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Acute pancreatitis markedly accelerates pancreatic cancer progression in mice expressing oncogenic Kras

Catherine Carrière^{1,†}, Alison L. Young¹, Jason R. Gunn¹, Daniel S. Longnecker², and Murray Korc^{1,†}

¹ Departments of Medicine, and Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH, 03755, and the Norris Cotton Comprehensive Cancer Center at Dartmouth Hitchcock Medical Center, Lebanon, NH, 03756, USA

² Department of Pathology, Dartmouth Medical School, Hanover, NH, 03755, USA

Abstract

Chronic pancreatitis increases by 16 fold the risk of developing pancreatic ductal adenocarcinoma (PDAC), one of the deadliest human cancers. It also appears to accelerate cancer progression in genetically engineered mouse models. We now report that in a mouse model where oncogenic Kras is activated in all pancreatic cell types, two brief episodes of acute pancreatitis caused rapid PanIN progression and accelerated pancreatic cancer development. Thus, a brief inflammatory insult to the pancreas, when occurring in the context of oncogenic Kras^{G12D}, can initiate a cascade of events that dramatically enhances the risk for pancreatic malignant transformation.

Keywords

Kras^{G12D}; PDAC; acute pancreatitis

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related death in the United States, with a median survival of 6 months [1]. PDAC is generally believed to arise predominantly through progression of pancreatic intraepithelial neoplasia (PanIN), ranging from low grade PanINs (termed PanIN1A, -1B) to high grade PanINs (termed PanIN-2, -3), to ductal adenocarcinoma [2]. PDAC is characterized by a high frequency of KRAS mutations at early stages and the accumulation over time of multiple additional genetic abnormalities [3]. Relevant mouse models of PDAC have been generated by targeting a conditionally mutated Kras allele (Kras^{G12D}) to early pancreatic progenitors, using Pdx1 (PK model) and Ptf1a promoters, and subsequently to all pancreatic cell types, underscoring the fact that oncogenic Kras is sufficient for PDAC initiation [4]. In these models, mouse PanIN (mPanIN) formation and progression faithfully recapitulated what is observed in human PDAC, and later in life (8–12 months old mice) these mouse models developed pancreatic cancer.

More recently, it was shown that activation of oncogenic Kras in pancreatic acinar (and in one model centroacinar as well) cells during embryogenesis was sufficient for PDAC initiation [5] [6] [7], and that subjecting the mice to chronic pancreatitis accelerated PDAC

[†]To whom correspondence may be addressed: Catherine Carriere or Murray Korc, 1 Medical Center Drive, Lebanon, NH 03756, Catherine.Carriere@Dartmouth.edu, Murray.Korc@Dartmouth.edu.

development [5]. In two mouse models, Kras oncogenic activation in adult acinar cells was sufficient for initiating PanIN formation but no pancreatic cancer was observed after one year [6;7]. However, in another model, oncogenic Kras activation in adult acinar/centroacinar cells yielded PDAC, but only when the mice were administered high doses of caerulein over many weeks resulting in a chronic pancreatitis like state with intense fibrosis and inflammatory cell infiltrates [5]. These results in mice are consistent with epidemiologic studies showing that patients suffering from chronic pancreatitis have a 16-fold increased risk of developing pancreatic cancer [8].

We now show that the PK mouse model, when subjected to two brief episodes of acute pancreatitis which do not induce chronic pancreatitis like changes, rapidly develops high grade PanINs and exhibits accelerated PDAC formation.

Material and methods

Mouse colony generation

The LSL-Kras^{G12D} (01XJ6 B6;129-Kras^{2^{tm4Tyj}}) mice were generated by D.A. Tuveson and T. Jacks [9] and obtained from MMHCC, NCI. The Pdx1-Cre mice were a gift from G. Gu [10]. All genotyping were done by PCR following the conditions of the providers.

