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Article

Stromal Elements Act to Restrain, Rather Than Support, Pancreatic Ductal Adenocarcinoma

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SUMMARY

Sonic hedgehog (Shh), a soluble ligand overexpressed by neoplastic cells in pancreatic ductal adenocarcinoma (PDAC), drives formation of a fibroblast-rich desmoplastic stroma. To better understand its role in malignant progression, we deleted Shh in a well-defined mouse model of PDAC. As predicted, Shh-deficient tumors had reduced stromal content. Surprisingly, such tumors were more aggressive and exhibited undifferentiated histology, increased vascularity, and heightened proliferation—features that were fully recapitulated in control mice treated with a Smoothened inhibitor. Furthermore, administration of VEGFR blocking antibody selectively improved survival of Shh-deficient tumors, indicating that Hedgehog-driven stroma suppresses tumor growth in part by restraining tumor angiogenesis. Together, these data demonstrate that some components of the tumor stroma can act to restrain tumor growth.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is notable for its profuse desmoplastic stroma comprised of activated fibroblasts, leukocytes, and extracellular matrix (Olive et al., 2009; Theunissen and de Sauvage, 2009). Studies utilizing in vitro assays and transplantation models have concluded that various stromal elements can enhance cancer cell proliferation and invasion (Hwang et al., 2008; Ikenaga et al., 2010; Lonardo et al., 2012; Vonlaufen et al., 2008; Xu et al., 2010). Various stromal cells can also contribute to immune suppression, further supporting tumor survival and growth. Together, these observations have led to the paradigm that tumor stroma functions to support and promote the growth of cancer (Hanahan and Weinberg, 2011). Based on this paradigm, the concept of "antistromal" therapy has emerged as a

Significance

Numerous therapies are being developed based on the premise that tumor stroma functions to promote cancer growth and invasion while simultaneously limiting the delivery of chemotherapy. Here, we demonstrate that depletion of stromal cells from pancreatic tumors—through genetic or pharmacological targeting of the Hh pathway—results in a poorly differentiated histology, increased vascularity and proliferation, and reduced survival. The study thus provides insight into the failure of Smoothened inhibitors, an antistromal therapy, in pancreatic cancer clinical trials. Moreover, we report that Hh-deficient tumors exhibit an increased sensitivity to VEGFR inhibition. Because poorly differentiated human pancreatic tumors are well-vascularized, in contrast to most pancreatic cancers, our results suggest that this patient subset may be susceptible to angiogenesis inhibitors.

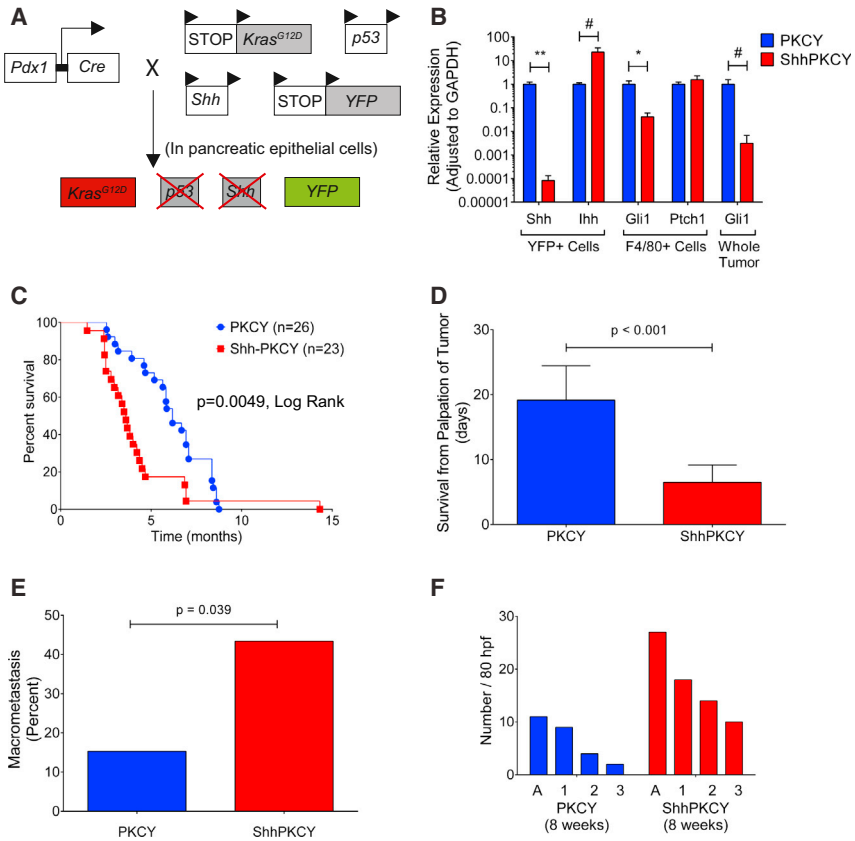

Figure 1. Sonic Hedgehog Behaves as a Tumor Suppressor in a Genetically Engineered Mouse Model of PDAC

(A) Schematic of the ShhPKCY mouse model used in this study, which employs the *Kras*^{G12D} (K), *Pdx1-Cre* (C), *p53* (P), *Rosa*^{YFP} (Y), and *Shh* alleles. Cre-mediated deletion results in simultaneous activation of *Kras*, deletion of one allele of *p53*, and both alleles of *Shh* and recombination of the *YFP* lineage label.

