Cancer Cell
Article

Stat3 and MMP7 Contribute to Pancreatic Ductal Adenocarcinoma Initiation and Progression

Akihisa Fukuda,[1,7] Sam C. Wang,[1,7] John P. Morris, IV,[1] Alexandra E. Folias,[1] Angela Liou,[1] Grace E. Kim,[2] Shizuo Akira,[3] Kenneth M. Boucher,[4,6] Matthew A. Firpo,[5,6] Sean J. Mulvihill,[5,6] and Matthias Hebrok[1,*]
[1]Diabetes Center, Department of Medicine
[2]Department of Pathology
University of California, San Francisco, San Francisco, CA 94143, USA
[3]Laboratory of Host Defense, WPI Immunology Frontier Research Center, Osaka University, Osaka 5650871, Japan
[4]Department of Oncological Sciences
[5]Department of Surgery
[6]Huntsman Cancer Institute
University of Utah, Salt Lake City, UT 84115, USA
[7]These authors contributed equally to this work
*Correspondence: mhebrok@diabetes.ucsf.edu
DOI 10.1016/j.ccr.2011.03.002

SUMMARY

Chronic pancreatitis is a well-known risk factor for pancreatic ductal adenocarcinoma (PDA) development in humans, and inflammation promotes PDA initiation and progression in mouse models of the disease. However, the mechanistic link between inflammatory damage and PDA initiation is unclear. Using a Kras-driven mouse model of PDA, we establish that the inflammatory mediator Stat3 is a critical component of spontaneous and pancreatitis-accelerated PDA precursor formation and supports cell proliferation, metaplasia-associated inflammation, and MMP7 expression during neoplastic development. Furthermore, we show that Stat3 signaling enforces MMP7 expression in PDA cells and that MMP7 deletion limits tumor size and metastasis in mice. Finally, we demonstrate that serum MMP7 level in human patients with PDA correlated with metastatic disease and survival.

INTRODUCTION

It is generally believed that pancreatic ductal adenocarcinoma (PDA) initiation commonly occurs with metaplastic conversion of normal cells to noninvasive ductal precursors such as pancreatic intraepithelial neoplasia (PanIN). Gain-of-function mutations in the Kras gene are nearly universal in human PDA (Almoguera et al., 1988), and pancreas-specific expression of mutant Kras in mice recapitulates the human PanIN-to-PDA sequence (Hingorani et al., 2003), suggesting that Kras mutation may represent an initiating event. Although both PanINs and PDA express ductal markers, mouse models have recently revealed that mutant Kras can reprogram acinar cells to enter the PanIN/PDA lineage in a process termed acinar to ductal metaplasia (ADM) (De La et al., 2008; Guerra et al., 2007; Habbe et al., 2008; Morris et al., 2010).

Chronic pancreatitis has been identified as risk factor for PDA development in humans (Lowenfels et al., 1993) and has been shown to significantly accelerate PanIN and PDA development in Kras-driven mouse models of PDA (Guerra et al., 2007). In addition acute pancreatitis can progress to chronic pancreatitis in human patients, often under conditions in which environmental insults such as alcohol and cigarette smoking are present (Lankisch et al., 2009). Notably, recent reports have shown that acute pancreatitis also markedly accelerates PanIN and PDA development in Kras-driven mouse models of PDA (Carriere et al., 2009; Morris et al., 2010).

Significance

Patients with pancreatic cancer have a less than 5% 5-year survival rate after diagnosis. Obstacles to improving outcomes include a lack of clear understanding of disease pathogenesis, poor early detection tools, the inherently aggressive biology of the tumor leading to early metastasis, and a lack of effective chemotherapeutics. In this study we establish the Stat3-MMP7 signaling axis as a key mediator in PDA biology both in the early and late-disease process, thus providing an attractive target for future therapeutic development. Furthermore, serum MMP7 levels in human patients with PDA were predictive of metastatic disease, therefore providing physicians and patients with a tool for guiding treatment plans.

Mouse models of acute pancreatitis have revealed that the exocrine pancreas possesses remarkable regenerative capacity after inflammatory damage. In response to acute pancreatitis induced by the cholecystokinin analog caerulein, acini transiently dedifferentiate into duct-like structures that express embryonic factors characteristic of pancreatic embryonic progenitors. In the absence of further damage, redifferentiation into functional acinar cells occurs rapidly within 1 week of caerulein treatment (De La and Murtaugh, 2009; Fendrich et al., 2008; Jensen et al., 2005; Morris et al., 2010; Siveke et al., 2008). In contrast, mutant Kras compromises the ability of acinar cells to regenerate following acute pancreatitis and locks damaged cells in a persistently dedifferentiated, ductal state that can rapidly give rise to PanINs (De La and Murtaugh, 2009; Morris et al., 2010). Thus, whereas pancreatitis provides a permissive environment for Kras-driven neoplasia, the molecular mechanisms supporting this state remain largely unknown.

Signal transducer and activator of transcription 3 (Stat3) integrates signals from cytokines and growth factors into transcriptional responses in target cells and may serve as a mediator of inflammation-associated processes, such as pancreatitis-driven PanIN development. Stat3 mediates a complex spectrum of cellular responses including inflammation, cell proliferation, and apoptosis (Levy and Lee, 2002). Stat3 also supports inflammation-associated tumorigenesis in the colon (Bollrath et al., 2009; Grivennikov et al., 2009), stomach (Ernst et al., 2008), and lung (Li et al., 2007). In the pancreas, Stat3 is aberrantly activated in human PDA, controls proliferation in PDA cell lines (Scholz et al., 2003), and is implicated in a mouse model of acinar to ductal reprogramming driven by persistent exocrine expression of the key pancreatic transcription factor Pdx1 (Miyatsuka et al., 2006). However, to our knowledge, the role of Stat3 in Kras-dependent initiation of the PanIN/PDA lineage has not been investigated.

Matrix metalloproteinase 7 (MMP7), also known as matrilysin, is a member of a family of zinc-dependent endopeptidases. MMP7 is expressed predominantly in epithelial cells of glandular tissue, unlike other MMPs, which are often stromally derived (Wilson et al., 1997). MMP7 is overexpressed in PanINs and PDA (Crawford et al., 2002; Yamamoto et al., 2001) and correlates with decreased survival and possibly tumor size, lymph node involvement, and distant metastasis (Fukushima et al., 2001; Jones et al., 2004; Yamamoto et al., 2001). Significantly, MMP7-deficient mice are resistant to acinar apoptosis and ADM in an obstructive-pancreatitis model (Crawford et al., 2002), and MMP7 is required for in vitro ADM driven by EGF signaling (Sawey et al., 2007). Other in vitro studies suggest that MMP7 is also involved in disease progression by dictating the invasive and metastatic capacity of PDA cells (Yamamoto et al., 2001). Interestingly, MMP7 is induced through a Stat3-dependent mechanism in prostate and breast cancer cell lines (Udayakumar et al., 2002; Yuan et al., 2008), but, to our knowledge, a role for MMP7 in Kras-driven PDA or whether the Stat3-MMP7 axis is maintained in PDA progression has not been investigated.

In this study we investigated the in vivo role of Stat3 in initiating the PanIN/PDA lineage in the setting of pancreatitis, tested for the presence of the Stat3-MMP7 signaling in the pancreas, and determined the role of MMP7 in PDA initiation and progression.

RESULTS

Persistent Stat3 Activation Is a Hallmark of Kras-Driven Ductal Metaplasia in Response to Acute Pancreatitis

To determine if Stat3 signaling is involved in the pancreatic response to inflammatory damage, we audited pancreatic Stat3 activation levels in response to acute pancreatitis. We induced acute pancreatitis with caerulein as described previously (Morris et al., 2010). Immunohistochemistry (IHC) revealed no active, phosphorylated Stat3 (p-Stat3) in control adult pancreas. In contrast, p-Stat3 was widely observed in the damaged exocrine compartment of control mice 1 day after caerulein injection. Corresponding with acinar regeneration, p-Stat3 staining was again generally absent in the exocrine pancreas by 7 days after caerulein treatment (Figures 1A–1D). Western blotting for p-Stat3 in pancreas lysates and quantitative RT-PCR (qPCR) for *Socs3*, a Stat3 target gene, revealed a similar pattern of transient activation (Figures 1I and 1J), suggesting that Stat3 signaling is active during pancreatic regeneration but quiescent in the undamaged pancreas.

We have previously shown that mutant Kras blocks acinar regeneration in favor of ductal reprogramming and PanIN formation after acute pancreatitis (Morris et al., 2010). Therefore, we examined Stat3 signaling during pancreatitis-induced PanIN development in mice expressing a conditional, constitutively active form of Kras ($Kras^{G12D}$) (Hingorani et al., 2003) targeted to pancreatic progenitors under the control of the *Ptf1a* promoter (*Ptf1a-Cre*) (Kawaguchi et al., 2002). In 6-week-old *Ptf1a-Cre; $Kras^{G12D}$* mice treated with PBS, p-Stat3 expression was absent in most morphologically normal acini and ducts but was readily detectable in spontaneously formed PanINs, directly associated stromal cells, and occasionally in acini surrounding the PanINs (Figure 1E). Similar to control mice, Stat3 activation was widely observed in epithelial cells in *Ptf1a-Cre; $Kras^{G12D}$* mice 1 day after caerulein treatment (Figures 1F, 1I, and 1K). However, in contrast to control mice, *Ptf1a-Cre; $Kras^{G12D}$* animals displayed persistent Stat3 activation 7 and 21 days postinjection (Figures 1G–1I and 1K) in the setting of metaplastic ducts and PanIN lesions, suggesting a role for Stat3 in reprogramming normal acinar cells into PDA precursors following inflammatory damage.

One possible explanation for persistent Stat3 activation in caerulein-treated *Ptf1a-Cre; $Kras^{G12D}$* mice is enforced expression of factors known to activate the JAK/STAT cascade. Previous studies have indicated an obligate role for IL-6 in Ras transformation, and that oncogenic Ras supports Stat3 activation by inducing the secretion of IL-6 (Ancrile et al., 2007). Similar to Stat3 signaling, we found that *IL-6* expression was only transiently upregulated after caerulein injection in wild-type mice, whereas *IL-6* was persistently expressed in *Ptf1a-Cre; $Kras^{G12D}$* animals (see Figure S1 available online). Therefore, persistent expression of *IL-6* may be involved in prolonged Stat3 activation in *Ptf1a-Cre; $Kras^{G12D}$* mice following inflammatory insults.