Acute pancreatitis induction

Mice were subjected to a series of seven hourly intraperitoneal injections of caerulein that was repeated 48 hours later, based on preliminary experiments that revealed the presence of edematous pancreatitis following each series of injections [11]. Caerulein (Sigma, St. Louis MO) was diluted in 6% dextran 70, 0.9% NaCl and injected at a dose of 50 µg/kg of body weight. At least 5 compound mutant animals per experiment were injected with caerulein in parallel with five single mutant (LSL-Kras^{G12D} only or Pdx1-Cre only) and wild type mice. A second group of compound mutant and control animals received injections of carrier buffer only. All animals were fasted for 12 hours before the experiment.

Histology and immunohistochemistry

Mice were perfused with PBS then 10% formalin/PBS. The pancreata were dissected and fixed overnight. For histology and immunostaining, pancreata were processed for paraffin embedding. Routine Hematoxylin and Eosin (H&E) staining was performed using standard procedures. For immunostaining, sections were deparaffinized, rehydrated and antigens were retrieved if required using a 2100-Retriever and antigen unmasking solution (Vector Laboratories). For cytokeratin 19 (CK19) immunostaining, Proteinase K antigen retrieval procedure was used. Immunostaining procedures were as described previously [12]. The antibodies and dilution used were: TromaIII (CK19 antibody developed by Rolf Kemler and obtained from Developmental Studies Hybridoma Bank, 1:10), Muc5a (Novocastra, 1:30), Ki67 (Novocastra, 1:200), Hes1 (a gift from Dr. T. Sudo, Toray Inc., Kamakura, Japan; 1:400), Pdx1 (Upstate, 1:5000), and SMA (Abcam, 1:100).

Results

Acute pancreatitis causes lesion progression

The mouse model used in this study is the PK model where the Pdx1-driven Cre recombinase starts being expressed in early pancreatic progenitors [10]. Following Cre mediated removal of a transcriptional stop region surrounded by LoxP sites (LoxP-Stop-LoxP or LSL), a mutated Kras allele is activated in these progenitors and subsequently in all pancreatic cells types [4;13]. By 2 months of age, the PK mice develop low-grade mPanINs in limited numbers, as well as very limited areas of acinar to ductal metaplasia (ADM). An

increase in lesions number but not in grade is observed at 6 months of age in conjunction with the appearance of extensive ADM lesions. After a lag phase of 8 to 12 months, the animals occasionally develop pancreatic cancer [4].

To determine whether acute pancreatitis could accelerate mPanIN progression and PDAC formation, mutant mice were subjected to 2 episodes of acute pancreatitis induced by caerulein, a cholecystokinin (CCK) analog that binds and activates the CCK receptor [11;14]. Despite the fact that the dosage of caerulein (50 ng/g of body weight/injection, administered by 7 intra-peritoneal injections and repeated 48 hours later) that we used in our experimental settings are relatively low compared to the dosage used in other publications (from 100–400 ng/g of body weight and twice 8 injections) [15] [16], we observed acute pancreatitis, as demonstrated by the presence of marked edema a few hours following the last caerulein injection. H&E staining of pancreas sections collected at different time points shows that at 24 hours, an important inflammatory infiltrate is present and acinar cells are rapidly dying, whereas one week later complete regeneration of the pancreas is observed (not shown) as described [15]. Three cohorts of 5 to 8 PK mutant animals at 2–3 months of age were subjected to two episodes of acute pancreatitis and sacrificed 30, 60 and 80 days later. As early as 1 month following the two acute pancreatitis episodes, all caerulein-treated PK pancreata presented numerous high grade mPanINs (Fig. 1C–D) as well as extensive ADM (Fig. 1B) while untreated PK pancreata showed only isolated and low-grade lesions and limited ADM (Fig. 1A). In all caerulein-treated control animals, carrying one or no mutation, the pancreata appeared to be normal with occasional inflammatory cells present at 1-month post treatment (not shown). There was no evidence for residual inflammation by 60 days. These data allowed us to conclude that two episodes of acute inflammation can cause rapid neoplastic progression in the pancreas even in the absence of chronic pancreatitis, but only in the context of *Kras* oncogenic activation.

