adult acinar cells: *K-Ras$^{+/LSLG12Vgeo}$*(KRas^{G12V}); *Elas-tTA/tetO-Cre*, (Guerra et al., 2007); *Ela-CreERT2$^{Tg/+}$* and *Mist1$^{CreERT2/+}$* (Habbe et al., 2008); *ElaCreERT* (De La O et al., 2008) and the *proCPA1CreER^{T2}* studied here. Different phenotypes, ranging from no lesion to high-grade mPanIN-3 formation, were observed in these strains (Table S7). The phenotypic diversity might be explained by several factors, including: 1) The *K-Ras$^{+/LSLG12Vgeo}$* strain lacks the 3′ UTR element which contains several regulatory sequences, including binding sites for the let-7 miRNA family that regulates both N-Ras and K-Ras (Johnson et al., 2005) and has been suggested to function in tumor suppression (Lee and Dutta, 2007; Mayr et al., 2007; Takamizawa et al., 2004; Yu et al., 2007). Thus, the more abundant lesion formation in the 10 day-old *K-Ras$^{+/LSLG12Vgeo}$; Elas-tTA/tetO-Cre* mice compared to the *proCPA1CreER^{T2};LSL-Kras^{G12D}* may result from more tightly controlled translational repression of Kras^{G12D} versus K-Ras$^{(G12V)}$. 2) The models utilize distinct *K-ras* activating mutations (G12V versus G12D) which might have an effect on the oncogenic potency of K-Ras (Bardeesy and Sharpless, 2006; Collado et al., 2005; Seeburg et al., 1984). 3) Recombination efficiency differences between the different strains may exist, either because of differences between Cre-driving promoters per se, mouse background, or different TM and doxycycline administration protocols. 4) The different mouse models may reflect a previously uncharacterized heterogeneity of acinar cells with respect to function and/or susceptibility to transformation. Nevertheless, the collective observations provide evidence that there are acinar cells in the adult pancreas sensitive to oncogenic K-Ras activation that can progress to at least mPanIN in the absence of overt tissue injury and that highly aggressive PDAC can be initiated from *proCPA1^{+}* cells, albeit with low penetrance.

When injury was induced before recombination, the *proCPA1CreER^{T2};LSL-Kras^{G12D}* and *RipCreER^{TM};LSL-Kras^{G12D}* mice became sensitive to Kras^{G12D} activation. Importantly, when we activated Kras^{G12D} first and then induced chronic pancreatitis, *RipCreER^{TM};LSL-Kras^{G12D}* mice still developed mPanINs. We found that under caerulein-only treatment conditions Cre recombinase activity was not detectable in the *proCPA1CreER^{T2}* strain, although, surprisingly, it could be detected in insulin^{+} and rare insulin^{-} cells in the *RipCreER^{TM}* strain. This result might reflect a differential sensitivity to caerulein of CreER^{T2} (Indra et al., 1999) in the *proCPA1CreER^{T2}* versus CreER™ (Danielian et al., 1998) in the *RipCreER^{TM}* strain. Alternatively, an increased amount of Cre protein might be produced by the *RipCreER^{TM}* transgene in comparison with the *proCPA1CreER^{T2}* knockin strain that might result in its greater sensitivity to caerulein. Regardless of the effects of caerulein, the vast majority of the mPanINs can be attributed to transformation of insulin^{+} cells, although it is possible that a few mPanINs are derived from insulin^{-} cells as well. Furthermore, 2/6 of the *RipCreER^{TM};LSL-Kras^{G12D};LSL-LacZ* and all *RipCreER^{TM};LSL-Kras^{G12D};p53$^{flox/flox}$;LSL-LacZ* mice (N=4) developed high grade mPanIN and tumors that resembled the most aggressive PDAC that arose in *Pdx1CreER^{TM}* and *proCPA1CreER^{T2}* compound mice. These tumors exhibited no morphologic or immunohistochemical evidence of neuroendocrine differentiation. In addition, the pre-invasive changes in these mice were of an exocrine phenotype (i.e. mPanIN) rather than endocrine. Taken together, these results show that insulin^{+} cells of the endocrine lineage of the adult pancreas can transdifferentiate and give rise to highly aggressive exocrine neoplasia (PDAC). Of note, LacZ^{+} cells were not detected in ductal metaplasia in TM/caerulein treated *RipCreER^{TM};LSL-LacZ* mice (data not shown), suggesting that the transdifferentiation of the insulin^{+} cells requires both injury and oncogenic K-Ras. Similar results were observed by Strobel et al. in *RipCreER^{TM};Z/AP* mice (Strobel et al., 2007). Interestingly, oncogenic K-Ras activation is rarely observed in human

endocrine tumors (Jonkers et al., 2007). This may in part be explained by reprogramming of endocrine cells to an exocrine fate upon injury and K-Ras activation.

The transdifferentiated cells in the *RipCreER^{TM};LSL-Kras^{G12D}* mice may be insulin$^+$ cells that reside either in and/or outside the islets of Langerhans. The physiological role of the insulin$^+$ cells that reside outside of the islets is largely unknown. However, the mPanINs in the caerulein+TM-treated mice were always observed outside the islets suggesting that these cells may be more susceptible to Kras^{G12D} than those that reside in the islets. Interestingly, neoplastic ducts were found within the islets of TM-treated *Pdx1CreER^{TM};LSL-Kras^{G12D}* mice but not when Kras^{G12D} was activated during development using the non-conditional *Pdx1Cre* strain (Hingorani et al., 2003). This may reflect a developmental compensation mechanism or result from mouse background differences.