Pancreatic Epithelial Stat3 Deletion Inhibits Both Spontaneous and Pancreatitis-Induced PanIN Formation

To establish if the Stat3 pathway is functionally relevant during PDA precursor formation, we generated mice expressing $Kras^{G12D}$ and conditional *Stat3* alleles (*Ptf1a-Cre; $Kras^{G12D}$;*

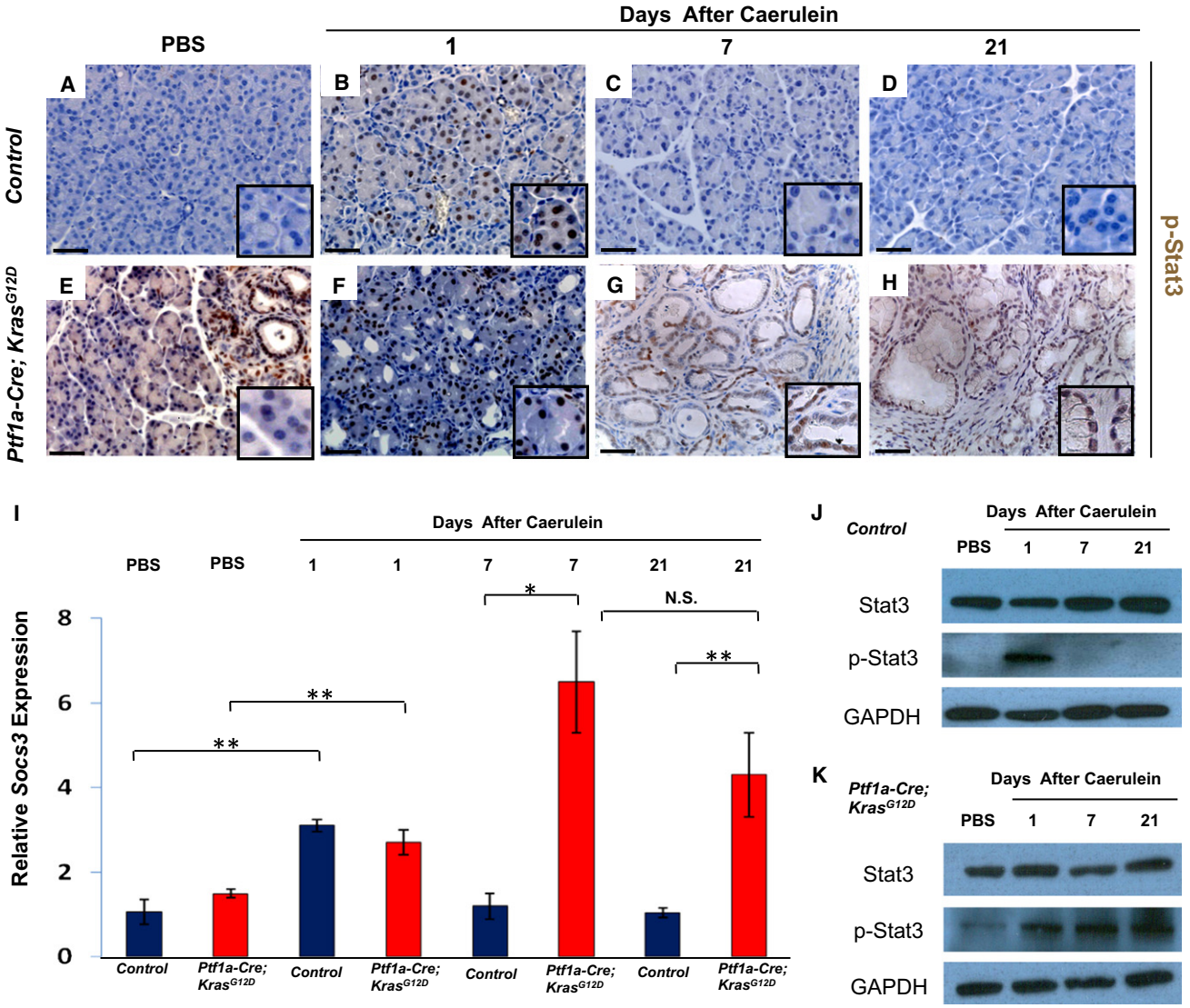

Figure 1. Persistent Stat3 Activation after Pancreatitis in Setting of Activated Kras

(A–H) IHC for p-Stat3 in control (A–D) or *Ptf1a-Cre; Kras*^{G12D} pancreata (E–H) after PBS and caerulein treatments. (A) PBS. (B) One day post-caerulein. (C) Seven days post-caerulein. (D) Twenty-one days post-caerulein. (E) PBS. Stat3 is activated only in the epithelial cells of spontaneously formed PanINs and surrounding stromal cells. (F) One day post-caerulein. (G) Seven days post-caerulein. Stat3 activation is maintained in metaplastic ducts in mice with activated Kras. (H) Twenty-one days post-caerulein. Stat3 activation is maintained in PanINs in mice with activated Kras.

(I) Stat3 target gene *Socs3* expression in pancreata by qPCR after caerulein injection. *Socs3* expression is maintained in mice with activated Kras signaling 7 and 21 days after caerulein. Mean ± SD (n = 3). *$p < 0.05$ and **$p < 0.01$. N.S., not significant.

(J) Western blot. p-Stat3 is observed only 1 day post-caerulein in control pancreata.

(K) Western blot. Sustained aberrant p-Stat3 post-caerulein in *Ptf1a-Cre; Kras*^{G12D} pancreata. Scale bars, 50 μm.

See also Figure S1.

Stat3$^{f/f}$) (Takeda et al., 1998). At both birth and adulthood (6 weeks), *Ptf1a-Cre; Stat3*$^{f/f}$ pancreata appeared normal, indicating that pancreatic Stat3 signaling was dispensable for pancreas development (data not shown). We analyzed 6-week-old *Ptf1a-Cre; Kras*^{G12D} and *Ptf1a-Cre; Kras*^{G12D}*; Stat3*$^{f/f}$ mice treated with PBS. Although we detected occasional PanINs in *Ptf1a-Cre; Kras*^{G12D}*; Stat3*$^{f/f}$ mice, the pancreatic area replaced by lesions staining with Alcian blue, which is a marker of intestinal mucin accumulation that characterizes human PanINs, was significantly smaller when compared to *Ptf1a-Cre; Kras*^{G12D} mice (Figures 2A–2C). These data suggest that Stat3 deletion interferes with Kras-driven spontaneous PanIN formation and that Stat3 signaling plays an intrinsic role in PanIN development in the absence of inflammatory insults.

Next, we investigated the role of epithelial Stat3 signaling on Kras-driven PanIN formation in the setting of acute pancreatitis. By 7 days after caerulein injection, the exocrine compartment of *Ptf1a-Cre; Kras*^{G12D} mice was nearly completely replaced by fibrosis and ductal structures (Figure 2D), as evident by rare expression of the acinar marker amylase (Figure 2H), and

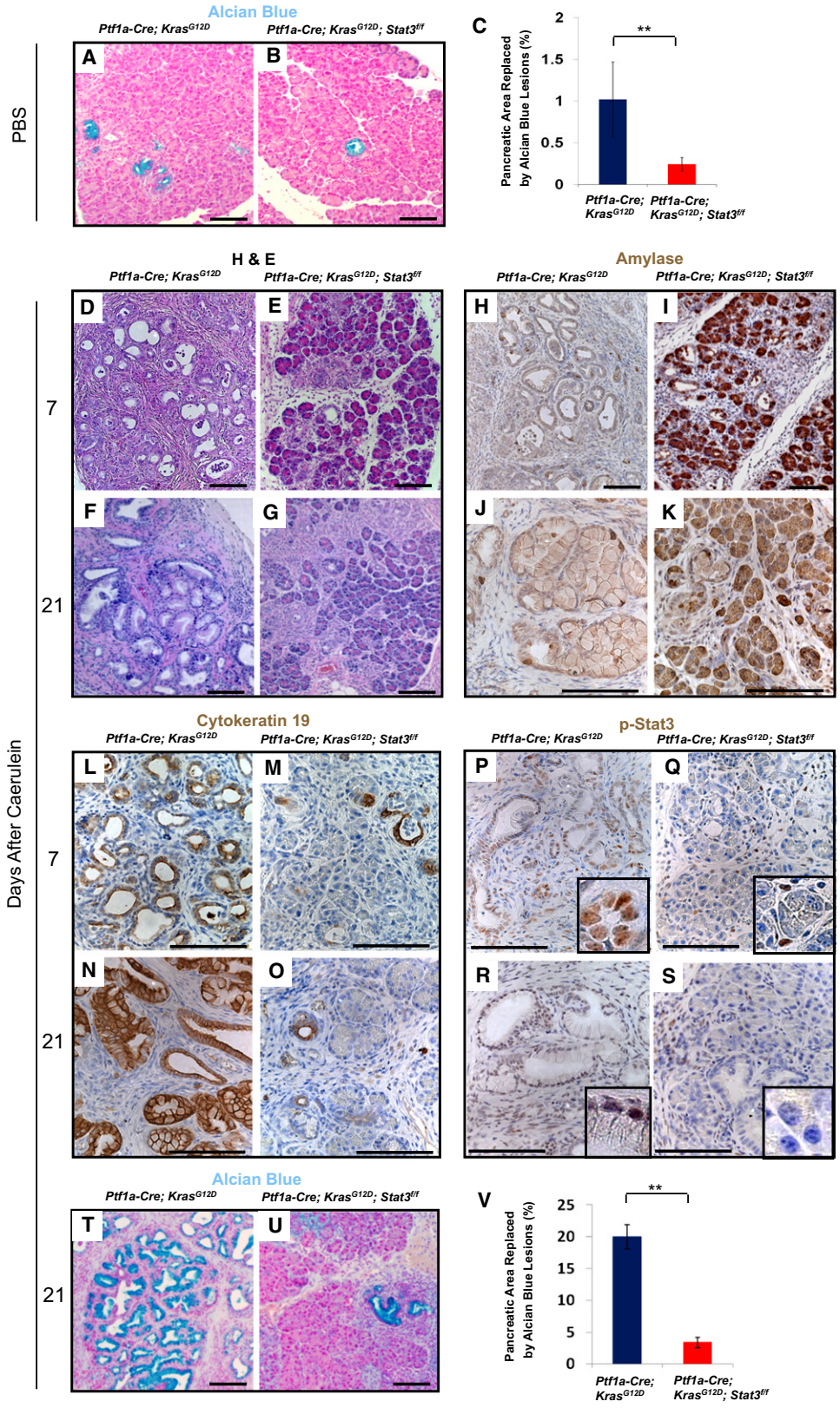

Figure 2. Stat3 Deletion Protects Against Spontaneous and Acute Pancreatitis-Accelerated PanIN Formation

Mice with activated Kras with or without Stat3 signaling (*Ptf1a-Cre; Kras^{G12D}* and *Ptf1a-Cre; Kras^{G12D}; Stat3$^{f/f}$*, respectively), were injected with PBS (A–C) or caerulein (D–V) and sacrificed either 7 or 21 days postinjection. The following stains were performed.

widespread expression of the duct marker cytokeratin 19 (CK 19) (Figure 2L). Ductal structures observed in *Ptf1a-Cre; Kras^{G12D}* mice 21 days after caerulein frequently stained positive with Alcian blue (Figure 2T). In contrast, pancreata of Stat3-deficient mice treated with caerulein did not display gross fibrosis at necropsy, maintained dramatically more amylase-positive parenchyma (Figures 2E, 2G, 2I, and 2K), and displayed fewer CK 19-positive duct structures (Figures 2M and 2O). The area of pancreas replaced by Alcian blue-positive lesions was also significantly reduced in Stat3-deficient mice (Figures 2T–2V). Although the frequency of PanINs was significantly different between the genotypes, PanINs observed in both genotypes were of equivalently low grades (PanIN 1A and 1B). As expected, Stat3 activation was maintained in metaplastic ducts in *Ptf1a-Cre; Kras^{G12D}* mice at both 7 and 21 days postinjection but was only observed in stromal cells surrounding PanIN lesions and areas of ductal metaplasia, but not in epithelial cells of acini, metaplastic ducts, or PanINs in *Ptf1a-Cre; Kras^{G12D}; Stat3$^{f/f}$* mice (Figures 2P–2S). Given that chronic pancreatitis is a significant risk factor for PDA in humans, we also tested the effects of chronic pancreatitis and found similarly protective effects of Stat3 deletion (Figures S2A–S2R). Summarily, these data establish Stat3 as a critical component of the permissive environment provided by pancreatitis to drive Kras-dependent PanIN formation.

We next used a cell culture model of acinar to ductal dedifferentiation to ask whether Stat3 mediates ductal reprogramming of acinar cells in a cell autonomous manner. Briefly, acinar clusters isolated from adult mice embedded in collagen I *trans*-differentiate into ductal structures in response to the EGF ligand, TGF-α (Means et al., 2005; Miyamoto et al., 2003). In support of our in vivo data, we found that *Ptf1a-Cre; Stat3$^{f/f}$* acinar explants formed significantly fewer CK19-positive ductal structures after TGF-α treatment, as compared to control acinar explants, suggesting that Stat3 contributes to acinar to ductal dedifferentiation in part through cell-autonomous mechanisms in vitro (Figures S2S–S2W).