As observed in human PDAC, while mPanIN-1 lesions exhibited a low index of proliferation, there was increased proliferation in mPanIN-2 and -3 as evidenced by increased Ki67 staining (Fig. 2A). mPanINs also expressed cytokeratin19 (CK19), underscoring their epithelial nature (Fig. 2B), and displayed mucus accumulation as shown by Muc5a expression (Fig. 2C). High-grade PanINs also exhibited the Notch signaling marker *Hes1* (Fig. 2D) and the early pancreatic progenitor marker *Pdx1* (Fig. 2E), both known to be reactivated in early stages of PDAC. ADM was also observed, as shown by the co-expression in the same cells of acinar (amylase) and ductal (CK19) markers (Fig. 2F), as observed by others [17].

Among the three groups of mice that were subjected to AP, two PK animals from the 80 days post caerulein group developed PDAC. The carcinomas were locally invasive, the cancer cells exhibited a ductal like morphology (Fig. 3A) and displayed increased proliferation (Fig. 3B). In addition, there were foci of cancer cells that were surrounded by a reactive stroma characterized by smooth muscle actin (SMA) expression (Fig. 3C), indicating the presence of activated pancreatic stellate cells.

Discussion

Caerulein-induced acute pancreatitis is a well-studied animal model in which caerulein normally causes a mild, edematous pancreatitis, which is self-limiting and highly reproducible. Initiation of acute pancreatitis in this model is mediated through premature intracellular activation of zymogens in the acinar cells, leading to acinar cell death and an inflammatory response associated with mild pancreatic edema [18]. While many acinar cells are lost in this process, full pancreatic function is restored approximately a week following caerulein treatment as the result of a robust acinar regenerative response.

In the present study we determined that two episodes of acute pancreatitis in the PK mouse model lead to a remarkable acceleration in the formation of high-grade mPanINs, reactivation of Notch signaling, and progression to PDAC. Extensive characterization of the events following caerulein-induced acute pancreatitis showed that during the 24 hours following an episode of acute pancreatitis, large numbers of acinar cells undergo apoptosis and that one week later, the acinar compartment is fully regenerated [15]. By contrast, ductal, centroacinar or endocrine cells do not exhibit an increase in apoptosis or proliferation [15]. Acute pancreatitis also causes a rapid activation of signaling pathways such as Notch and Hedgehog, which are known to be upregulated in PDAC early lesions [15;19]. Taken together with the present findings, these observations indicate that acute pancreatitis-induced acinar cell regeneration and associated alterations in key signaling pathways, in conjunction with oncogenic Kras activation, combine to markedly increase the propensity for pancreatic malignant transformation. Our findings thus suggest that PanINs and PDAC may arise from the acinar cell compartment of the exocrine pancreas.

Several recent reports support the concept that acinar cells could give rise to PDAC. First, restricted activation of a mutated Kras allele in acinar cells (and centroacinar cells in some cases), using the elastase or the Mist1 promoters for Cre expression, leads to mPanIN development in adult mice [6;7] and infrequent invasive PDAC [5]. Second, activation of Kras^{G12D} in the Nestin lineage, constituted essentially of acinar cells, leads to the development of mPanINs in similar number and grade that was seen in the PK model [20]. Third, chronic pancreatitis induced by repeated injections of caerulein over many weeks causes PDAC progression in mice carrying an activated Kras^{G12V} allele in adult acinar/centroacinar cells [5]. Fourth, ductal cells, ADM lesions and islet cells display cilia; by contrast, pancreatic acinar cells, all grades of PanINs, and pancreatic cancer cells are characterized by the absence of cilia in both humans and mouse models [21], suggesting that PanINs, PDAC, and acinar cells have a common origin. Moreover, lineage tracing experiments suggest that new acinar cells originate from preexisting acinar cells [16;22;23]. Taken together, these observations support the hypothesis that induction of acinar cell proliferation by pancreatic injury such as an episode of acute pancreatitis, when occurring in the context of an activated mutated Kras allele, can lead to deregulated growth and progression to PDAC.