Caerulein might promote Kras^{G12D}-induced transformation by several mechanisms. It is possible that a rare population of *proCPA1*- and/or *insulin*-expressing progenitor cells that are normally susceptible to Kras^{G12D} oncogenic effect needs to be amplified by tissue injury for the appearance of the phenotype. Another possibility is that injury-induced regeneration or the secretion of inflammatory cytokines leads to the proliferation of stem cell/facultative stem cells that are most probably Pdx1$^+$. Upon the initiation of differentiation of such cells, a subset might transiently express either *proCPA1CreER^{T2}* or *RipCreER^{TM}* and, thus, activate Kras^{G12D} upon TM administration. However, our results provide evidence that *proCPA1*- and insulin-expressing cells can form mPanINs, favoring the hypothesis that inflammation and tissue injury facilitate PDAC development by promoting reprogramming of differentiated cells rather than by stem cell mobilization. The mechanism through which such a reprogramming event might occur requires further investigation. However, our study and those of others suggest a role for the Notch signaling pathway in this process. Caerulein-derived acinar-ductal metaplasia is associated with Notch pathway up-regulation (Gomez et al., 2004; Jensen et al., 2005; Siveke et al., 2008). We also found Notch to be activated in mPanINs derived from Caerulein/TM –*RipCreER^{TM};LSL-Kras^{G12D}* mice. Re-activation of the Notch signaling pathway, which is important for cell fate decisions and maintenance of undifferentiated stem/progenitor cells during embryogenesis (Artavanis-Tsakonas et al., 1999), may be a mechanism by which differentiated cells can revert to a stem/ progenitor fate or to convert to a cell type that is sensitive to Kras^{G12D} activity. It has been recently shown that Notch and K-Ras reprogram acinar cells to mPanINs (De La O et al., 2008). The precise mechanism of, and requirement for, interaction between chronic pancreatitis, Notch and K-Ras activation for mPanIN formation from different pancreatic cells still need to be determined.

These data add to the increasing body of evidence pointing to a remarkable plasticity of pancreatic adult differentiated cells (De La O et al., 2008; Habbe et al., 2008; Shen et al., 2000; Zhou et al., 2008) and questions the existence of a pancreatic "stem cell" as well as its relevance for PDAC. Our findings suggest that Kras^{G12D} regulates the differentiation status of pancreatic epithelial progenitor/differentiated cells to a certain extent and that in combination with non-genetic stress such as inflammation, this is further accelerated. More specifically, a multipotent *Pdx1$^+$ Insulin$^-$* cell may exist in the adult pancreas. This cell type may be the source of the endocrine cells within mPanINs and ductal metaplasia observed in the TM treated *Pdx1CreER^{TM};LSL-Kras^{G12D}* mice and the endocrine cells arising from the ductal lining after pancreatic duct ligation (Xu et al., 2008). It is possible that this cell type is activated only in certain settings such as injury and neoplasia and may be particularly susceptible to Kras^{G12D}-induced proliferation and differentiation. It might also be the source of the ductal structures located in the islets, if it resides both in the ductal lining as well as the islets or if it migrates to the islets under certain conditions. Alternatively, these structures may arise from a differentiated cell, such as the carbonic anhydrase II$^+$ cell that give rise to

endocrine and exocrine cells after birth and injury (Inada et al., 2008), upon Kras[G12D] activation.

Our study reveals the involvement of different cell types in PDAC initiation and provides a series of models to explore the biology and treatment of PDAC. With the advances in the ability to reprogram adult pancreatic cells in a controlled manner (Zhou et al., 2008), we expect that a better understanding of the reprogramming and transforming events attributed to Kras[G12D] activation in combination with chronic inflammation will lead to the development of new therapeutic modalities to prevent PanIN formation or to repair the diseased/damaged tissue.

Experimental Procedures

Mouse strains

All animal studies and procedures were approved by the MIT Institutional Animal Care and Use Committee. Research was conducted in compliance with the Animal Welfare Act Regulations and other Federal statutes relating to animals and experiments involving animals and adheres to the principles set forth in the Guide for Care and Use of Laboratory Animals, National Research Council, 1996. The *LSL-Kras^{G12D}* strain (Jackson et al., 2001) was crossed to the following strains: *Pdx1CreER^{TM}* (Gu et al., 2002), *proCPA1CreER^{T2}* (Zhou et al., 2007), *RipCreER^{TM}* (Dor et al., 2004), *Trp53^{flox}* (Jonkers et al., 2001) and *Ink4A/Arf^{flox}* (Aguirre et al., 2003). Primers used for genotyping by PCR are listed in Table S8 and details of reactions are available upon request. Cre strains were crossed to *R26-LSL-LacZ* mice (Jackson Laboratories) to determine Cre expression patterns.

Histopathology, Immunohistochemistry (IHC) and immunofluorescence (IF)

Histopathologic analysis of pancreata was carried out by two pathologists (G.C.C. and E.L.S.). mPanIN lesions and PDAC were graded according to consensus criteria for mouse models of pancreatic cancer (Hruban et al., 2006). We carried out IHC and IF analyses according to manufacturer's recommendations, typically using a modified citric acid unmasking protocol followed by standard detection with 3,3-diaminobenzidine (DAB) using a kit from Vector Laboratories. In some cases, secondary antibodies were conjugated to AlexaFluor 594 (Invitrogen) and nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI). We used the following primary antibodies: insulin (Zymed), glucagon (Abcam), PYY (RDI), Pdx1 (Gift of Dr. Christopher V Wright), CPA1 (Chemicon) and β-galactosidase (AbD Serotec). IF staining was performed as described (Jackson et al., 2001). Triple-color microscopy and imaging were performed with a Nikon Eclipse E600 and a Spot cooled CCD camera and software. Images were processed with Adobe Photoshop.

β-Galactosidase histochemistry

Pancreata were dissected and fixed in 4% PFA, incubated in 0.5M Sucrose and frozen in OCT. β-galactosidase activity was assayed as describe (Paratore et al., 2002).

Tamoxifen treatment

Mice were treated with TM (Sigma) by intraperitoneal injections (I.P.) of 9mg/40g body weight in corn oil, one every other day for a total of 3 injections. For oral delivery, TM was dissolved in a 0.5% Carboxymethyl cellulose, 0.4% Tween 80, 0.9% NaCl solution.

Caerulein treatment

Mice were I.P. injected with 0.1 ml of a 50μg/ml Caerulein (Sigma) in saline solution 5 times per week.

Pancreata size measurement

Pancreata areas were determined using Bioquant Image Analysis software in manual measurement mode.