To determine the long-term effects of Stat3 deletion on Kras-driven neoplasia, we analyzed *Ptf1a-Cre; Kras^{G12D}* and *Ptf1a-Cre; Kras^{G12D}; Stat3$^{f/f}$* 8 months after acute pancreatitis. Although no PDA was found in either group, *Ptf1a-Cre; Kras^{G12D}* mice appeared to have marked pancreatic exocrine insufficiency, as demonstrated by steatorrhea and significant loss of body weight (Figures S2X–S2F′). Histology revealed that the pancreatic epithelium was replaced almost entirely by PanINs. In contrast, Stat3-deficient mice maintained body weight comparable to controls, did not have steatorrhea, and retained significantly more normal exocrine parenchyma than *Ptf1a-Cre; Kras^{G12D}* animals (Figures S2X–S2F′). Thus, the protective effect of Stat3 deletion persists even months after acute damage. Notably, PanIN 1B was the highest grade of lesion seen in either genotype, suggesting that additional molecular events critical to PanIN and PDA progression, such as the loss of tumor suppressor genes, had not yet occurred over the course of our aging experiments.

Stat3 Supports Persistent Cell Proliferation during Kras-Driven PanIN Development following Acute Pancreatitis

Activated Stat3 signaling has been shown to regulate cellular proliferation. To determine whether pancreatic Stat3 deletion affects cell proliferation during Kras-driven metaplasia and PanIN development after pancreatitis, we determined the proliferative index of ductal cells, acinar cells, and PanIN lesions. We found that there were significantly more amylase^{+}/Ki67^{+} acinar cells 2 days after caerulein and significantly more CK19^{+}/Ki67^{+} ductal cells 2 and 7 days after caerulein in *Ptf1a-Cre; Kras* mice compared to *Ptf1a-Cre; Kras^{G12D}; Stat3$^{f/f}$* animals (Figures 3A–3P). Thus, loss of Stat3 impairs proliferation during Kras-driven pancreatic metaplasia. In contrast the proliferative capacity of the few lesions positive for Alcian blue observed in *Ptf1a-Cre; Kras^{G12D}; Stat3$^{f/f}$* animals was not altered when compared to controls (Figures 3Q–3X). Staining for cleaved caspase-3 showed that there were few apoptotic cells in all three genotypes at both time points (data not shown), suggesting that contribution of apoptosis is relatively minimal during pancreatitis-promoted ductal reprogramming. Collectively, these data demonstrate that Stat3 supports persistent cell proliferation that characterizes Kras-dependent acinar metaplasia and PanIN formation.

Stat3 Contributes to Inflammation Associated with Kras-Driven Metaplasia after Injury

Because Stat3 is a critical mediator of inflammation during neoplasia (Yu et al., 2009), we used FACS analysis to evaluate whether Stat3 deletion altered the initial inflammatory response during Kras-driven metaplasia following injury. One day after caerulein injection, the percentage of total infiltrating immune cells (CD45^{+} cells) was higher in *Ptf1a-Cre; Kras^{G12D}* pancreata compared to tissue from control and *Ptf1a-Cre; Kras^{G12D}; Stat3$^{f/f}$* mice (Figure 4A). Additionally, an increase in the CD11b^{+} Gr1^{+} myeloid subset (known to contain myeloid-derived suppressor cells and immature myeloid cells), as well as an increased trend in infiltrating B (B220^{+}) and T (Thy1.2^{+}) lymphocytes, was seen in *Ptf1a-Cre; Kras^{G12D}* mice compared to both control and Stat3-deficient mice (Figures S3A–S3G). Although additional analysis is necessary to refine the identity and function

(A and B) Alcian blue stain showing spontaneously formed PanINs.
(C) Alcian blue-positive lesion in total pancreas area of *Ptf1a-Cre; Kras^{G12D}* (n = 6) and *Ptf1a-Cre; Kras^{G12D}; Stat3$^{f/f}$* (n = 3) mice. Mean ± SD. **p < 0.01.
(D–G) H&E. Fewer ductal and more acinar structures are observed in Stat3-deficient mice.
(H–K) Amylase. Stat3-deficient mice retained larger areas of amylase-expressing epithelium.
(L–O) CK19. Mice with Stat3-intact display more CK19-positive ductal structures.
(P–S) p-Stat3.
(T–U) Alcian blue stain, 21 days post-caerulein.
(V) Alcian blue-positive lesion in total pancreas area of *Ptf1a-Cre; Kras^{G12D}* and *Ptf1a-Cre; Kras^{G12D}; Stat3$^{f/f}$* mice. Mean ± SD (n = 3). **p < 0.01. Scale bars, 100 μm.
See also Figure S2.

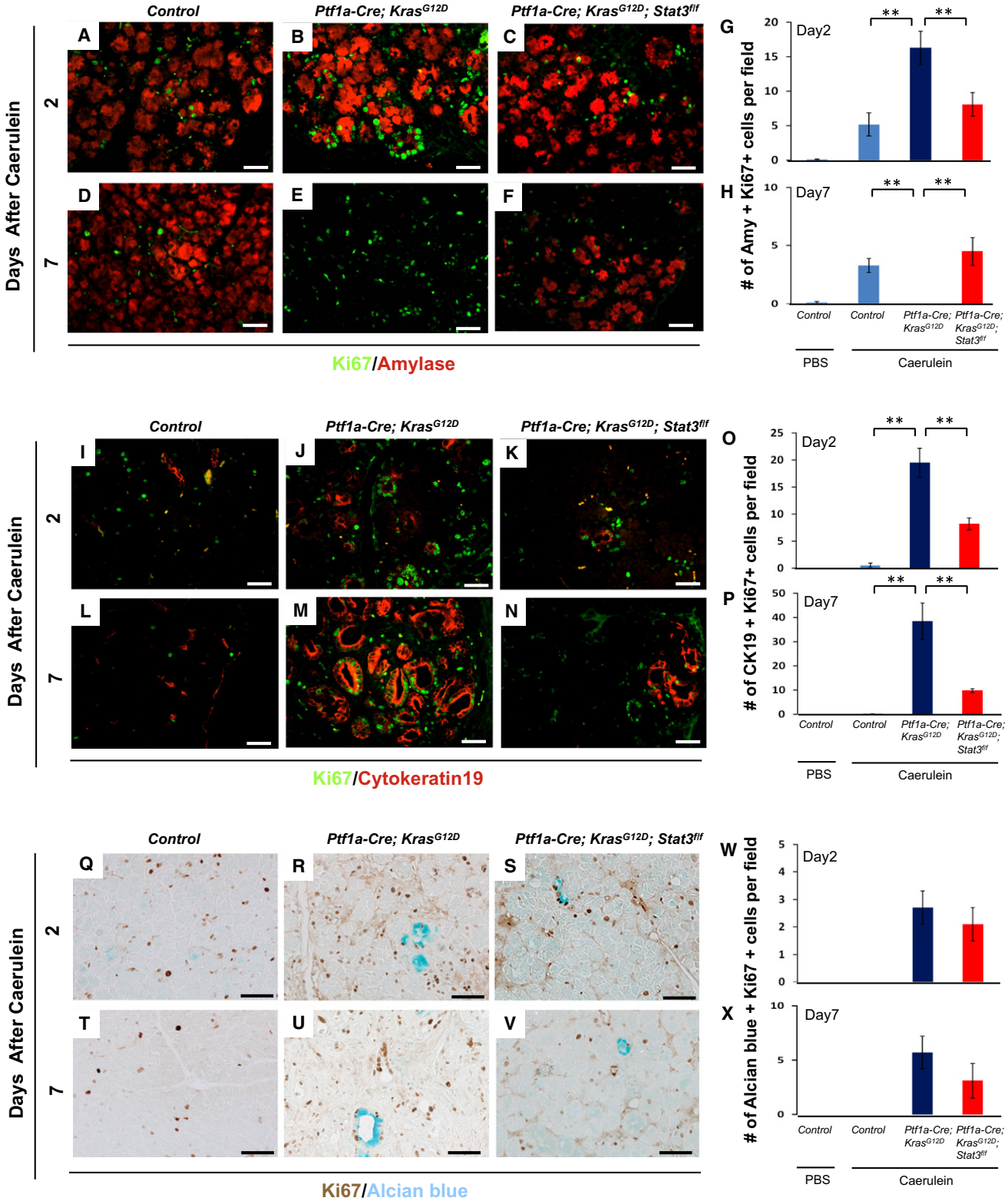

Figure 3. Stat3 Supports Cell Proliferation during Kras-Driven PanIN Development following Acute Pancreatitis

Costaining of Ki67 with amylase (A–F), CK 19 (I–N), or Alcian blue (Q–V) in pancreata from control, *Ptf1a-Cre; Kras^{G12D}*, and *Ptf1a-Cre; Kras^{G12D}; Stat3$^{f/f}$* mice 2 or 7 days postinjection. (A–C, I–K, and Q–S) Two days post-caerulein. (D–F, L–N, and T–V) Seven days post-caerulein. Quantification of double-positive cells displaying Ki67 with amylase (G and H), cytokeratin19 (O and P), or Alcian blue (W and X), per 400× field. Mean ± SD (n = 3). **p < 0.01. (G, O, and W) Two days post-caerulein. (H, P, and X) Seven days post-caerulein. Scale bars, 50 μm.

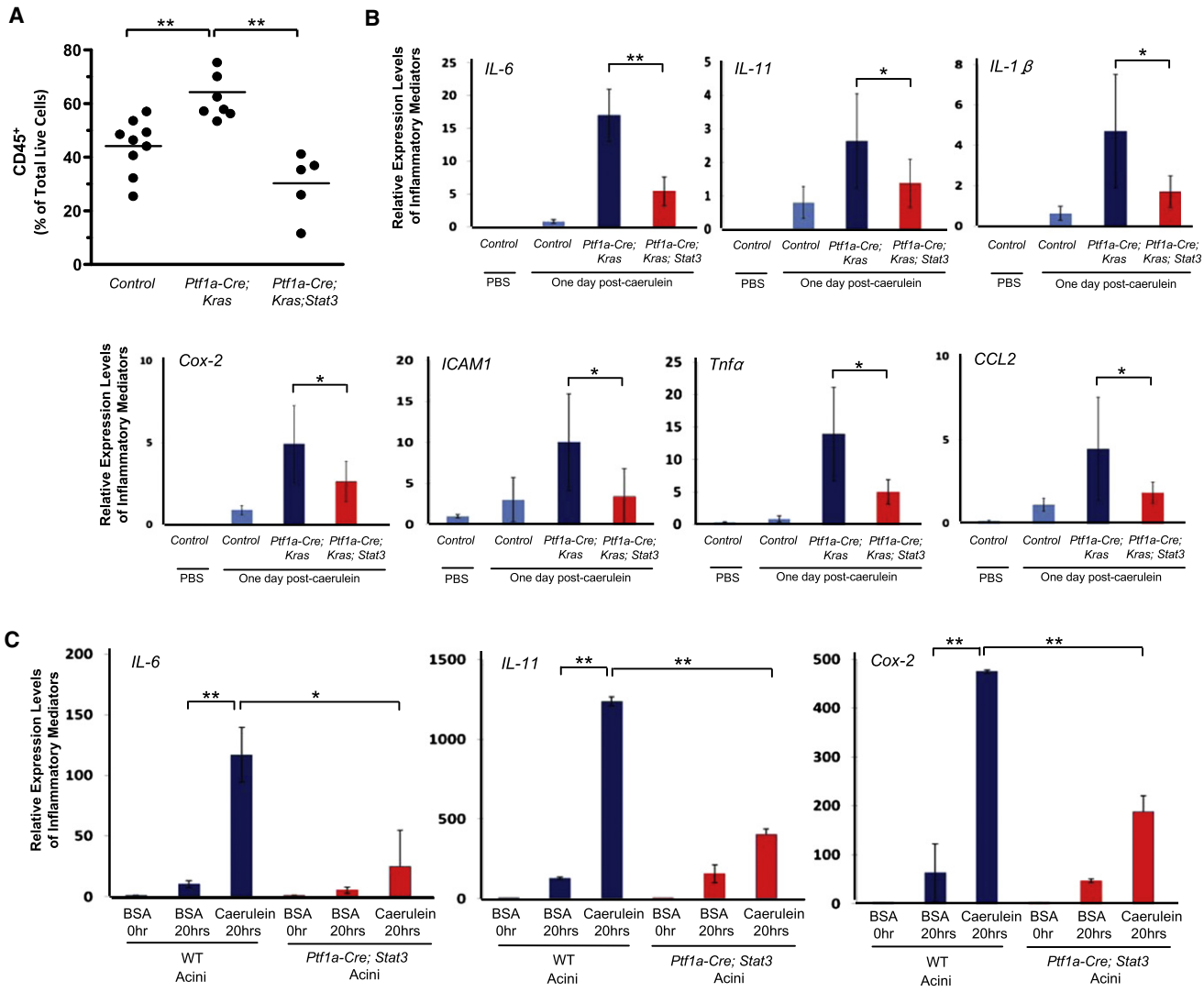

Figure 4. Elimination of Pancreatic Stat3 Alters Inflammatory Response of Mutant Kras Mice after Injury

(A) The percentage of $CD45^+$ immune cells among total live cells present in the pancreas was quantified using flow cytometry. Data from three independent experiments are shown using control (n = 9), *Ptf1a-Cre; Kras^{G12D}* (n = 8), and *Ptf1a-Cre; Kras^{G12D}; Stat3$^{f/f}$* (n = 5) mice.