(B) Confirmation of Shh knockdown in ShhPKCY animals. Quantitative PCR analysis of Hedgehog signaling components in YFP$^+$ sorted pancreatic epithelial derived cells and F4/80$^+$ cells from tumors as well as whole tumor derived from PKCY (blue) and ShhPKCY (red) mice (n = 5 for each group; bars represent means ± SD).

(C) Kaplan-Meier survival analysis for PKCY (n = 26) and ShhPKCY mice (n = 23). $p < 0.005$ by Mantel-Cox (log rank) test.

(D) Survival of mice from first clinical palpation of tumor. Presence of tumor was confirmed by ultrasound. Bars represent means ± SD; $p < 0.001$.

(E) Fraction of mice with any macrometastatic lesion by visual inspection at the time of tissue harvest by genotype (n = 26 and 23 for PKCY and ShhPKCY mice, respectively). $p = 0.039$.

(F) Quantitation of acinar to ductal metaplasia (bar A) and PanIN lesions by grade (bars 1–3) in 8-week-old PKCY and ShhPKCY mice. Eighty nonoverlapping high powered fields in which pancreas tissue covered at least 90% of the entire field were analyzed (n = 3 for each group).

Data are presented as the aggregate number of ADMs and PanINs (by grade) for each genotype. #, $p < 0.05$; *, $p < 0.01$; **, $p < 0.001$ by two-tailed Student's t test. See also Figure S1.

promising, albeit unproven, therapeutic approach (Engels et al., 2012).

The Hedgehog (Hh) signaling pathway contributes to stromal desmoplasia in multiple solid tumor systems. Although normally absent in the adult pancreas, this developmental morphogen pathway is reactivated during inflammation and neoplasia. Both sonic hedgehog (Shh) ligand and downstream signaling are induced de novo in preneoplastic lesions and increase significantly during PDAC progression as the stromal compartment enlarges (Thayer et al., 2003). Although ectopic activation of Hh signaling within pancreatic epithelial cells can accelerate tumorigenesis (Mao et al., 2006; Morton et al., 2007; Pasca di Magliano et al., 2006), deletion of the Hh signaling mediator Smoothened (Smo) from the epithelium has no impact on PDAC progression (Nolan-Stevaux et al., 2009). Hence, canonical Hh signaling in PDAC is likely to occur in a paracrine fashion, whereby Shh ligand secreted from epithelial cells activates Smo-dependent downstream signaling in adjacent stromal cells, promoting desmoplasia (Bailey et al., 2008; Tian et al., 2009). The notion that Hh-dependent tumor stroma facilitates tumorigenesis is supported by the finding that inhibiting Hh signaling retards pancreatic tumor growth and metastasis in transplantation models (Bailey et al., 2008; Feldmann et al., 2008a, 2008b) and through our own study of the effects of acute inhibition of Smo in genetically engineered mouse models (Olive et al., 2009). In this study, we sought to interrogate the role of the tumor stroma by using both genetic deletion and long-term pharmacologic inhibition to eliminate stroma-promoting Hh signaling.

RESULTS

Shh Loss Accelerates PDAC Progression

To explore the role of paracrine Hh signaling in an autochthonous mouse model of PDAC, we conditionally deleted *Shh*, the predominant Hh ligand expressed in the diseased pancreas, by breeding Shh^{fl} alleles into the *Pdx1-Cre;Kras*$^{LSL\text{-}G12D/+}$*;p53*$^{fl/+}$*;Rosa26*$^{LSL\text{-}YFP/+}$ (PKCY) model (Rhim et al., 2012). Because *Pdx1-Cre* mediates recombination exclusively in the epithelial cells of the pancreas (Rhim et al., 2012), this combination of alleles results in the simultaneous activation of mutant *Kras* and deletion of *Shh* and *p53* within this tissue compartment (Figure 1A). *Shh* deletion had no effect on pancreatic development (Figure S1A available online), and the resulting *Shh*$^{fl/fl}$*;Pdx1-Cre;Kras*$^{LSL\text{-}G12D/+}$*;p53*$^{fl/+}$*;Rosa26*$^{LSL\text{-}YFP}$ (ShhPKCY) mice were born at expected Mendelian ratios and were phenotypically normal at birth.

To confirm the deletion of *Shh* in the pancreatic epithelial compartment, we performed transcriptional analysis on FACS-sorted yellow fluorescent protein (YFP$^+$) cells from 10- to 16-week-old PKCY and ShhPKCY mice (Rhim et al., 2012). As predicted, Shh transcripts were markedly reduced in YFP$^+$ pancreatic epithelial cells from ShhPKCY mice (Figure 1B).