Molecular analysis

For verification of Cre-mediated recombination, DNA was prepared from wild-type tails and LCM mPanINs of Caerulein &TM treated *proCPA1CreER^T2^;LSL-Kras^G12D^* and *RipCreER^TM^;LSL-Kras^G12D^* mice. PCR was performed with primers flanking the Lox–Stop–Lox cassette (sequences available in Table S8 and on http://web.mit.edu/jacks-lab/protocols_table.html). Wild-type *K-ras, 2Lox and 1Lox -K-ras^G12D^* alleles were detected, yielding 620-bp, 510-bp and ~1100-bp products, respectively. LCM and DNA isolation were performed using the Veritas Microdissection System and the PicoPure™ DNA Extraction Kit from Molecular Devices, respectively.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

Aguirre AJ, Bardeesy N, Sinha M, Lopez L, Tuveson DA, Horner J, Redston MS, DePinho RA. Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. Genes Dev 2003;17:3112–3126. [PubMed: 14681207]

Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. Science 1999;284:770–776. [PubMed: 10221902]

Bardeesy N, DePinho RA. Pancreatic cancer biology and genetics. Nat Rev Cancer 2002;2:897–909. [PubMed: 12459728]

Bardeesy N, Sharpless NE. RAS unplugged: negative feedback and oncogene-induced senescence. Cancer Cell 2006;10:451–453. [PubMed: 17157783]

Berman DM, Karhadkar SS, Maitra A, Montes De Oca R, Gerstenblith MR, Briggs K, Parker AR, Shimada Y, Eshleman JR, Watkins DN, Beachy PA. Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. Nature 2003;425:846–851. [PubMed: 14520411]

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 2007;104:4437–4442. [PubMed: 17360542]

Collado M, Gil J, Efeyan A, Guerra C, Schuhmacher AJ, Barradas M, Benguria A, Zaballos A, Flores JM, Barbacid M, et al. Tumour biology: senescence in premalignant tumours. Nature 2005;436:642. [PubMed: 16079833]

Danielian PS, Muccino D, Rowitch DH, Michael SK, McMahon AP. Modification of gene activity in mouse embryos in utero by a tamoxifen-inducible form of Cre recombinase. Curr Biol 1998;8:1323–1326. [PubMed: 9843687]

De La O JP, Emerson LL, Goodman JL, Froebe SC, Illum BE, Curtis AB, Murtaugh LC. Notch and Kras reprogram pancreatic acinar cells to ductal intraepithelial neoplasia. Proc Natl Acad Sci U S A 2008;105:18907–18912. [PubMed: 19028876]

Dor Y, Brown J, Martinez OI, Melton DA. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature 2004;429:41–46. [PubMed: 15129273]

Gomez G, Englander EW, Wang G, Greeley GH Jr. Increased expression of hypoxia-inducible factor-1alpha, p48, and the Notch signaling cascade during acute pancreatitis in mice. Pancreas 2004;28:58–64. [PubMed: 14707731]

Grippo PJ, Nowlin PS, Demeure MJ, Longnecker DS, Sandgren EP. Preinvasive pancreatic neoplasia of ductal phenotype induced by acinar cell targeting of mutant Kras in transgenic mice. Cancer Res 2003;63:2016–2019. [PubMed: 12727811]

Gu G, Dubauskaite J, Melton DA. Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. Development 2002;129:2447–2457. [PubMed: 11973276]

Guerra C, Schuhmacher AJ, Canamero M, Grippo PJ, Verdaguer L, Perez-Gallego L, Dubus P, Sandgren EP, Barbacid M. Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. Cancer Cell 2007;11:291–302. [PubMed: 17349585]

Habbe N, Shi G, Meguid RA, Fendrich V, Esni F, Chen H, Feldmann G, Stoffers DA, Konieczny SF, Leach SD, Maitra A. 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 2008;105:18913–18918. [PubMed: 19028870]

Hingorani SR, Petricoin EF, Maitra A, Rajapakse V, King C, Jacobetz MA, Ross S, Conrads TP, Veenstra TD, Hitt BA, et al. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell 2003;4:437–450. [PubMed: 14706336]

Hoorens A, Prenzel K, Lemoine NR, Kloppel G. Undifferentiated carcinoma of the pancreas: analysis of intermediate filament profile and Ki-ras mutations provides evidence of a ductal origin. J Pathol 1998;185:53–60. [PubMed: 9713360]

Hruban RH, Adsay NV, Albores-Saavedra J, Anver MR, Biankin AV, Boivin GP, Furth EE, Furukawa T, Klein A, Klimstra DS, et al. Pathology of genetically engineered mouse models of pancreatic exocrine cancer: consensus report and recommendations. Cancer Res 2006;66:95–106. [PubMed: 16397221]

Hruban RH, Adsay NV, Albores-Saavedra J, Compton C, Garrett ES, Goodman SN, Kern SE, Klimstra DS, Kloppel G, Longnecker DS, et al. Pancreatic intraepithelial neoplasia: a new nomenclature and classification system for pancreatic duct lesions. Am J Surg Pathol 2001;25:579–586. [PubMed: 11342768]

Inada A, Nienaber C, Katsuta H, Fujitani Y, Levine J, Morita R, Sharma A, Bonner-Weir S. Carbonic anhydrase II-positive pancreatic cells are progenitors for both endocrine and exocrine pancreas after birth. Proc Natl Acad Sci U S A 2008;105:19915–19919. [PubMed: 19052237]

Indra AK, Warot X, Brocard J, Bornert JM, Xiao JH, Chambon P, Metzger D. Temporally-controlled site-specific mutagenesis in the basal layer of the epidermis: comparison of the recombinase activity of the tamoxifen-inducible Cre-ER(T) and Cre-ER(T2) recombinases. Nucleic Acids Res 1999;27:4324–4327. [PubMed: 10536138]

Jackson EL, Willis N, Mercer K, Bronson RT, Crowley D, Montoya R, Jacks T, Tuveson DA. Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. Genes Dev 2001;15:3243–3248. [PubMed: 11751630]

Jaffee EM, Hruban RH, Canto M, Kern SE. Focus on pancreas cancer. Cancer Cell 2002;2:25–28. [PubMed: 12150822]

Jensen JN, Cameron E, Garay MV, Starkey TW, Gianani R, Jensen J. Recapitulation of elements of embryonic development in adult mouse pancreatic regeneration. Gastroenterology 2005;128:728–741. [PubMed: 15765408]

Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert KL, Brown D, Slack FJ. RAS is regulated by the let-7 microRNA family. Cell 2005;120:635–647. [PubMed: 15766527]

Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 2008;321:1801–1806. [PubMed: 18772397]

Jonkers J, Meuwissen R, van der Gulden H, Peterse H, van der Valk M, Berns A. Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. Nat Genet 2001;29:418–425. [PubMed: 11694875]

Jonkers YM, Ramaekers FC, Speel EJ. Molecular alterations during insulinoma tumorigenesis. Biochim Biophys Acta 2007;1775:313–332. [PubMed: 17572302]

Koizumi M, Doi R, Toyoda E, Masui T, Tulachan SS, Kawaguchi Y, Fujimoto K, Gittes GK, Imamura M. Increased PDX-1 expression is associated with outcome in patients with pancreatic cancer. Surgery 2003;134:260–266. [PubMed: 12947327]

Lee YS, Dutta A. The tumor suppressor microRNA let-7 represses the HMGA2 oncogene. Genes Dev 2007;21:1025–1030. [PubMed: 17437991]

Li D, Xie K, Wolff R, Abbruzzese JL. Pancreatic cancer. Lancet 2004;363:1049–1057. [PubMed: 15051286]

Mayr C, Hemann MT, Bartel DP. Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. Science 2007;315:1576–1579. [PubMed: 17322030]

Mills, SE. Histology for Pathologists. third. Philadelphia: Lippincott Williams & Wilkins; 2007.

Miyamoto Y, Maitra A, Ghosh B, Zechner U, Argani P, Iacobuzio-Donahue CA, Sriuranpong V, Iso T, Meszoely IM, Wolfe MS, et al. Notch mediates TGF alpha-induced changes in epithelial differentiation during pancreatic tumorigenesis. Cancer Cell 2003;3:565–576. [PubMed: 12842085]

Paratore C, Eichenberger C, Suter U, Sommer L. Sox10 haploinsufficiency affects maintenance of progenitor cells in a mouse model of Hirschsprung disease. Hum Mol Genet 2002;11:3075–3085. [PubMed: 12417529]

Prasad NB, Biankin AV, Fukushima N, Maitra A, Dhara S, Elkahloun AG, Hruban RH, Goggins M, Leach SD. Gene expression profiles in pancreatic intraepithelial neoplasia reflect the effects of Hedgehog signaling on pancreatic ductal epithelial cells. Cancer Res 2005;65:1619–1626. [PubMed: 15753353]

Rozenblum E, Schutte M, Goggins M, Hahn SA, Panzer S, Zahurak M, Goodman SN, Sohn TA, Hruban RH, Yeo CJ, Kern SE. Tumor-suppressive pathways in pancreatic carcinoma. Cancer Res 1997;57:1731–1734. [PubMed: 9135016]

Seeburg PH, Colby WW, Capon DJ, Goeddel DV, Levinson AD. Biological properties of human c-Ha-ras1 genes mutated at codon 12. Nature 1984;312:71–75. [PubMed: 6092966]

Shen CN, Slack JM, Tosh D. Molecular basis of transdifferentiation of pancreas to liver. Nat Cell Biol 2000;2:879–887. [PubMed: 11146651]

Shields JM, Pruitt K, McFall A, Shaub A, Der CJ. Understanding Ras: 'it ain't over 'til it's over'. Trends Cell Biol 2000;10:147–154. [PubMed: 10740269]

Siveke JT, Lubeseder-Martellato C, Lee M, Mazur PK, Nakhai H, Radtke F, Schmid RM. Notch signaling is required for exocrine regeneration after acute pancreatitis. Gastroenterology 2008;134:544–555. [PubMed: 18242220]

Slack JM. Developmental biology of the pancreas. Development 1995;121:1569–1580. [PubMed: 7600975]

Stanger BZ, Stiles B, Lauwers GY, Bardeesy N, Mendoza M, Wang Y, Greenwood A, Cheng KH, McLaughlin M, Brown D, et al. Pten constrains centroacinar cell expansion and malignant transformation in the pancreas. Cancer Cell 2005;8:185–195. [PubMed: 16169464]

Stoffers DA, Heller RS, Miller CP, Habener JF. Developmental expression of the homeodomain protein IDX-1 in mice transgenic for an IDX-1 promoter/lacZ transcriptional reporter. Endocrinology 1999;140:5374–5381. [PubMed: 10537169]

Strobel O, Dor Y, Stirman A, Trainor A, Fernandez-del Castillo C, Warshaw AL, Thayer SP. Beta cell transdifferentiation does not contribute to preneoplastic/metaplastic ductal lesions of the pancreas by genetic lineage tracing in vivo. Proc Natl Acad Sci U S A 2007;104:4419–4424. [PubMed: 17360539]

Swift GH, Liu Y, Rose SD, Bischof LJ, Steelman S, Buchberg AM, Wright CV, MacDonald RJ. An endocrine-exocrine switch in the activity of the pancreatic homeodomain protein PDX1 through formation of a trimeric complex with PBX1b and MRG1 (MEIS2). Mol Cell Biol 1998;18:5109–5120. [PubMed: 9710595]

Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, Harano T, Yatabe Y, Nagino M, Nimura Y, et al. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res 2004;64:3753–3756. [PubMed: 15172979]

Thayer SP, di Magliano MP, Heiser PW, Nielsen CM, Roberts DJ, Lauwers GY, Qi YP, Gysin S, Fernandez-del Castillo C, Yajnik V, et al. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. Nature 2003;425:851–856. [PubMed: 14520413]

Tuveson DA, Zhu L, Gopinathan A, Willis NA, Kachatrian L, Grochow R, Pin CL, Mitin NY, Taparowsky EJ, Gimotty PA, et al. Mist1-KrasG12D knock-in mice develop mixed differentiation metastatic exocrine pancreatic carcinoma and hepatocellular carcinoma. Cancer Res 2006;66:242–247. [PubMed: 16397237]

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 1998;115:1254–1262. [PubMed: 9797382]

Wang L, Heidt DG, Lee CJ, Yang H, Logsdon CD, Zhang L, Fearon ER, Ljungman M, Simeone DM. Oncogenic function of ATDC in pancreatic cancer through Wnt pathway activation and beta-catenin stabilization. Cancer Cell 2009;15:207–219. [PubMed: 19249679]