Recovery from acute pancreatitis in humans does not necessarily lead to irreversible damage and is associated with pancreatic regeneration [24]. The most frequent causes for acute pancreatitis in Western countries are gallstone disease and alcohol consumption [25]. Heavy alcohol consumption, independently of chronic pancreatitis, has been associated with an increased incidence of PDAC [26]. Conceivably, alcohol may exert this effect by precipitating acute pancreatitis. It is not clear, however, whether such brief episodes of acute pancreatitis act by promoting a pro-inflammatory environment in the pancreas, by inducing bursts of acinar cell regeneration, or by upregulating signaling pathways and transcription factors involved in embryonic development of the pancreas and in PDAC [15]. Irrespective of the mechanisms, if confirmed in future studies, the present findings suggest that prevention of acute pancreatitis may be an important strategy for decreasing the incidence of PDAC.

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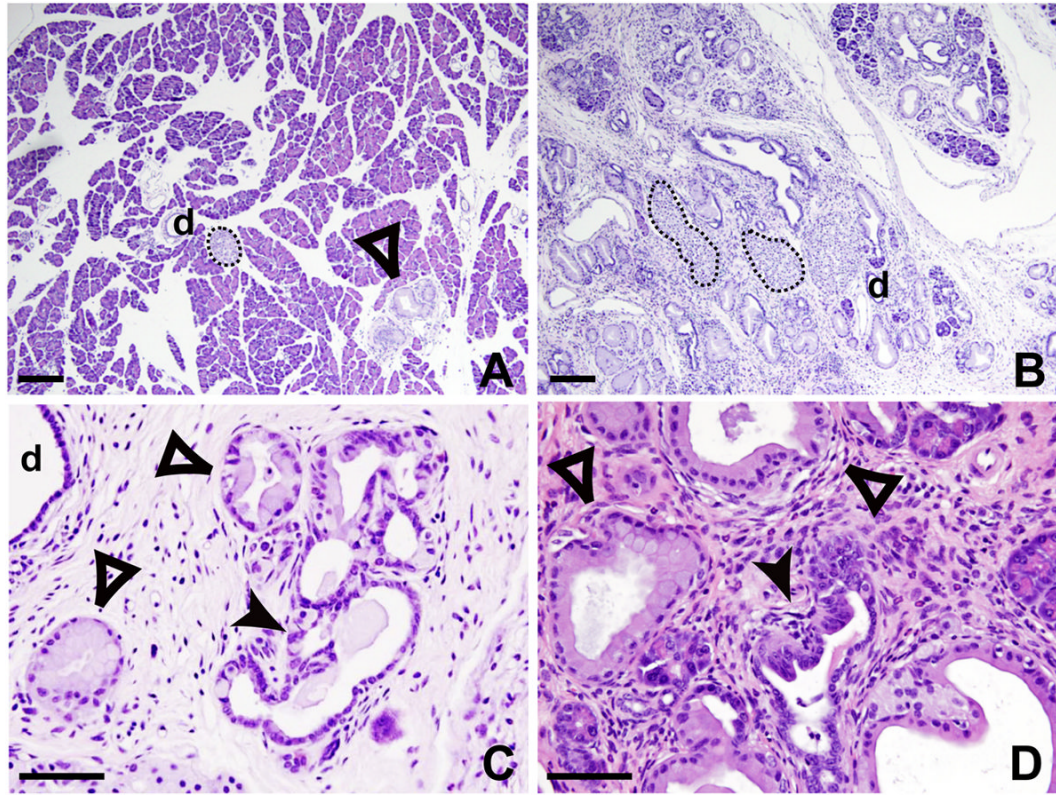


Figure 1.

