Senescence-associated SIN3B promotes inflammation and pancreatic cancer progression

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Pancreatic ductal adenocarcinoma (PDAC) is strikingly resistant to conventional therapeutic approaches. We previously demonstrated that the histone deacetylase–associated protein SIN3B is essential for oncogene-induced senescence in cultured cells. Here, using a mouse model of pancreatic cancer, we have demonstrated that SIN3B is required for activated KRAS-induced senescence in vivo. Surprisingly, impaired senescence as the result of genetic inactivation of Sin3B was associated with delayed PDAC progression and correlated with an impaired inflammatory response. In murine and human pancreatic cells and tissues, levels of SIN3B correlated with KRAS-induced production of IL-1α. Furthermore, evaluation of human pancreatic tissue and cancer cells revealed that Sin3B was decreased in control and PDAC samples, compared with samples from patients with pancreatic inflammation. These results indicate that senescence-associated inflammation positively correlates with PDAC progression and suggest that SIN3B has potential as a therapeutic target for inhibiting inflammation-driven tumorigenesis.

Introduction
Pancreatic ductal adenocarcinoma (PDAC) is a deadly disease, with a median survival of approximately 6 months. Although surgery offers the potential for long-term survival, the typical presentation of advanced disease at diagnosis often precludes surgery as an option (1). Thus, it is important to understand the molecular bases for progression of the disease in order to devise improved approaches for early intervention. Molecular pathology studies of human specimens and the development of genetically engineered mouse models have demonstrated that PDAC arises from noninvasive precursor lesions known as pancreatic intraepithelial neoplasia (PanIN), which are driven by activating KRas mutations (1–5). While endogenous expression of oncogenic KRas (KRasG12D) leads to PanIN lesions in the mouse, the progression to PDAC requires additional mutations in genes such as p53 or Ink4a/Arf. These mutations promote cellular proliferation in the presence of an activated oncogene in vitro and are detected in high-grade PanIN lesions and PDAC in human specimens (2, 4, 6–8).

Cellular senescence is an irreversible cell-cycle arrest triggered by different stimuli, including oxidative stress, DNA damage, and oncogene activation, that prevents damaged or mutated cells from proliferating uncontrollably (9). Senescence is associated with low proliferation in a wide variety of cancer preneoplastic lesions, including lung adenoma, melanocytic naevi, and PanIN (8, 10–12). Through its ability to drive a potent cell-cycle exit, cellular senescence has long been considered a tumor-suppressive mechanism (9, 13, 14). Recently, it was demonstrated that senescent cells secrete a specific set of proinflammatory cytokines, including ILs (such as IL-1α, IL-1β, IL-6, and IL-8), chemokines, and growth factors, known collectively as the senescence-associated secretory phenotype (SASP) (15–17). In contrast with the tumor-suppressing impact of senescence-associated cell-cycle arrest, in vitro studies suggest that the SASP may promote a protumorigenic microenvironment (18–21). This notion is particularly relevant to PDAC, as its progression is intimately linked to inflammation (1, 22–26). The cellular factors that contribute to the oncogene-driven inflammation in pancreatic cells remain for the most part unknown, but recent studies have implicated IL-1α expression as an inducer of constitutive NF-κB activation and subsequent inflammation (27–30). Identifying unsuspected druggable targets driving KRAS-induced inflammatory response could lead to the development of novel therapeutic approaches for targeting the disease at stages where treatment may be most effective.

SIN3 proteins are noncatalytic scaffolding proteins that serve as evolutionarily conserved components of the histone deacetylase HDAC1/2 transcriptional repression complex (31, 32). We have recently demonstrated that mouse embryonic fibroblasts genetically inactivated for Sin3B are refractory to quiescence as well as oncogene-induced senescence (33–35). In addition, SIN3B levels are significantly upregulated in preneoplastic senescent lesions in a mouse model of PDAC (34). Unlike most perturbations that bypass oncogene-induced senescence, Sin3B inactivation is not sufficient to sensitize to oncogenic RAS-induced transformation, providing an experimental context in which these processes are uncoupled (34). Therefore, genetic inactivation of Sin3B represents a unique opportunity to dissect the physiological relevance of cellular senescence in pancreatic cancer progression. Using this approach, we demonstrate here that the inactivation of Sin3B in the pancreas prevents oncogenic KRAS-induced senescence, correlating with a defect in the proinflammatory phenotype, ultimately resulting in delayed pancreatic cancer progression.