Wu KL, Gannon M, Peshavaria M, Offield MF, Henderson E, Ray M, Marks A, Gamer LW, Wright CV, Stein R. Hepatocyte nuclear factor 3beta is involved in pancreatic beta-cell-specific transcription of the pdx-1 gene. Mol Cell Biol 1997;17:6002–6013. [PubMed: 9315659]

Xu X, D'Hoker J, Stange G, Bonne S, De Leu N, Xiao X, Van De Casteele M, Mellitzer G, Ling Z, Pipeleers D, et al. Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 2008;132:197–207. [PubMed: 18243096]

Yoshida T, Hanahan D. Murine pancreatic ductal adenocarcinoma produced by in vitro transduction of polyoma middle T oncogene into the islets of Langerhans. Am J Pathol 1994;145:671–684. [PubMed: 7521578]

Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, Huang Y, Hu X, Su F, Lieberman J, Song E. let-7 regulates self renewal and tumorigenicity of breast cancer cells. Cell 2007;131:1109–1123. [PubMed: 18083101]

Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature 2008;455:627–632. [PubMed: 18754011]

Zhou Q, Law AC, Rajagopal J, Anderson WJ, Gray PA, Melton DA. A multipotent progenitor domain guides pancreatic organogenesis. Dev Cell 2007;13:103–114. [PubMed: 17609113]
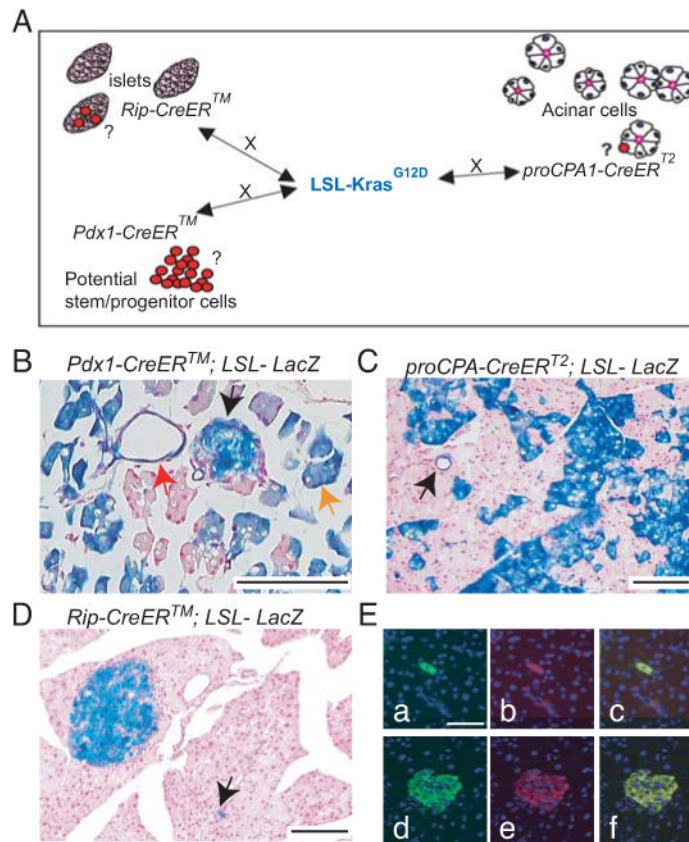
Figure 1. Kras^{G12D} activation in distinct cell populations of the adult pancreas

A: Diagram describing the different mouse strains used in the study. The *LSL-Kras^{G12D}* strain was crossed to the insulin$^+$ cells specific *RipCreER^{TM}* strain (Dor et al., 2004); to the acinar *proCPA1CreER^{T2}* strain (Zhou et al., 2007) and to the putative stem/ progenitor cell specific *Pdx1CreER^{TM}* strain (Gu et al., 2002).

B-D: X-gal stained pancreata of TM-treated *Pdx1CreER^{TM};LSL-LacZ* (B), *proCPA1CreER^{T2};LSL-LacZ* (C), *RipCreER^{TM};LSL-LacZ* (D). Note specific recombination in islet (black arrow), acinar (orange arrow) and ductal (red arrow) cells (B), acinar cells and a duct (C and arrow in C, respectively) and in the islets of Langerhans and scattered extrainsular endocrine cells (D and arrow in D, respectively).

E: LacZ and insulin CoIF staining of pancreas derived from a TM-treated *RipCreER^{TM};LSL-LacZ*. Note double staining of a single cell (top row) and of cells within the islets of Langerhan (lower row) (a)+(d): Insulin, (b)+(e) LacZ, (c)+(f) merge
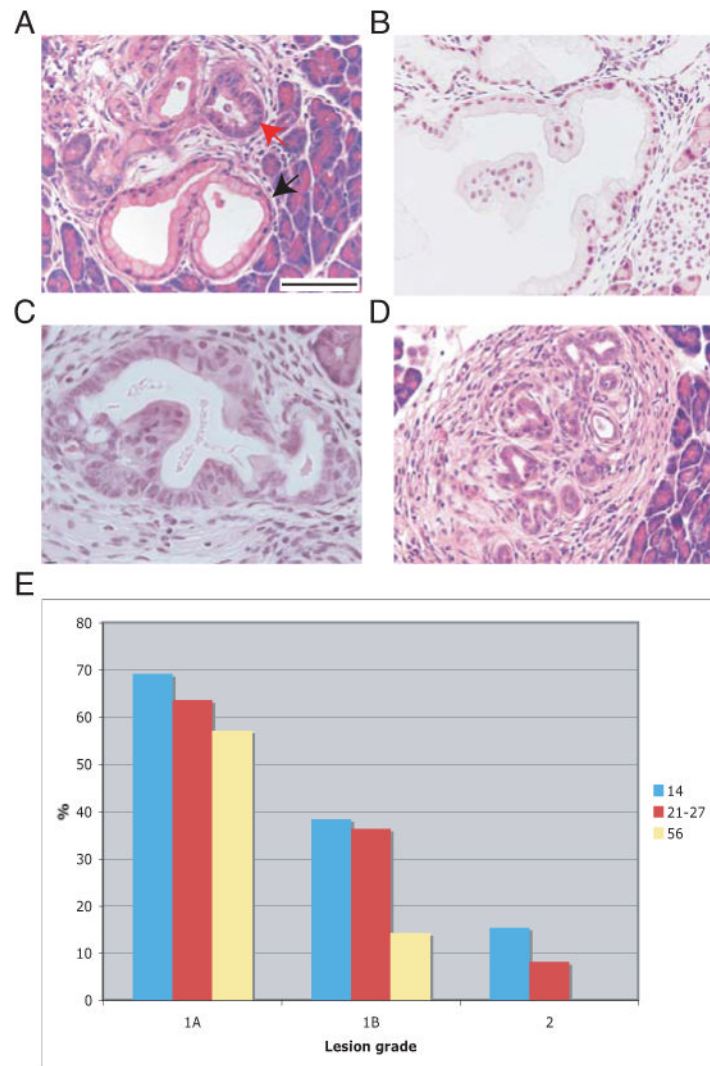
Bars: 100 µm.