PanIN progression 2 months following acute pancreatitis episodes. H&E staining of untreated (A) and caerulein treated NK pancreata (B–D). While untreated pancreata display rare lesions (A), treated pancreata show extensive ADM and high number of mPanIN-1 (B), as well as high-grade lesions mPanIN-2 (C) and -3 (D). Islets (dotted outlines), normal ducts (d), mPanIN-1 (empty arrowheads), and mPanIN-2 and -3 (solid arrowheads) are readily seen. Scale bar: 100 μ m for A–B, 20 μ m for C–D.

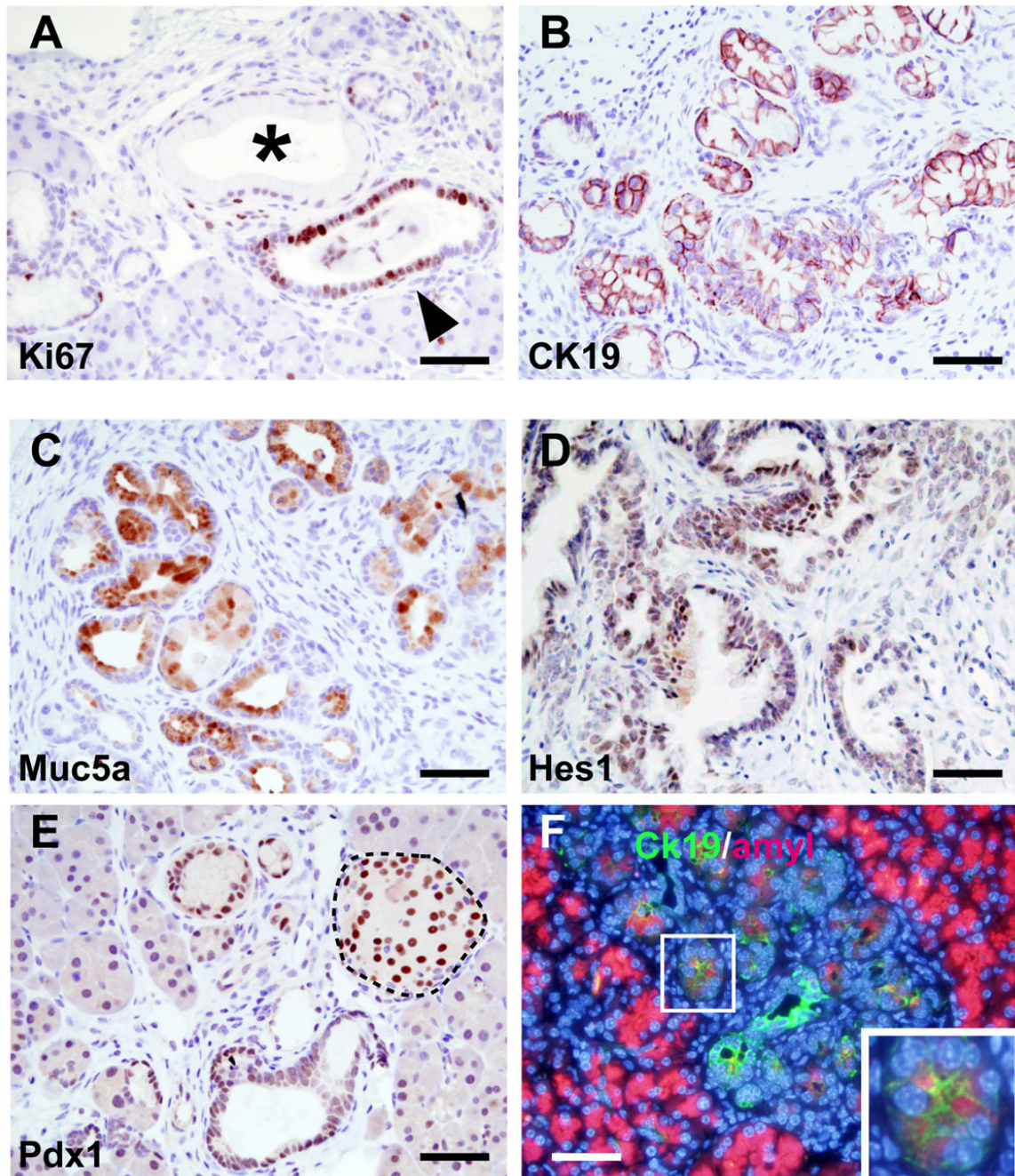


Figure 2.