(B) Relative expression of inflammatory mediators (*IL-6*, *IL-11*, *IL-1β*, *Cox-2*, *ICAM1*, *Tnfα*, and *CCL2*) in pancreata as measured by qPCR from control (n = 5), *Ptf1a-Cre; Kras^{G12D}* (n = 8), and *Ptf1a-Cre; Kras^{G12D}; Stat3$^{f/f}$* (n = 8) mice 1 day after caerulein injection. Mean ± SD.

(C) Relative expression of inflammatory mediators and Stat3 target genes (*IL-6*, *IL-11*, and *Cox-2*) in cultured primary acini isolated from wild-type and *Ptf1a-Cre; Stat3$^{f/f}$* mice 20 hr after treatment with BSA or 10 nM caerulein. Mean ± SD (n = 3). *p < 0.05, **p < 0.01. (*Ptf1a-Cre; Kras*, *Ptf1a-Cre; Kras; Stat3*, and *Ptf1a-Cre; Stat3* represent *Ptf1a-Cre; Kras^{G12D}*, *Ptf1a-Cre; Kras^{G12D}*, and *Ptf1a-Cre; Kras^{G12D}; Stat3$^{f/f}$* mice, respectively).

See also Figure S3.

of these immune subsets, these data indicate that the lack of Stat3 signaling during initiation of Kras-driven metaplasia decreases total inflammatory infiltration and alters immune cell composition.

To better understand how Stat3 might contribute to metaplasia-associated inflammation, we determined the expression of cytokines in pancreata from *Ptf1a-Cre; Kras^{G12D}* and *Ptf1a-Cre; Kras^{G12D}; Stat3$^{f/f}$* mice 1 day after caerulein. qPCR for Stat3 target genes, including cytokines (*IL-6*, *IL-1β*, *IL-11*), the inflammatory mediator *ICAM1*, and the prostaglandin-producing enzyme *Cox-2* (Yu et al., 2009), as well as other inflammatory mediators associated with pancreatitis (the cytokine *Tnfα*, and the chemokine *CCL2*) (Malka et al., 2000; Marra, 2005), revealed significantly higher levels in the pancreata of *Ptf1a-Cre; Kras^{G12D}* mice compared to those from *Ptf1a-Cre; Kras^{G12D}; Stat3$^{f/f}$* mice (Figure 4B). Because immune cells present in tissue inflammation produce cytokines (Shrivastava and Bhatia, 2010), these differences in cytokine expression may reflect the distinct inflammatory composition observed in each genotype. However, acini are known to represent an epithelial source of cytokines during caerulein pancreatitis (Bhatia et al., 2002; Blinman et al., 2000; Grady et al., 1997). To determine if the

differences in cytokine expression observed in bulk tissue may in part be due to epithelial Stat3-dependent function, we measured the expression of the Stat3-dependent genes *IL6*, *IL-11*, and *Cox-2* in primary cultured acini treated with caerulein isolated from control and *Ptf1a-Cre; Stat3$^{f/f}$* mice. Interestingly, we found a significant decrease in expression of these factors in caerulein-treated acini lacking Stat3 compared with control (Figure 4C). Taken together, our data demonstrate that epithelial Stat3 modulates the initial immune response, as well as contributes to the cytokine milieu present during initiation of Kras-driven metaplasia.

Stat3 Supports MMP7 Expression during Kras-Driven PanIN Development following Acute Pancreatitis

Because Stat3 appears to be critical for Kras-dependent PanIN development, we searched for possible downstream signaling factors supported by Stat3. Crawford et al. (2002) have previously shown that *MMP7* is expressed in the exocrine compartment in response to obstructive pancreatitis induced by pancreatic ductal ligation. Furthermore, in vitro studies in prostate and breast cancer cell lines have implicated MMP7 as a downstream target of Stat3 signaling (Udayakumar et al., 2002; Yuan et al., 2008). To determine if there is an in vivo connection between Stat3 and MMP7 during regeneration following acute pancreatitis or in Kras-driven metaplasia and PanIN development, we stained for MMP7 in pancreas from control, *Ptf1a-Cre; Kras^{G12D}*, and *Ptf1a-Cre; Kras^{G12D}; Stat3$^{f/f}$* mice after caerulein treatment. In the absence of damage, MMP7 was not expressed in control mice or the normal parenchyma of *Ptf1a-Cre; Kras^{G12D}* mice but was seen in spontaneously formed PanINs (data not shown). In control mice, MMP7 was expressed 2 days posttreatment but absent 7 days following injection (Figures 5A and 5D). In contrast, MMP7 expression in *Ptf1a-Cre; Kras^{G12D}* mice was detectable 2 days after caerulein treatment and remained prominent 7 days postinjection (Figures 5B and 5E). This differential MMP7 expression pattern between the genotypes mirrored Stat3 activation profiles after pancreatitis. Intriguingly, there was significantly less MMP7 staining, as well as *MMP7* expression by qPCR, in Stat3-deficient pancreata compared to *Ptf1a-Cre; Kras^{G12D}* mice at both time points (Figures 5C and 5F–5H). No differences were detected in the expression of other *MMPs*, including *MMP2*, *MMP3*, *MMP9*, and *MMP14* between *Ptf1a-Cre; Kras^{G12D}* and Stat3-deficient mice (Figure S4). These data indicate that Stat3 activity regulates MMP7 expression during ductal reprogramming in vivo, suggesting a potential role for MMP7 in PanIN development following pancreatitis.

To test the hypothesis that MMP7 is a critical downstream effector of Stat3 during pancreatitis-driven PanIN development, we compared PanIN formation in *Pfta1-Cre; Kras^{G12D}* mice containing heterozygous or homozygous germline deletion of *MMP7* (*Pfta1-Cre; Kras^{G12D}; MMP7^{Het}* and *Pfta1-Cre; Kras^{G12D}; MMP7^{KO}*, respectively). Unlike the effect of Stat3 deletion, we observed no significant difference in PanIN frequency, regardless of MMP7 expression (Figure 5I). These results were validated using another cohort of mice based on the Pdx1 promoter (data not shown). Therefore, whereas Stat3 supports MMP7 expression during Kras-driven neoplasia in the pancreas, MMP7 does not appear to be a critical Stat3 effector for ductal metaplasia and PanIN development.

MMP7 Expression Is Controlled by Stat3 in PDA Cells, and MMP7 Deletion Limits Tumor Size and Metastasis

Although MMP7 signaling does not appear to be essential to pancreatitis-induced PanIN formation, previous in vitro work using various cancer cell lines suggests that MMP7 may play a role in cancer progression, including metastasis (Adachi et al., 1999; Yamamoto et al., 2001). Therefore, we examined if the positive relationship between Stat3 and MMP7 observed during preneoplastic initiation persists in PDA cells and whether MMP7 itself plays a role in PDA progression and tumor biology. First, we screened seven human PDA cell lines for *MMP7* expression and found that CFPAC-1 and Colo357 expressed *MMP7* at readily detectable levels (arbitrarily determined as absolute Ct $\leq$ 35 by qPCR; Figure S5A). We treated Colo357 and CFPAC-1 cells with compound S3I-201, which inhibits Stat3 activity by binding to the Stat3 SH2 domain and preventing homodimerization and activation of Stat3 (Lin et al., 2009; Siddiquee et al., 2007). A concentration-dependent decrease in both p-Stat3 levels and *MMP7* expression was observed in Colo357 cells treated with the inhibitor (Figure 6A). CFPAC-1 cells also displayed significant reduction in both p-Stat3 and MMP7 expression at all concentrations tested (Figure S5B), indicating that Stat3 signaling dynamically controls *MMP7* expression in PDA cells.

Because Stat3 is active and continues to support MMP7 expression in PDA cells, we investigated if MMP7 itself contributes to PDA biology. We analyzed the effect of MMP7 deletion on a number of Kras-driven mouse models of PDA. First, we observed *Pdx1-Cre^{Late}; Kras^{G12D}; MMP7^{KO}* and *Pdx1-Cre^{Late}; Kras^{G12D}; MMP7^{Het}* animals until a censor date of 13 months (expression pattern of *Pdx-Cre^{Late}* mice previously described in Heiser et al. [2006]). We found that all *Pdx1-Cre^{Late}; Kras^{G12D}; MMP7^{Het}* mice developed PDA (four of eight *MMP7^{Het}* mice succumbed to PDA before censor, and the other four animals had PDA at the time of sacrifice), whereas four of 11 *Pdx1-Cre^{Late}; Kras^{G12D}; MMP7^{KO}* mice (36.3%) were PDA free (Figure 6B). The highest lesion grades in these animals were PanIN 1 in one mouse and PanIN 2 in three mice. These results suggest that MMP7 impacts PDA progression in vivo.

Next, we performed a more extensive analysis to test at what stage MMP7 deletion impacts PDA progression in transgenic mice fated to develop PDA with 100% penetrance due to pancreatic deletion of one allele of p53 (Bardeesy et al., 2006) (*Pdx1-Cre^{Late}; Kras^{G12D}; p53$^{f/+}$; MMP7^{Het} or MMP7^{KO}*). We found that tumors identified at the time of death in *Pdx1-Cre^{Late}; Kras^{G12D}; p53$^{f/+}$; MMP7^{Het}* mice were significantly larger than tumors observed in *Pdx1-Cre^{Late}; Kras^{G12D}; p53$^{f/+}$; MMP7^{KO}* mice (mean diameter of 1.6 cm compared to 1.0 cm, Figures 6C–6E). Furthermore, there were clear differences in disease stage between the *MMP7^{Het}* and *MMP7^{KO}* groups. Although *Pdx1-Cre^{Late}; Kras^{G12D}; p53$^{f/+}$; MMP7^{Het}* mice demonstrated the usual stigmata of end-stage PDA in the form of ascites, regional disease, and distant metastasis in a majority of cases, metastasis was dramatically reduced in *Pdx1-Cre^{Late}; Kras^{G12D}; p53$^{f/+}$; MMP7^{KO}* mice (Figures 6D and 6E and Table 1; Figures S5H and S5I). No *MMP7^{KO}* mice had obvious metastasis to lymph nodes, whereas 58% of *MMP7^{Het}* mice had lymph node involvement. Similarly, whereas only 12.5% of *MMP7^{KO}* mice had hepatic involvement, 66.7% of *MMP7^{Het}* mice had liver