Figure 2. Kras^{G12D} activation by *Pdx1CreER^{TM}* causes mPanIN formation and ductal metaplasia
A-D: H&E-stained sections of *Pdx1CreER^{TM};LSL-Kras^{G12D}* mice 20 (A) and 120 (B-D) days after TM administration. A. mPanIN1A (black arrow). Note flat columnar epithelium with mucin accumulation and basally oriented nuclei. Red arrow: ductal metaplasia. B. mPanIN1B. Note papillary architecture with small, basally oriented nuclei. C. mPanIN2. Note papillary architecture, moderate nuclear pleomorphism and partial loss of polarity. D. Ductal metaplasia. Bars: 100 μm.
E: Percentage of mice that developed different grades of mPanINs upon Kras^{G12D} activation at the described postnatal dates
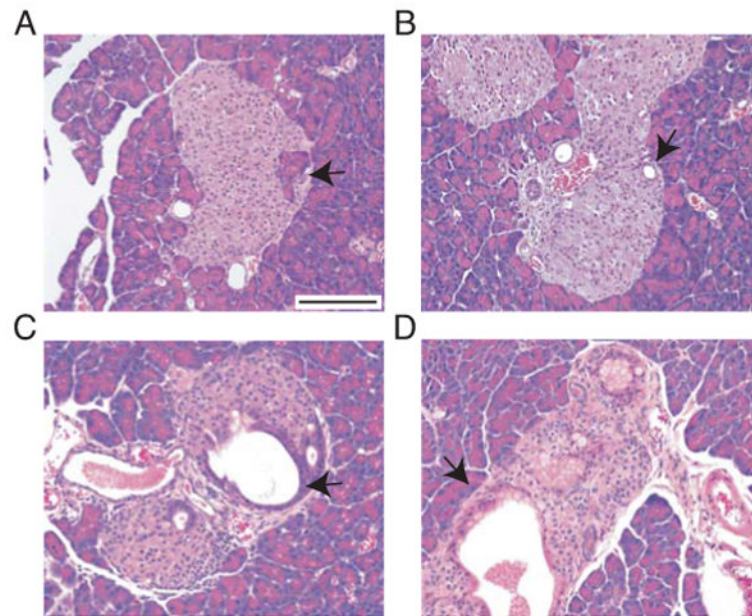
Figure 3. Kras^{G12D} activation by *Pdx1CreER^{TM}* causes intra-islet ductal/ acinar lesions
A-D: H&E-stained sections of *Pdx1CreER^{TM};LSL-Kras^{G12D}* mice 10 (A, B) 20 (C) and 40 (D) days after TM administration. Acinar cells (arrow in A) and small ducts (arrow in B) embedded within the islets. Enlarged ducts within the islets (arrow in C). Mucin-producing elongated cells in ductal structures in the islets (arrow in D).
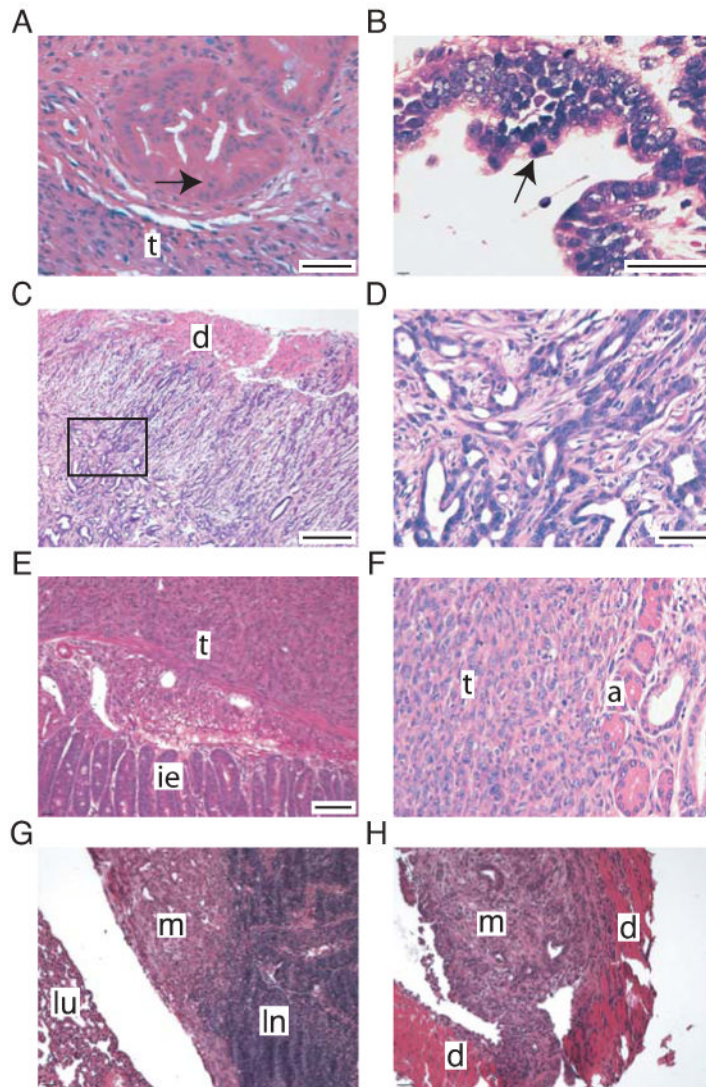Bars: 50 μm.