High grade PanIN characterization following acute pancreatitis. Ki67 staining shows a clear increase in proliferation in high-grade lesions (A) when compared to mPanIN-1 (*). High-grade PanINs express CK19 (B) and Muc5a (C). Markers of early progenitors Hes1, panel D) and Pdx1 (panel E) are reactivated; as expected, Pdx1 is also seen in the islet cells (dotted outline). ADM is observed as shown by coexpression of CK19 (green) and Amylase (red) in same cells (see inset). Scale bar: 20μm.

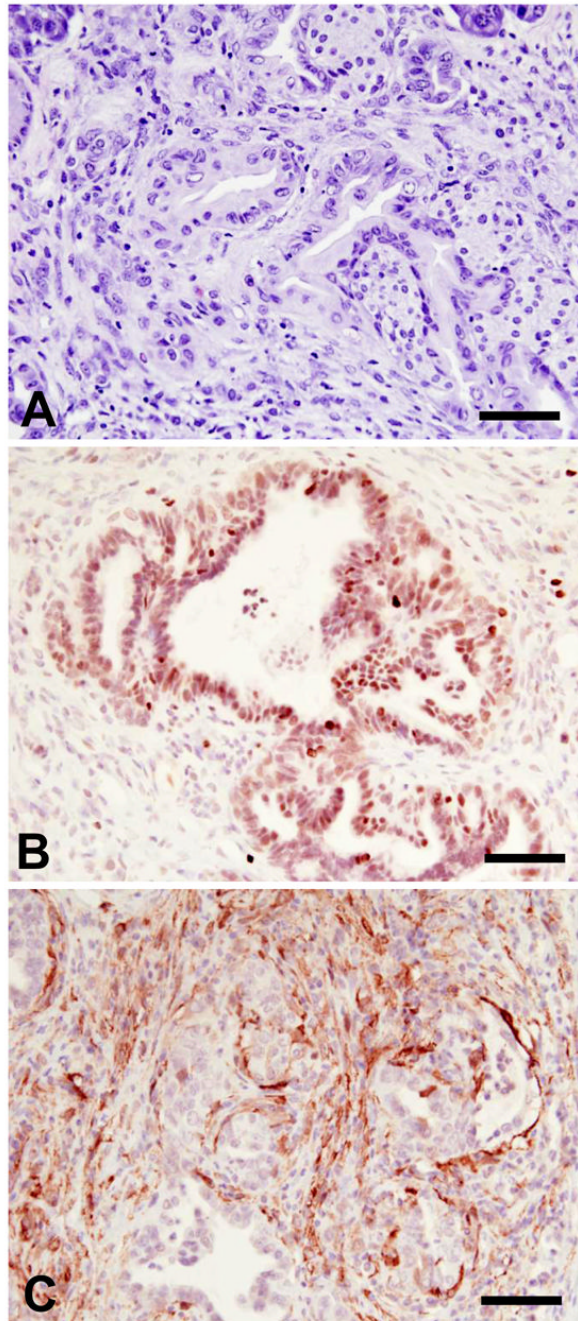


Figure 3.

PK mice develop PDAC following acute pancreatitis. H&E staining of ductal adenocarcinoma (A) reveals the presence of many nuclei that are enlarged, pleomorphic and crowded, with the cancer cells exhibiting a ductal-like morphology. Adenocarcinoma cells are highly proliferative as shown by Ki67 expression (B). They are surrounded by fibroblasts characterized by SMA expression (C). Scale bar: 20µm.