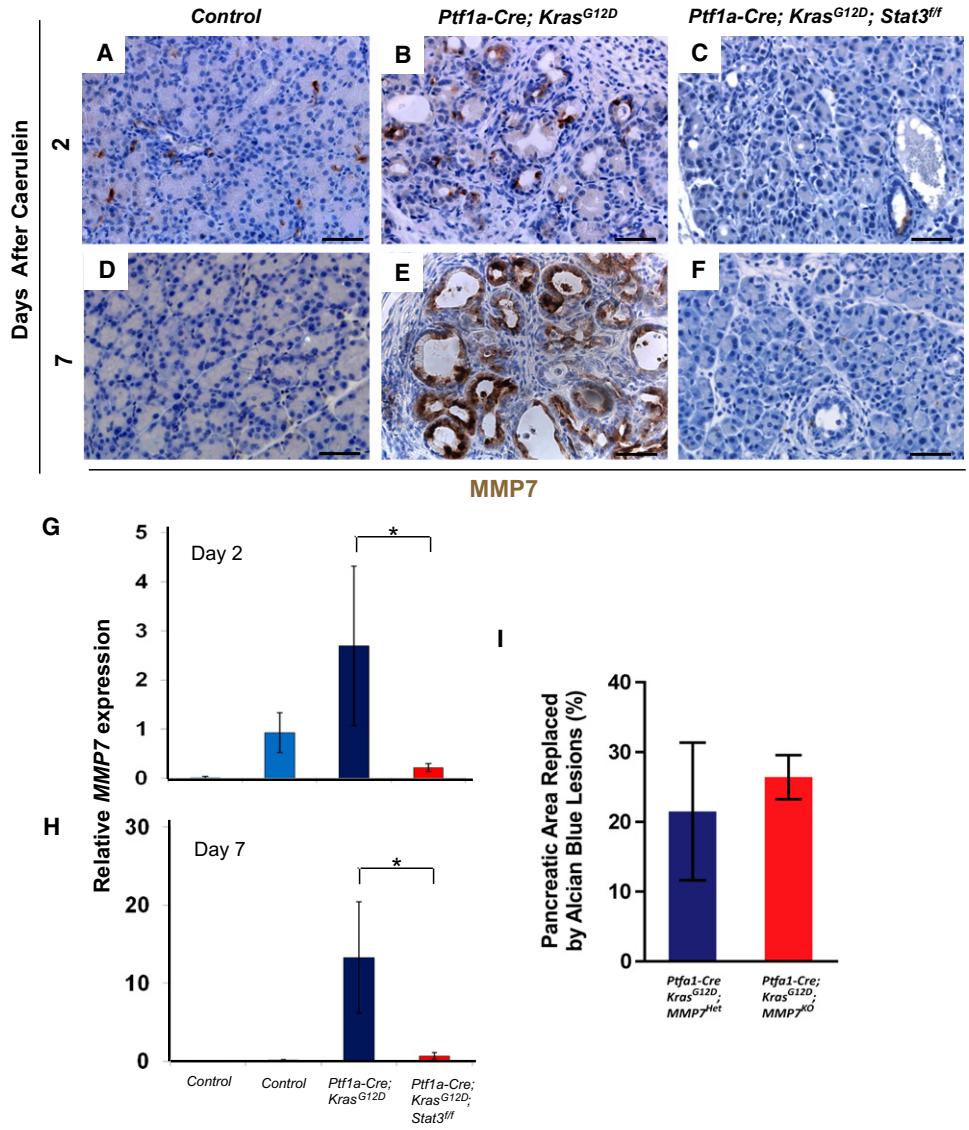

Figure 5. Stat3 Regulates MMP7 Expression during Kras-Driven PanIN Development following Acute Pancreatitis

(A–F) IHC for MMP7 in pancreata from control, *Ptf1a-Cre; Kras*^{G12D}, and *Ptf1a-Cre; Kras*^{G12D}*; Stat3*$^{f/f}$ mice, 2 or 7 days post-caerulein injections. (A–C) Two days post-caerulein. (D–F) Seven days post-caerulein.

(G and H) qPCR for *MMP7* expression. Mean ± SD (n = 3). *p < 0.05.

(I) Alcian blue-positive lesion in total pancreas area of *Ptf1a-Cre; Kras*^{G12D}*; MMP7*^{Het} (n = 7) and *Ptf1a-Cre; Kras*^{G12D}*; MMP7*^{KO} (n = 4) mice. Mean ± SD. Scale bars, 50 μm.

See also Figure S4.

metastasis. These striking differences suggest that MMP7 contributes to PDA progression by supporting tumor size and metastasis.

Despite the profound differences in disease staging between the *MMP7*^{Het} and *MMP7*^{KO} cohorts, there was surprisingly no difference in overall survival between mice in the two groups (Figure S5C). Although these results may, at first glance, imply that *MMP7* loss results in increased malignancy (i.e., death despite lower tumor burden), a more detailed analysis of the cohorts suggested otherwise. First, despite the lack of difference in overall survival, three of nine mice with complete *MMP7* loss survived beyond 36 weeks, whereas no *Pdx1-Cre*^{Late}*; Kras*^{G12D}*; p53*$^{f/+}$*; MMP7*^{Het} mice survived beyond 35 weeks and with the majority succumbing before 30 weeks (Figure S5C). Also, five of nine *Pdx1-Cre*^{Late}*; Kras*^{G12D}*; p53*$^{f/+}$*; MMP7*^{KO} mice appeared to have died from functional obstructive disease in the setting of relatively small primary tumors. Three mice, including one long-term survivor (Mouse 221, 37.9 weeks, Table S1), had a massively dilated stomach and duodenum with a relatively small pancreatic head tumor located at the transition point (Figures 6E; Figure S5E). Another long-term survivor (Mouse 328, 36.1 weeks, Table S1) had biliary outlet disease that resulted in jaundice and grossly dilated gallbladder and common bile duct that measured 5 mm (Figures S5F and

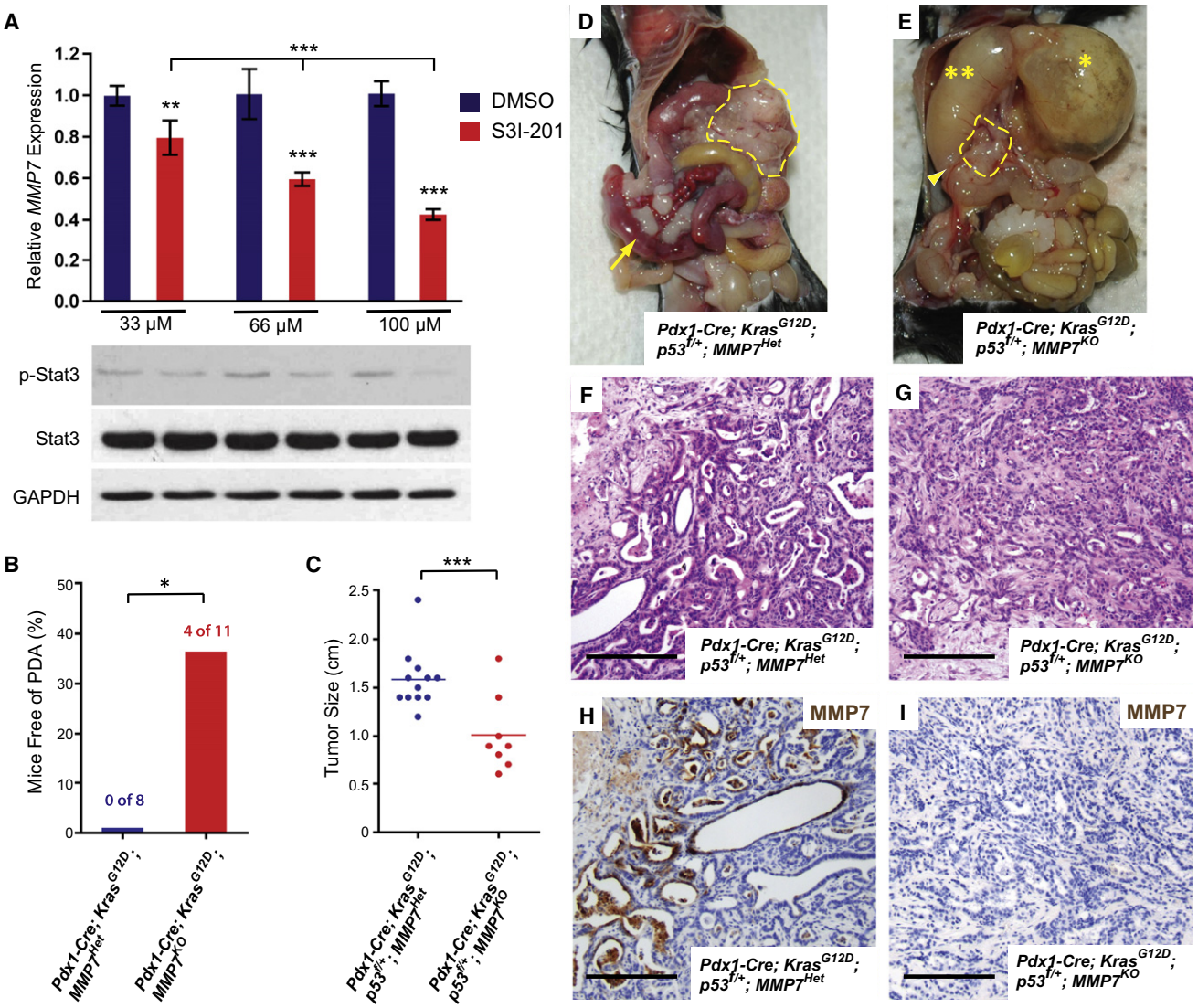

Figure 6. MMP7 Expression Is Mediated by Stat3 in PDA Cells and Contributes to Tumor Size and Metastasis

(A) Colo357 cells treated with compound S3I-201, a specific Stat3 inhibitor, or DMSO. Both *MMP7* (qPCR) and p-Stat3 (western blot) expression showed dose-dependent reduction. Mean ± SD ($n \geq 3$). **$p < 0.01$, ***$p < 0.001$.

(B) Number of *Pdx1-Cre; Kras^{G12D}; MMP7^{Het}* (n = 8) or *Pdx1-Cre; Kras^{G12D}; MMP7^{KO}* (n = 11) mice that were free of PDA found at time of death or at time of sacrifice at censor date. *$p \leq 0.05$ (chi-square test).

(C–I) Characterization of *Pdx1-Cre; Kras^{G12D}; p53$^{f/+}$; MMP7^{Het}* and *Pdx1-Cre; Kras^{G12D}; p53$^{f/+}$; MMP7^{KO}* mice. (C) Size of primary tumor at death (cm). Solid bar denotes mean. ***$p < 0.001$. (D) Representative photograph of disease seen in *Pdx1-Cre; Kras^{G12D}; p53$^{f/+}$; MMP7^{Het}* cohort. Large pancreatic head mass is outlined in yellow dashed line. Yellow arrow points to mesenteric lymphadenopathy. Liver nodules and ascites were also seen at necropsy in this mouse. (E) *Pdx1-Cre; Kras^{G12D}; p53$^{f/+}$; MMP7^{KO}* mouse. Yellow dashed line outlines relatively smaller pancreatic mass. Single asterisk (*) denotes massively dilated stomach, and double asterisks (**) denote dilated proximal duodenum. Arrowhead points to transition point (located next to tumor) from dilated proximal duodenum to decompressed distal bowel. There was no liver disease or obvious lymphadenopathy. (F and G) H&E. Histology from both genotypes is similar. (H) IHC for MMP7 *Pdx1-Cre; Kras^{G12D}; p53$^{f/+}$; MMP7^{Het}*, seen in patchy distribution. (I) No MMP7 staining in *Pdx1-Cre; Kras^{G12D}; p53$^{f/+}$; MMP7^{KO}* tumors. Scale bars, 100 μm. See also Figure S5.