Results
Genetic inactivation of Sin3B delays progression of KRASG12D-driven pancreatic lesions. To examine the potential significance of SIN3B upregulation in PanIN lesions (34), mice carrying a Sin3B condi-
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The national allele were first crossed with transgenic mice expressing the Cre recombinase under the control of the pancreas-specific p48 promoter (35, 36). Sin3B flox/+ p48-Cre and Sin3B flox/+ p48-Cre animals (hereafter referred to as Sin3B+/– and Sin3B–/–) were born at the expected ratio (data not shown). Sin3B p+/– animals were used as controls, as heterozygote animals are phenotypically indistinguishable from Sin3B+/+ animals through 16 months of age (data not shown and ref. 35). Similar to the control littermates, Sin3B+/– animals exhibited no gross abnormalities up to 1 year of age and presented normal pancreatic morphology (Supplemental Figure 1A and data not shown; supplemental material available online with this article; doi:10.1172/JCI72619DS1). Transcript analysis and immunohistochemistry (IHC) confirmed the efficient Sin3B inactivation in Sin3B–/– pancreata (Supplemental Figure 1, A and B). Of note, the residual Sin3B expression detected in Sin3B+/– pancreata likely reflects the heterogeneity of the pancreas tissue, which contains circulating blood cells that are not affected by p48-Cre–induced deletion (Supplemental Figure 1B). Finally, the exocrine and endo-

Figure 1
Genetic inactivation of Sin3B delays progression of KRASG12D-driven PanINs. (A) Representative 6-month-old pancreata from Sin3B+/– KrasG12D and Sin3B–/– KrasG12D mice. (B) H&E staining in 8- and 24-week-old Sin3B+/– KrasG12D and Sin3B–/– KrasG12D pancreata. (C) Immunohistochemistry for CK19 in 8-week-old Sin3B+/– KrasG12D and Sin3B–/– KrasG12D pancreata. (D) Alcian blue staining for mucin in 8-week-old Sin3B+/– KrasG12D and Sin3B–/– KrasG12D pancreata. (E) Number of duct-like structures per field in 6- to 8-week-old (6/8 weeks) and 24-week-old pancreata. Black bars represent Sin3B+/– KrasG12D pancreas and gray bars represent Sin3B–/– KrasG12D pancreas. (F) Number of normal ducts (ND), metaplastic lesions (ML), PanIN1 (P1), PanIN2 (P2), and PanIN3 (P3) per field in 6- to 8-week-old mice. Black bars represent Sin3B+/– KrasG12D pancreas, and gray bars represent Sin3B–/– KrasG12D pancreas. (G) Number of normal ducts, metaplastic lesions, PanIN1, PanIN2, and PanIN3 per field in 24-week-old mice. Black bars represent Sin3B+/– KrasG12D pancreas, and gray bars represent Sin3B–/– KrasG12D pancreas. (H) Kaplan-Meier Survival curve of Sin3B+/– KrasG12D mice (black, n = 42) and Sin3B–/– KrasG12D mice (red, n = 26). P < 0.05 at 300 days.
crine functions of the pancreas appeared largely unaffected by the genetic inactivation of Sin3B, as evidenced by the production of amylase and insulin in both Sin3Bp+/– and Sin3Bp–/– pancreata (Supplemental Figure 1, C and D). Thus, SIN3B appears largely dispensable for the development and normal function of the pancreas.

We next investigated whether Sin3B inactivation affects the progression of KRasG12D-driven PanINs by crossing Sin3Bp–/– mice with Cre-inducible Lox-STOP-Lox-KRasG12D mice (37). All genotypes (including Sin3Bp+/– KRaspG12D and Sin3Bp–/– KRaspG12D mice) were detected at the expected ratio (data not shown), and efficient Sin3B deletion was confirmed (Supplemental Figure 1, E and F). While the pancreata of 24-week-old Sin3Bp+/– KRaspG12D mice was granular with abundant pale nodules throughout, signaling the presence of numerous metaplastic and PanIN lesions, the pancreata of their Sin3B-deleted littermates (Sin3Bp–/– KRaspG12D) exhibited normal gross appearance (Figure 1A).Histologic examination of additional animals at different time points revealed PanINs surrounded by extensive fibrosis as early as 6 to 8 weeks and progressively higher grade lesions in Sin3Bp+/– KRaspG12D mice (Figure 1B), as previously reported (2). In stark contrast, pancreata from Sin3Bp–/– KRaspG12D mice comprised of mostly normal acini with rare metaplastic areas and early PanINs, up to 24 weeks of age (Figure 1B) (n > 10 mice for each genotype). These morphologic findings were corroborated by staining for CK19 and Alcian blue, which together mark mucin-containing PanIN cells (Figure 1, C and D). Quantification of CK19-positive structures indicated significantly fewer duct-like structures at 6 to 8 weeks (Figure 1, E and F) and a delay in the progression of the pancreatic lesions at 24 weeks (Figure 1, E and G) in Sin3Bp–/– KRaspG12D mice compared with their Sin3Bp+/– KRaspG12D littermates. Furthermore, the pancreata of Sin3Bp+/– KRaspG12D animals older than 6 months of age also displayed a significantly higher percentage of normal acini compared with Sin3Bp–/– KRaspG12D animals (P < 0.001) (Supplemental Table 2). Finally, mortality was also significantly delayed upon Sin3B deletion in KRas-expressing mice (Figure 1H, P < 0.05 at 300 days, and Supplemental Table 1). Of note, Sin3Bp–/– KRaspG12D mice finally developed PDAC later in life, as indicated by the drop in viability in these mice past 1 year (Supplemental Tables 1–3). Strikingly, most of the tumor cells in the Sin3Bp–/– KRaspG12D PDAC expressed SIN3B (Supplemental Figure 1G). Along with the near complete deletion of SIN3B observed in these tumors, most tumor cells were still stained with anti-SIN3B antibody despite the deletion of SIN3B, strongly suggesting that this protein might not be essential for the malignant transformation of pancreatic ducts.