Figure 4. Kras^{G12D} activation in combination with p53 or Ink4A/Arf loss in *Pdx1*$^+$ cells of the adult pancreas results in mPanIN3 and PDAC development

A-E, G-H: H&E-stained sections of mPanIN3 and PDACs derived from *Pdx1CreER^{TM};LSL-Kras^{G12D}; Trp53$^{flox/flox}$* mice.

A-B: mPanIN3 lesions. Note adjacent tumor (t) and high grade features, including cribriform architecture (A) and apical mitosis (B). Arrows point to mitotic figures.

C-D: Invasive, moderately differentiated PDAC (Note invasion through the duodenal wall (d) in C). D: enlarged box in C.

E: Undifferentiated PDAC. Tumor (t) invades submucosa of the small intestine and abuts intestinal epithelium (ie).

F: H&E-stained section of undifferentiated PDAC (t) and residual normal acini (a) derived from a *Pdx1CreER^{TM};LSL-Kras^{G12D}; Ink4A/Arf$^{flox/flox}$* mouse.

G-H: Metastatic PDAC. G: Metastasis (m) to mediastinal lymph node (ln) with adjacent lung (lu). H: Metastasis to diaphragm (d).
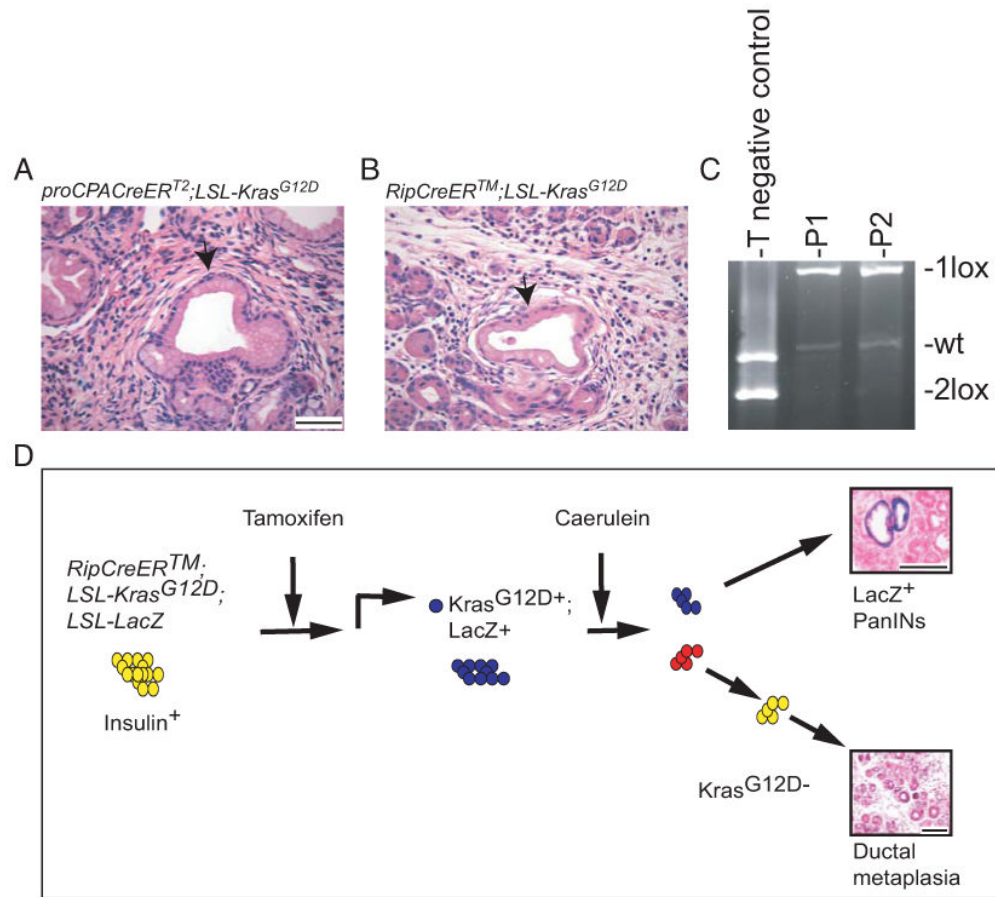
Bars: A, B: 100 μm; C 200 μm; D, F 50 μm; E,G,H: 200 μm.

Figure 5. Chronic pancreatitis promotes mPanIN formation in mouse models largely refractory to Kras^{G12D} activation alone

A-B: H&E staining of pancreata derived from Caerulein and TM-treated *proCPA1CreER^{T2};LSL-Kras^{G12D}* (A) and *RipCreER^{TM};LSL-Kras^{G12D}* mice (B). Note mPanIN formation (arrows). Bars: 50 μm

C: PCR of tail (T) and laser captured microdissected mPanINs DNA from Caerulein+ TM-treated *proCPA1CreER^{T2};LSL-Kras^{G12D}* (P1) and *RipCreER^{TM};LSL-Kras^{G12D}* (P2) mice. The recombined allele (1lox) is present in the mPanINs DNA but not in the tail DNA of compound mutant mice.