S5G). Four of these five *MMP7^{KO}* animals did not have any evidence of distant disease and in all likelihood would have survived longer if not for these functional obstructions of the gastrointestinal tract. In contrast only two of ten *Pdx1-Cre^{Late}; Kras^{G12D}; p53$^{f/+}$; MMP7^{Het}* mice had significant obstruction, but one of these also had disseminated disease (Mouse 344, Table S1). Finally, the potential for relatively longer survival in mice with MMP7 deletion was confirmed in a second cohort of mice utilizing *Ptf1a-Cre* to activate expression of mutant Kras in the context of one deleted allele of p53 (*Ptf1a-Cre; Kras^{G12D}; p53$^{f/+}$; MMP7^{Het} or MMP7^{KO}*). Two out of four of these *Ptf1a-Cre; Kras^{G12D}; p53$^{f/+}$; MMP7^{KO}* mice survived beyond 34 weeks, whereas no *MMP7^{Het}* mice reached even 29 weeks of age (Figure S5D). This suggests that complete loss of *MMP7* may extend

Table 1. Disease Spectrum of *Pdx1-Cre; Kras^{G12D}; p53$^{f/+}$* Mice with Heterozygous or Homozygous *MMP7* Deletion

	Median Age at Death (week)	Mean Tumor Size (cm)	Duo/Stomach Obstruction	Biliary Obstruction	LN Disease[a]	Liver Mets[a]
Pdx1-Cre; Kras^{G12D}; p53$^{f/+}$; MMP7^{Het} (n = 12)	27.5	1.6 ± 0.3	8.3%	8.3%	58.3%	66.7%
Pdx1-Cre; Kras^{G12D}; p53$^{f/+}$; MMP7^{KO} (n = 8)	30.5	1.0 ± 0.4	37.5%	12.5%	0%	12.5%

See also Table S1. Duo, duodenum.
[a] $p < 0.05$, by chi-square test.

life span beyond the time frame observed in mice with intact MMP7 activity.

Although we observed significant differences in disease stage in our studies, we saw no differences in tumor histology in any of the cohorts we followed. All mice analyzed developed tumors that can be generally described as moderately to poorly differentiated (Figures 6F and 6G), regardless of MMP7 status. Of interest, MMP7 staining in *Pdx1-Cre^{Late}; Kras^{G12D}; p53$^{f/+}$; MMP7^{Het}* mice was seen in a patchy distribution throughout the tumor localizing to the luminal surfaces of glandular structures, consistent with staining patterns seen in human PDA (Figure 6H; Figure S5J). As expected, no MMP7 expression was found in PDA observed in *Pdx1-Cre^{Late}; Kras^{G12D}; p53$^{f/+}$; MMP7^{KO}* mice (Figure 6I).

Serum MMP7 Predicts Metastatic Disease in Humans

Previous studies with human PDA samples have shown that MMP7 levels correlate with tumor stage (Fukushima et al., 2001; Yamamoto et al., 2001) and overall survival (Jones et al., 2004; Yamamoto et al., 2001). To study the potential utility of serum MMP7 to provide preoperative clinical information, we accessed the University of Utah Huntsman Cancer Institute database and identified 101 patients with PDA in whom serum MMP7 levels were measured.

When patients were grouped by tumor class, all patients without lymph node involvement (N0) had serum MMP7 levels below 20.2 ng/ml, whereas patients with metastatic disease (M1; stage IV; please see Table S2 for the TNM staging system for PDA) had significantly higher levels (Figures 7A and 7B). However, regional lymph node involvement (N1; stage IIB) did not correlate to MMP7 levels, even though some patients with N1 did have MMP7 levels above 20.2 ng/ml.

Serum MMP7 levels were a significant predictor of survival, independent of whether MMP7 was used as a continuous or discrete variable, with 20 ng/ml as the threshold value (Table S3). With one exception, patients with MMP7 above this value had higher than expected risk of earlier death. Patients with PDA with MMP7 serum levels below 20 ng/ml had a median survival of 329 days, whereas those with levels above the threshold had a median survival of 114.5 days (Table S3). Kaplan-Meier survival analysis confirmed significantly decreased survival in the high-serum MMP7 group (Figure 7C; Table S4).

We found that tumor stage, distant metastasis, tumor resectability, and whether the patient received treatment were all favorable predictors of survival in univariate analyses (Table S3). Interestingly, serum MMP7 levels remained a significant predictor of survival, even after stratifying for treatment type and tumor class in multivariate analysis (Table S5). These data suggest that serum levels of MMP7 may be a useful clinical tool to predict survival in patients suffering from PDA.

DISCUSSION

Stat3 Contributes to Pancreatic Cancer Initiation

The reprogramming of normal pancreatic cells into those with a neoplastic fate is a key step in PDA initiation and deserves

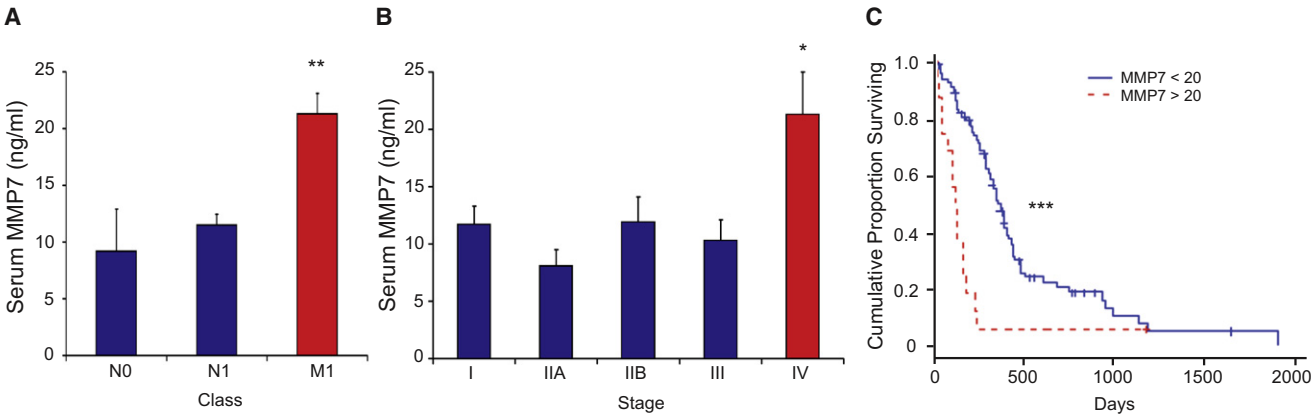

Figure 7. Serum MMP7 Level Predicts Metastatic Disease in Humans

(A and B) Serum MMP7 levels in patients with PDA. Mean ± SEM. (A) Patients stratified by class. N0, no regional lymph node involvement; N1, regional lymph node involvement; M1, distant metastasis. **$p < 0.01$. (B) Patients stratified by stage. (See Table S1 for definition of stage.) *$p < 0.05$ versus stages IIA, IIB, and III. (C) Kaplan-Meier curves showing significantly lower survival in patients with elevated serum MMP7 (red) than with low MMP7 (blue). Threshold is 20 ng/ml. ***$p < 0.0001$ by Mantel-Cox log rank test.

See also Tables S2–S5.

closer study. Mouse models have revealed that mutant Kras significantly alters pancreatic plasticity, and can drive acinar cells toward a ductal fate capable of giving rise to PDA (De La et al., 2008; Guerra et al., 2007; Habbe et al., 2008; Morris et al., 2010). Chronic pancreatitis is a potent risk factor for PDA development in humans, and pancreatitis induction in Kras-driven mouse models of PDA has revealed that inflammatory damage can promote (Guerra et al., 2007) and/or accelerate (Carriere et al., 2009; Morris et al., 2010) PanIN and PDA formation. Kras appears to drive acinar-derived PanIN development following pancreatitis by blocking normal regeneration in favor of a persistently dedifferentiated, ductal state that is permissive for PanIN formation. This finding suggests that assuming a dedifferentiated state may be a rate-limiting step during Kras-driven neoplastic reprogramming in the pancreas. In this study we induced both acute and chronic pancreatitis in transgenic mice expressing an oncogenic Kras allele in the context of epithelial Stat3 deficiency and found that these animals formed significantly fewer PanINs and retained more normal parenchyma than mice that had intact Stat3 signaling. These data implicate Stat3 as an important accomplice to Kras in diverting normal regeneration and maintaining the dedifferentiated ductal state initiated by pancreatitis.

Also, we found that in the presence of mutant Kras, Stat3 supports metaplasia-associated inflammation in a manner that is distinct from what is observed during normal pancreatic regeneration. We found that pancreatic epithelial Stat3 deletion results in attenuated inflammatory cell infiltration and reduced expression of proinflammatory cytokines. We speculate that the reduced cytokine expression in *Ptf1a-Cre; Kras^{G12D}; Stat3$^{f/f}$* mice is due to a combination of cell-autonomous effects of Stat3 deletion in the pancreatic epithelium and a reduced number of infiltrating inflammatory cells. Therefore, our data suggest that Stat3 plays both a critical cell autonomous role in maintaining the proliferative, persistently dedifferentiated epithelial state permissive for PDA precursor formation and also contributes to metaplasia-associated inflammation. Further work is required to enhance our understanding of how Stat3 activation contributes to immune cell recruitment. Also, aberrant cytokine expression has been shown to enhance tumorigenesis (Ernst et al., 2008), and additional studies are required to determine how epithelial-derived, Stat3-dependent cytokines, and those produced by infiltrating immune cells, contribute to PanIN development and PDA progression. Another unresolved question concerns the roles of the distinct immune cell types infiltrating during Kras-driven pancreatitis-induced PanIN formation. Summarily, these findings demonstrate that Stat3 activation in pancreatic epithelial cells plays a significant role linking pancreatitis and PDA initiation in vivo.

Although we have focused our efforts on caerulein-induced pancreatitis, it would be interesting to investigate whether Stat3 signaling is also important in other models of pancreatic damage. These include hereditary pancreatitis caused by a missense mutation in the trypsin gene (PRSS1) (Archer et al., 2006) or cytokine-induced pancreatitis caused by ectopic expression of interleukin-1β (Marrache et al., 2008). Another potential avenue of investigation is whether Stat3 interacts with the primary cilia, which are the cellular appendages known to regulate Hedgehog signaling. The elimination of the primary cilia produces pancreatic lesions that resemble those found in patients with chronic pancreatitis or cystic fibrosis (Cano et al., 2006).

MMP7 Contributes to PDA Size and Metastasis

Similar to findings obtained from other organs, we confirmed that Stat3 controls MMP7 expression in PDA cell lines. In this study we used several genetic models to demonstrate that MMP7 has a functional role in PDA progression by predominantly affecting tumor size and metastasis. Furthermore, we and others have found that MMP7 levels correlate with both tumor stage and survival (Jones et al., 2004; Yamamoto et al., 2001).

The mechanisms by which MMP7 affects PDA tumor size and metastasis need to be clarified in future studies. However, based on the number of substrates that MMP7 can act upon, multiple pathways may be involved. Epithelial basement membrane and extracellular components are MMP7 targets (Remy et al., 2006), suggesting that PDA cells lacking in MMP7 may not be able to escape the primary tumor site. Another possibility is the need for MMP7 activity for proper seeding at a potential metastatic site (Lynch et al., 2005). MMP7 can also influence the immune system by controlling chemokine gradients to affect neutrophil infiltration (Li et al., 2002). Such effects on immune cells may help propagate the feed-forward loop established by aberrant Stat3 signaling and perhaps contribute to the exuberant desmoplastic reaction seen in PDA. Finally, MMP7 may also affect the tumor angiogenic response because MMP7 knockout mice develop prostate cancers that have fewer tumor blood vessels that are also smaller in diameter (Littlepage et al., 2010). Future studies will have to address the exact mechanisms by which MMP7 functions in the context of PDA, a feat that is complicated by the observation that MMP7 is expressed only regionally throughout the tumors, suggestive of local differences in the malignant nature of individual cells throughout a tumor. The concept of the ''invasive front'' that possesses more malignant and metastatic potential was recently reviewed (Christofori, 2006), and it is conceivable that metastases first arose from these nests of MMP7-positive cells. This highly speculative concept is an intriguing idea that could be tested with the development of lineage-tracing systems dependent on MMP7 expression.

Clinical Implications

Our study implicates both Stat3 and MMP7 as potential targets for adjuvant therapy development. The involvement of Stat3 in PDA initiation highlights the potential of Stat3 as a therapeutic target for preventing inflammation-associated PDA formation at the earliest stage. A recent study confirmed the potential utility of targeting the Stat3 pathway by showing that JAK2 kinase inhibition and Stat3 knockdown both abrogated tumor xenograft growth in a variety of solid-organ cancers, including pancreas (Hedvat et al., 2009). The role of Stat3 in pancreatic cancer progression and metastasis in vivo requires future studies, though there are in vitro data showing the importance of Stat3 to PDA cell growth (Scholz et al., 2003).

The significant reduction of metastatic disease seen in *MMP^{KO}* mice has profound translational implications for patients with PDA. Currently, only about 20% of patients undergoing PDA resections survive beyond 5 years (Yeo et al., 1995). Our study

suggests that MMP7 inhibition may be a potential adjuvant therapy to block disease progression after resection. However, previous clinical trials involving MMP inhibitors have yielded disappointing results (Coussens et al., 2002). Part of the failure may have been because clinical studies enrolled patients with advanced-stage cancers, whereas successful results in mice occurred with treatment starting at earlier disease stage. It is possible that the beneficial effect of MMP7 inhibition is greater in prevention of progression of subclinical disease as in the adjuvant setting than in reduction of size of gross metastatic disease. Furthermore, some MMPs may have tumor-suppressive effects that could be compromised by nonspecific pan-MMP inhibition (Kessenbrock et al., 2010), suggesting that targeting specific MMPs may be a more effective therapeutic strategy.

Using a large repository of serum samples from human patients with PDA, we found that serum MMP7 levels significantly correlate with metastatic disease and survival, albeit with low sensitivity. This finding may be able to help guide patients and physicians with diagnostic and prognostic information to predict the survival benefit before patients undergo PDA resections. These considerations are particularly important because pancreatic resections entail complicated and lengthy operations that not only require months of recovery but carry significant risk for complications and death (Winter et al., 2006). Serum MMP7 levels might also be considered in decisions regarding postoperative adjuvant chemoradiation therapy after resection.

Recent sequencing studies have revealed that PDA cells possess a bewildering array of mutations (Jones et al., 2008). In order to develop improved therapeutics, it is essential to identify critical signaling nodes that PDA cells depend upon for growth and spread. Our study implicating Stat3 and MMP7 as key mediators of PDA initiation and progression not only points to potentially novel targets for future therapies but also demonstrates the utility of the Kras/inflammation system as an in vivo model to dissect complex signaling pathways important to pancreatic carcinogenesis.

EXPERIMENTAL PROCEDURES

Mice

Experimental animals were generated by crossing *Ptf1a-Cre* (Dr. C. Wright, Vanderbilt University, Nashville, TN) (Kawaguchi et al., 2002), *Pdx1-Cre*^{Late} (Dr. P. Herrera, University of Geneva Medical School, Geneva) to mice expressing LSL-*Kras*^{G12D} (Dr. D. Tuveson, Cancer Research UK Cambridge Research Institute, Cambridge) (Hingorani et al., 2003), and *Stat3*^{flox} (Takeda et al., 1998), MMP7^{KO} (Crawford et al., 2002), or p53^{flox} (Bardeesy et al., 2006). Transgenic mice were in a mixed background. Littermates not expressing Cre as well as *Ptf1a-Cre* and *Stat3*$^{f/+}$ mice of the same age were used as control. All mouse experiments were performed under the approval of the UCSF IACUC.

Caerulein Treatment

Acute pancreatitis was induced by intraperitoneal (i.p.) caerulein injections (50 μg/kg; Sigma): one injection per hour for six injections and repeated 2 days later. Chronic pancreatitis was induced by 3 or 4 weeks of caerulein injections as previously described (Martinez-Romero et al., 2009; Strobel et al., 2007) with the following modifications. The 3-week protocol was six hourly i.p. injections (50 μg/kg) per day, 3 days per week, for 3 weeks. The 4-week protocol was one i.p. injection per day (70 μg/kg), 5 days per week, for 4 weeks. Animals were sacrificed 3 days after the final injection in the both chronic pancreatitis models.

Primary Acinar Tissue Culture

Primary acinar tissue cultures were prepared by modifying published protocols (Means et al., 2005). Transdifferentiation events were induced by addition of TGF-α (50 ng/ml; Chemicon International). Cultures were maintained in a 37°C and 5% CO_2 incubator for 5 days with medium replaced on days 1 and 3. Cultured acini were treated with 10 nM caerulein for 20 hr.

Human Serum MMP7 Measurements

Serum samples were obtained from 101 consecutive subjects with histologically or cytologically confirmed PDA for whom pretreatment serum samples were available. The cohort consisted of 46 women (median age 69 years, range 43–86) and 55 men (median age 64 years, range 42–86). Whole blood was collected prior to the patients receiving treatment. Serological MMP7 measurements were performed by ELISA (Quantikine Human MMP7 (total) Immunoassay; R&D Systems) according to the manufacturer recommendations except that serum samples were diluted 4-fold with sample diluent. All human subjects gave informed consent, and the study was approved by the Institutional Review Board of the University of Utah.

Statistical Analyses

Data are presented as mean ± SD or SEM as noted and subjected to unpaired Student's t test, unless otherwise noted. A p value < 0.05 was considered to be significant. Statistical analysis of clinical variables and serum MMP7 level methods are described in Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures, five tables, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.ccr.2011.03.002.

ACKNOWLEDGMENTS

We thank all of our colleagues who shared the indicated mouse lines as detailed in the Experimental Procedures section, Cecilia Austin for tissue processing and preparation, and all Hebrok lab members for helpful discussion. Imaging experiments were supported by the UCSF Diabetes and Endocrinology Research Center. Work in Hebrok Laboratory was supported by the Pancreatic Cancer Action Network and the NIH (CA112537). A.F. was supported by a postdoctoral Research Fellowship of the Japan Society for the Promotion of Science, the Klein Family Foundation, and the National Pancreas Foundation. S.C.W. was supported by the NIH under Ruth L. Kirschstein National Research Service Award F32 from the National Cancer Institute and the American College of Surgeons Resident Research Scholarship. Work at the University of Utah was supported by the NIH (R03 CA115225 to S.J.M. and P30CA042014 to the Huntsman Cancer Institute for core facilities), the Huntsman Cancer Institute Pancreas Cancer Research Program, and the Huntsman Cancer Foundation. The authors declare that no conflicts of interest exist.

Received: May 7, 2010
Revised: December 10, 2010
Accepted: March 1, 2011
Published: April 11, 2011

REFERENCES

Adachi, Y., Yamamoto, H., Itoh, F., Hinoda, Y., Okada, Y., and Imai, K. (1999). Contribution of matrilysin (MMP-7) to the metastatic pathway of human colorectal cancers. Gut *45*, 252–258.

Almoguera, C., Shibata, D., Forrester, K., Martin, J., Arnheim, N., and Perucho, M. (1988). Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. Cell *53*, 549–554.

Ancrile, B., Lim, K.H., and Counter, C.M. (2007). Oncogenic Ras-induced secretion of IL6 is required for tumorigenesis. Genes Dev. *21*, 1714–1719.

Archer, H., Jura, N., Keller, J., Jacobson, M., and Bar-Sagi, D. (2006). A mouse model of hereditary pancreatitis generated by transgenic expression of R122H trypsinogen. Gastroenterology *131*, 1844–1855.

Bardeesy, N., Aguirre, A.J., Chu, G.C., Cheng, K.H., Lopez, L.V., Hezel, A.F., Feng, B., Brennan, C., Weissleder, R., Mahmood, U., et al. (2006). Both p16(Ink4a) and the p19(Arf)-p53 pathway constrain progression of pancreatic adenocarcinoma in the mouse. Proc. Natl. Acad. Sci. USA *103*, 5947–5952.

Bhatia, M., Brady, M., Kang, Y.K., Costello, E., Newton, D.J., Christmas, S.E., Neoptolemos, J.P., and Slavin, J. (2002). MCP-1 but not CINC synthesis is increased in rat pancreatic acini in response to cerulein hyperstimulation. Am. J. Physiol. Gastrointest. Liver Physiol. *282*, G77–G85.

Blinman, T.A., Gukovsky, I., Mouria, M., Zaninovic, V., Livingston, E., Pandol, S.J., and Gukovskaya, A.S. (2000). Activation of pancreatic acinar cells on isolation from tissue: cytokine upregulation via p38 MAP kinase. Am. J. Physiol. Cell Physiol. *279*, C1993–C2003.

Bollrath, J., Phesse, T.J., von Burstin, V.A., Putoczki, T., Bennecke, M., Bateman, T., Nebelsiek, T., Lundgren-May, T., Canli, O., Schwitalla, S., et al. (2009). gp130-mediated Stat3 activation in enterocytes regulates cell survival and cell-cycle progression during colitis-associated tumorigenesis. Cancer Cell *15*, 91–102.

Cano, D.A., Sekine, S., and Hebrok, M. (2006). Primary cilia deletion in pancreatic epithelial cells results in cyst formation and pancreatitis. Gastroenterology *131*, 1856–1869.

Carriere, C., Young, A.L., Gunn, J.R., Longnecker, D.S., and Korc, M. (2009). Acute pancreatitis markedly accelerates pancreatic cancer progression in mice expressing oncogenic Kras. Biochem. Biophys. Res. Commun. *382*, 561–565.

Christofori, G. (2006). New signals from the invasive front. Nature *441*, 444–450.

Coussens, L.M., Fingleton, B., and Matrisian, L.M. (2002). Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science *295*, 2387–2392.

Crawford, H.C., Scoggins, C.R., Washington, M.K., Matrisian, L.M., and Leach, S.D. (2002). Matrix metalloproteinase-7 is expressed by pancreatic cancer precursors and regulates acinar-to-ductal metaplasia in exocrine pancreas. J. Clin. Invest. *109*, 1437–1444.

De La, O.J.P., and Murtaugh, L.C. (2009). Notch and Kras in pancreatic cancer: at the crossroads of mutation, differentiation and signaling. Cell Cycle *8*, 1860–1864.

De La, O.J.P., Emerson, L.L., Goodman, J.L., Froebe, S.C., Illum, B.E., Curtis, A.B., and Murtaugh, L.C. (2008). Notch and Kras reprogram pancreatic acinar cells to ductal intraepithelial neoplasia. Proc. Natl. Acad. Sci. USA *105*, 18907–18912.

Ernst, M., Najdovska, M., Grail, D., Lundgren-May, T., Buchert, M., Tye, H., Matthews, V.B., Armes, J., Bhathal, P.S., Hughes, N.R., et al. (2008). STAT3 and STAT1 mediate IL-11-dependent and inflammation-associated gastric tumorigenesis in gp130 receptor mutant mice. J. Clin. Invest. *118*, 1727–1738.

Fendrich, V., Esni, F., Garay, M.V., Feldmann, G., Habbe, N., Jensen, J.N., Dor, Y., Stoffers, D., Jensen, J., Leach, S.D., and Maitra, A. (2008). Hedgehog signaling is required for effective regeneration of exocrine pancreas. Gastroenterology *135*, 621–631.

Fukushima, H., Yamamoto, H., Itoh, F., Nakamura, H., Min, Y., Horiuchi, S., Iku, S., Sasaki, S., and Imai, K. (2001). Association of matrilysin mRNA expression with K-ras mutations and progression in pancreatic ductal adenocarcinomas. Carcinogenesis *22*, 1049–1052.

Grady, T., Liang, P., Ernst, S.A., and Logsdon, C.D. (1997). Chemokine gene expression in rat pancreatic acinar cells is an early event associated with acute pancreatitis. Gastroenterology *113*, 1966–1975.

Grivennikov, S., Karin, E., Terzic, J., Mucida, D., Yu, G.Y., Vallabhapurapu, S., Scheller, J., Rose-John, S., Cheroutre, H., Eckmann, L., and Karin, M. (2009). IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. Cancer Cell *15*, 103–113.