D: Suggested model of Caerulein/ inflammation–induced mPanIN formation in TM-treated *RipCreER^{TM};LSL-Kras^{G12D};LSL-LacZ* mice. TM administration results in recombination-induced activation of Kras^{G12D} and LacZ expression in insulin$^+$ cells of the adult mouse. This recombination is permanent and marks both the cells and their progeny. Upon caerulein treatments, if insulin$^+$ cells are targeted for transformation by Kras^{G12D}, they will give rise to LacZ$^+$ mPanINs. Otherwise, only LacZ$^-$ caerulein-induced metaplasia will form. Bars: A, B 50 μm; D, 100 μm.
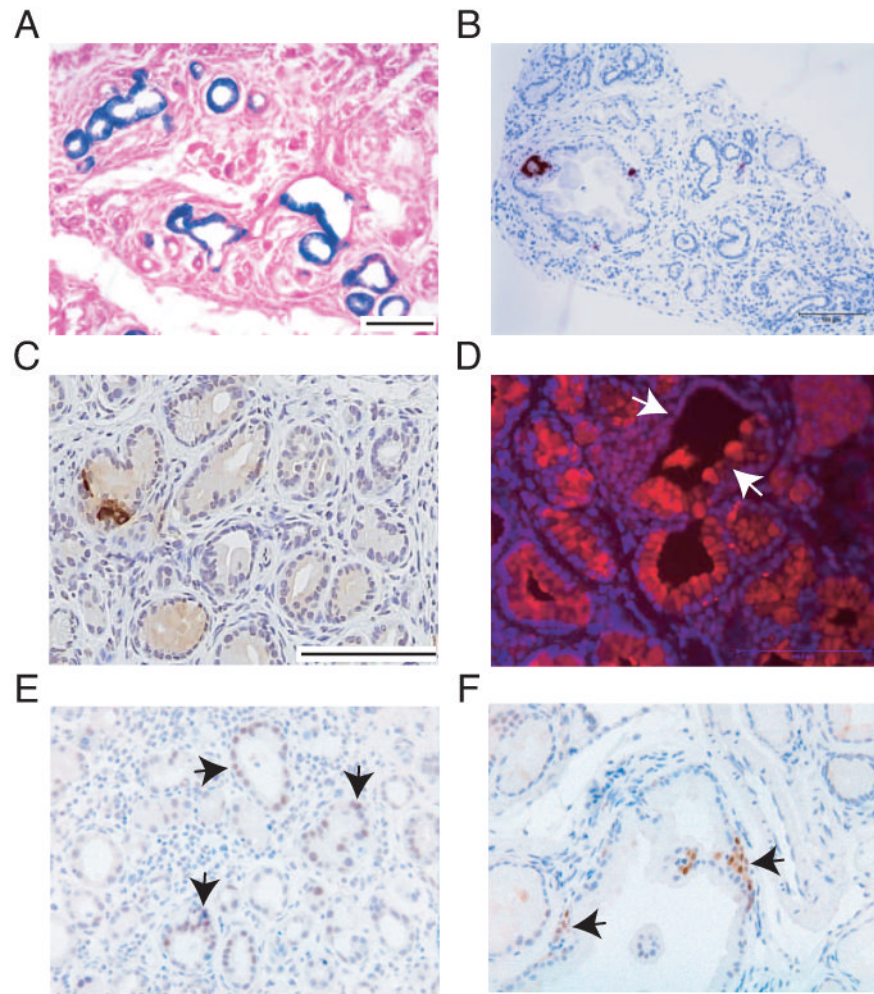
Figure 6. Characterization of mPanINs derived from TM and then caerulein treated *proCPA1CreER^T2;LSL-Kras^G12D* and *RipCreER^TM;LSL-Kras^G12D* mice
A-C, E: X-gal (A) insulin (B, C) and Pdx1 (E) staining of pancreata derived from *RipCreER^TM;LSL-LacZ;LSL-Kras^G12D* mice. Note X-gal and Pdx1 positive staining in mPanINs (A and black arrows in E, repectively) and negative staining for insulin in most mPanINs (B-C)
D, F: IF staining for CPA1 and IHC staining for Pdx1 of pancreata derived from *proCPA1CreER^T2;LSL-Kras^G12D* mice. Note positive and negative CPA1 staining in the same mPanIN (white arrows in D) and positive staining for Pdx1 (black arrows in F).
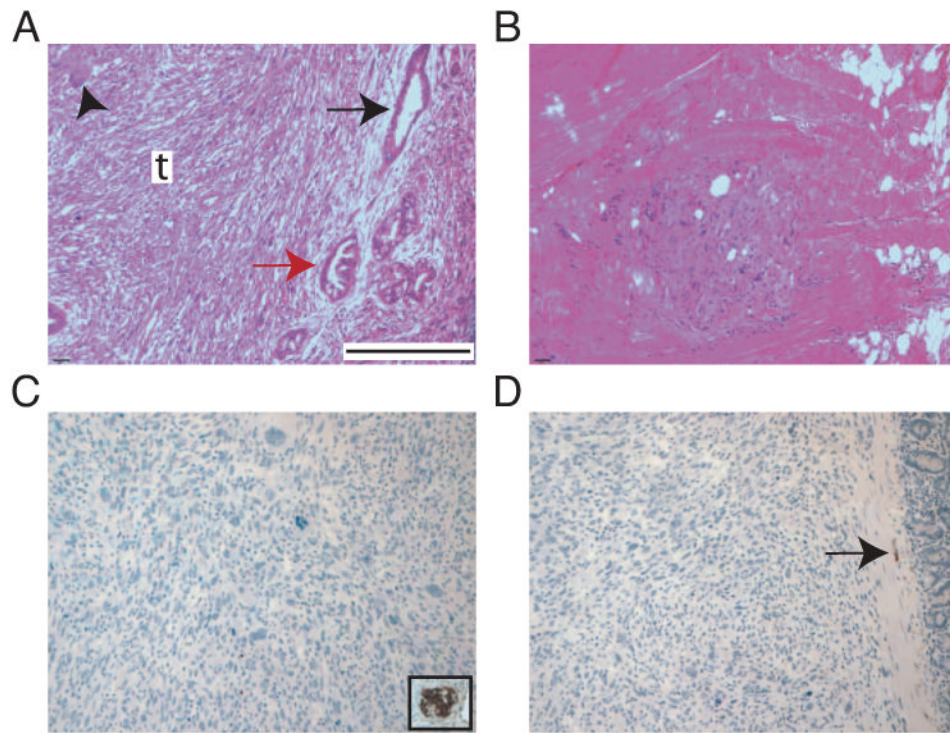Bars: A, B 100 μm; C-F 100 μm.

Figure 7. Kras^G12D activation and p53 loss followed by chronic pancreatitis results in mPanIN2-3 and PDAC development from insulin+ cells

A-D: Pancreata derived from *RipCreER^TM;LSL-Kras^G12D; Trp53^flox/flox* mice treated with TM followed by caerulein.

A: Multiple mPanINs (grade 2-3, black and red arrows, respecively) and undifferentiated carcinoma (t). Arrowhead: giant cell of invasive carcinoma.

B: Metastatic carcinoma in the diaphragm.

C: Negative immunostain for insulin in carcinoma. Inset: positive insulin immunostain in non-neoplastic islet.

D: Negative immunostain for synaptophysin in carcinoma. Note positive staining in axon located in the smooth muscle of the small intestine (arrow).

Bars: A-D: 400 μm.