Guerra, C., Schuhmacher, A.J., Canamero, M., Grippo, P.J., Verdaguer, L., Perez-Gallego, L., Dubus, P., Sandgren, E.P., and Barbacid, M. (2007). Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. Cancer Cell *11*, 291–302.

Habbe, N., Shi, G., Meguid, R.A., Fendrich, V., Esni, F., Chen, H., Feldmann, G., Stoffers, D.A., Konieczny, S.F., Leach, S.D., and Maitra, A. (2008). Spontaneous induction of murine pancreatic intraepithelial neoplasia (mPanIN) by acinar cell targeting of oncogenic Kras in adult mice. Proc. Natl. Acad. Sci. USA *105*, 18913–18918.

Hedvat, M., Huszar, D., Herrmann, A., Gozgit, J.M., Schroeder, A., Sheehy, A., Buettner, R., Proia, D., Kowolik, C.M., Xin, H., et al. (2009). The JAK2 inhibitor AZD1480 potently blocks Stat3 signaling and oncogenesis in solid tumors. Cancer Cell *16*, 487–497.

Heiser, P.W., Lau, J., Taketo, M.M., Herrera, P.L., and Hebrok, M. (2006). Stabilization of beta-catenin impacts pancreas growth. Development *133*, 2023–2032.

Hingorani, S.R., Petricoin, E.F., Maitra, A., Rajapakse, V., King, C., Jacobetz, M.A., Ross, S., Conrads, T.P., Veenstra, T.D., Hitt, B.A., et al. (2003). Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell *4*, 437–450.

Jensen, J.N., Cameron, E., Garay, M.V., Starkey, T.W., Gianani, R., and Jensen, J. (2005). Recapitulation of elements of embryonic development in adult mouse pancreatic regeneration. Gastroenterology *128*, 728–741.

Jones, L.E., Humphreys, M.J., Campbell, F., Neoptolemos, J.P., and Boyd, M.T. (2004). Comprehensive analysis of matrix metalloproteinase and tissue inhibitor expression in pancreatic cancer: increased expression of matrix metalloproteinase-7 predicts poor survival. Clin. Cancer Res. *10*, 2832–2845.

Jones, S., Zhang, X., Parsons, D.W., Lin, J.C., Leary, R.J., Angenendt, P., Mankoo, P., Carter, H., Kamiyama, H., Jimeno, A., et al. (2008). Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science *321*, 1801–1806.

Kawaguchi, Y., Cooper, B., Gannon, M., Ray, M., MacDonald, R.J., and Wright, C.V. (2002). The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. Nat. Genet. *32*, 128–134.

Kessenbrock, K., Plaks, V., and Werb, Z. (2010). Matrix metalloproteinases: regulators of the tumor microenvironment. Cell *141*, 52–67.

Lankisch, P.G., Breuer, N., Bruns, A., Weber-Dany, B., Lowenfels, A.B., and Maisonneuve, P. (2009). Natural history of acute pancreatitis: a long-term population-based study. Am. J. Gastroenterol. *104*, 2797–2805, quiz 2806.

Levy, D.E., and Lee, C.K. (2002). What does Stat3 do? J. Clin. Invest. *109*, 1143–1148.

Li, Q., Park, P.W., Wilson, C.L., and Parks, W.C. (2002). Matrilysin shedding of syndecan-1 regulates chemokine mobilization and transepithelial efflux of neutrophils in acute lung injury. Cell *111*, 635–646.

Li, Y., Du, H., Qin, Y., Roberts, J., Cummings, O.W., and Yan, C. (2007). Activation of the signal transducers and activators of the transcription 3 pathway in alveolar epithelial cells induces inflammation and adenocarcinomas in mouse lung. Cancer Res. *67*, 8494–8503.

Lin, L., Amin, R., Gallicano, G.I., Glasgow, E., Jogunoori, W., Jessup, J.M., Zasloff, M., Marshall, J.L., Shetty, K., Johnson, L., et al. (2009). The STAT3 inhibitor NSC 74859 is effective in hepatocellular cancers with disrupted TGF-beta signaling. Oncogene *28*, 961–972.

Littlepage, L.E., Sternlicht, M.D., Rougier, N., Phillips, J., Gallo, E., Yu, Y., Williams, K., Brenot, A., Gordon, J.I., and Werb, Z. (2010). Matrix metalloproteinases contribute distinct roles in neuroendocrine prostate carcinogenesis, metastasis, and angiogenesis progression. Cancer Res. *70*, 2224–2234.

Lowenfels, A.B., Maisonneuve, P., Cavallini, G., Ammann, R.W., Lankisch, P.G., Andersen, J.R., Dimagno, E.P., Andrén-Sandberg, A., and Domellöf, L. (1993). Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. N. Engl. J. Med. *328*, 1433–1437.

Lynch, C.C., Hikosaka, A., Acuff, H.B., Martin, M.D., Kawai, N., Singh, R.K., Vargo-Gogola, T.C., Begtrup, J.L., Peterson, T.E., Fingleton, B., et al. (2005). MMP-7 promotes prostate cancer-induced osteolysis via the solubilization of RANKL. Cancer Cell *7*, 485–496.

Malka, D., Vasseur, S., Bodeker, H., Ortiz, E.M., Dusetti, N.J., Verrando, P., Dagorn, J.C., and Iovanna, J.L. (2000). Tumor necrosis factor alpha triggers

antiapoptotic mechanisms in rat pancreatic cells through pancreatitis-associated protein I activation. Gastroenterology *119*, 816–828.

Marra, F. (2005). Renaming cytokines: MCP-1, major chemokine in pancreatitis. Gut *54*, 1679–1681.

Marrache, F., Tu, S.P., Bhagat, G., Pendyala, S., Osterreicher, C.H., Gordon, S., Ramanathan, V., Penz-Osterreicher, M., Betz, K.S., Song, Z., and Wang, T.C. (2008). Overexpression of interleukin-1beta in the murine pancreas results in chronic pancreatitis. Gastroenterology *135*, 1277–1287.

Martinez-Romero, C., Rooman, I., Skoudy, A., Guerra, C., Molero, X., Gonzalez, A., Iglesias, M., Lobato, T., Bosch, A., Barbacid, M., et al. (2009). The epigenetic regulators Bmi1 and Ring1B are differentially regulated in pancreatitis and pancreatic ductal adenocarcinoma. J. Pathol. *219*, 205–213.

Means, A.L., Meszoely, I.M., Suzuki, K., Miyamoto, Y., Rustgi, A.K., Coffey, R.J., Jr., Wright, C.V., Stoffers, D.A., and Leach, S.D. (2005). Pancreatic epithelial plasticity mediated by acinar cell transdifferentiation and generation of nestin-positive intermediates. Development *132*, 3767–3776.

Miyamoto, Y., Maitra, A., Ghosh, B., Zechner, U., Argani, P., Iacobuzio-Donahue, C.A., Sriuranpong, V., Iso, T., Meszoely, I.M., Wolfe, M.S., et al. (2003). Notch mediates TGF alpha-induced changes in epithelial differentiation during pancreatic tumorigenesis. Cancer Cell *3*, 565–576.

Miyatsuka, T., Kaneto, H., Shiraiwa, T., Matsuoka, T.A., Yamamoto, K., Kato, K., Nakamura, Y., Akira, S., Takeda, K., Kajimoto, Y., et al. (2006). Persistent expression of PDX-1 in the pancreas causes acinar-to-ductal metaplasia through Stat3 activation. Genes Dev. *20*, 1435–1440.

Morris, J.P., 4th, Cano, D.A., Sekine, S., Wang, S.C., and Hebrok, M. (2010). beta-catenin blocks Kras-dependent reprogramming of acini into pancreatic cancer precursor lesions in mice. J. Clin. Invest. *120*, 508–520.

Remy, L., Trespeuch, C., Bachy, S., Scoazec, J.Y., and Rousselle, P. (2006). Matrilysin 1 influences colon carcinoma cell migration by cleavage of the laminin-5 beta3 chain. Cancer Res. *66*, 11228–11237.

Sawey, E.T., Johnson, J.A., and Crawford, H.C. (2007). Matrix metalloproteinase 7 controls pancreatic acinar cell transdifferentiation by activating the Notch signaling pathway. Proc. Natl. Acad. Sci. USA *104*, 19327–19332.

Scholz, A., Heinze, S., Detjen, K.M., Peters, M., Welzel, M., Hauff, P., Schirner, M., Wiedenmann, B., and Rosewicz, S. (2003). Activated signal transducer and activator of transcription 3 (STAT3) supports the malignant phenotype of human pancreatic cancer. Gastroenterology *125*, 891–905.

Shrivastava, P., and Bhatia, M. (2010). Essential role of monocytes and macrophages in the progression of acute pancreatitis. World J. Gastroenterol. *16*, 3995–4002.

Siddiquee, K., Zhang, S., Guida, W.C., Blaskovich, M.A., Greedy, B., Lawrence, H.R., Yip, M.L., Jove, R., McLaughlin, M.M., Lawrence, N.J., et al. (2007). Selective chemical probe inhibitor of Stat3, identified through structure-based virtual screening, induces antitumor activity. Proc. Natl. Acad. Sci. USA *104*, 7391–7396.

Siveke, J.T., Lubeseder-Martellato, C., Lee, M., Mazur, P.K., Nakhai, H., Radtke, F., and Schmid, R.M. (2008). Notch signaling is required for exocrine regeneration after acute pancreatitis. Gastroenterology *134*, 544–555.

Strobel, O., Dor, Y., Stirman, A., Trainor, A., Fernandez-del Castillo, C., Warshaw, A.L., and Thayer, S.P. (2007). Beta cell transdifferentiation does not contribute to preneoplastic/metaplastic ductal lesions of the pancreas by genetic lineage tracing in vivo. Proc. Natl. Acad. Sci. USA *104*, 4419–4424.

Takeda, K., Kaisho, T., Yoshida, N., Takeda, J., Kishimoto, T., and Akira, S. (1998). Stat3 activation is responsible for IL-6-dependent T cell proliferation through preventing apoptosis: generation and characterization of T cell-specific Stat3-deficient mice. J. Immunol. *161*, 4652–4660.

Udayakumar, T.S., Stratton, M.S., Nagle, R.B., and Bowden, G.T. (2002). Fibroblast growth factor-1 induced promatrilysin expression through the activation of extracellular-regulated kinases and STAT3. Neoplasia *4*, 60–67.

Wilson, C.L., Heppner, K.J., Labosky, P.A., Hogan, B.L., and Matrisian, L.M. (1997). Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin. Proc. Natl. Acad. Sci. USA *94*, 1402–1407.

Winter, J.M., Cameron, J.L., Campbell, K.A., Arnold, M.A., Chang, D.C., Coleman, J., Hodgin, M.B., Sauter, P.K., Hruban, R.H., Riall, T.S., et al. (2006). 1423 pancreaticoduodenectomies for pancreatic cancer: a single-institution experience. J. Gastrointest. Surg. *10*, 1199–1210.

Yamamoto, H., Itoh, F., Iku, S., Adachi, Y., Fukushima, H., Sasaki, S., Mukaiya, M., Hirata, K., and Imai, K. (2001). Expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in human pancreatic adenocarcinomas: clinicopathologic and prognostic significance of matrilysin expression. J. Clin. Oncol. *19*, 1118–1127.

Yeo, C.J., Cameron, J.L., Lillemoe, K.D., Sitzmann, J.V., Hruban, R.H., Goodman, S.N., Dooley, W.C., Coleman, J., and Pitt, H.A. (1995). Pancreaticoduodenectomy for cancer of the head of the pancreas. 201 patients. Ann. Surg. *221*, 721–731.

Yu, H., Pardoll, D., and Jove, R. (2009). STATs in cancer inflammation and immunity: a leading role for STAT3. Nat. Rev. Cancer *9*, 798–809.

Yuan, G., Qian, L., Shi, M., Lu, F., Li, D., Hu, M., Yu, M., Shen, B., and Guo, N. (2008). HER2-dependent MMP-7 expression is mediated by activated STAT3. Cell. Signal. *20*, 1284–1291.