Figure 2
Sin3B deletion does not affect the ADM process in a cell-autonomous manner. (A) H&E staining of paraffin sections of pancreas obtained from 8- and 24-week-old Sin3Bp+/– KRaspG12D and Sin3Bp–/– KRaspG12D mice. (B) Immunofluorescence on cryogenic sections of 6-week-old Sin3Bp+/– KRaspG12D and Sin3Bp–/– KRaspG12D pancreata using anti-α-amylase antibody (red). Nuclei are counterstained with DAPI in blue. (C) Immunofluorescence on cryogenic sections of Sin3Bp+/– KRaspG12D pancreas using anti-α-amylase antibody (red) and anti-CK19 antibody (green). Nuclei are counterstained with DAPI (blue). (D) Immunofluorescence on the 3D structure obtained from acinar cells isolated from Sin3Bp+/– KRaspG12D and Sin3Bp–/– KRaspG12D pancreata 1 day (d) and 5 days after plating in 3D culture using anti-α-amylase antibody (red) and anti-CK19 antibody (green). Nuclei are counterstained with topoisomerase (TOPRO, blue). (E) Number of sphere-like structures obtained from acinar cells isolated from Sin3Bp+/– KRaspG12D (black bars, n = 3) and Sin3Bp–/– KRaspG12D (gray bars, n = 3) pancreata at 1 day, 3 days, and 5 days after plating in 3D culture. Scale bars: 50 μm (A, B); 25 μm (C, D).
in younger mice (Supplemental Figure 1, E and F), this observation suggests that Sin3B inactivation is potently counterselected to allow PDAC progression. Thus, these results strongly support the notion that SIN3B promotes KRAS-driven cancer progression upon KRAS activation.

Sin3B deletion does not affect the ADM process in a cell-autonomous manner. Recent lineage-tracing studies indicate that the majority of human and mouse PanIN lesions result from the transdifferentiation of acinar cells into ductal cells through a process known as acinar-to-ductal metaplasia (ADM) (38–40). Based on our finding that KRAS-expressing, Sin3B-deleted pancreata contained significantly more acini and fewer PanINs compared with their Sin3B-expressing littermates, we investigated whether the ADM process was impaired. Histologic observations (Figure 2A) and the overall reduction of amylase staining (Figure 2B) strongly suggested that ADM had already occurred and was completed by 8 weeks of age in Sin3Bp+/–KRaspG12D pancreata. In contrast, ADM was still observed in 8-week-old Sin3Bp–/–KRaspG12D animals, as evidenced by frequent coexpression of amylase and CK19, suggesting that SIN3B delays the initiation or impairs the maintenance of ADM lesions in vivo (Figure 2C). To assess whether SIN3B regulates ADM in a cell-autonomous manner, acinar cells from 5 week-old Sin3B p+/– KRaspG12D mice and their Sin3B p–/– KRaspG12D littermates (Supplemental Figure 2A) were cultured using a 3D matrix (41). Amylase expression was detectable in acini of both genotypes upon initial isolation, and these cells progressively underwent ADM, as evidenced by an increase in CK19 staining and formation of sphere-like structures by day 5 (Figure 2D). The efficiency of sphere formation was not

**Figure 3**

Sin3B deletion impairs oncogene KRAS-induced senescence in vivo. (A) Quantitative PCR for Dec1, p15INK4B, p53, and p21 mRNA expression in pancreata obtained from 6- to 8-week-old Sin3Bp+/– KraspG12D (black bars, n = 3) and Sin3Bp–/– KraspG12D (gray bars, n = 3) mice. Sin3Bp–/– KraspG12D expression is relative to Sin3Bp+/– KraspG12D expression. *P < 0.05; **P < 0.0001. (B) Cryogenic sections stained for SA-β-gal in Sin3Bp+/– KraspG12D and Sin3Bp–/– KraspG12D pancreata. (C) Immunochemistry for p-HP1γ on paraffin sections of 8-week-old Sin3Bp+/– KraspG12D and Sin3Bp–/– KraspG12D pancreata. (D) Immunochemistry for DEC1 on paraffin sections of 8-week-old Sin3Bp+/– KraspG12D and Sin3Bp–/– KraspG12D pancreata. Arrows indicate positively stained cells. Scale bars: 50 μm. (E) Percentage of DEC1-positive ductal cells at 6 to 8 weeks and at 24 weeks in Sin3Bp+/– KraspG12D and Sin3Bp–/– KraspG12D pancreata. ND, not detected. *P < 0.05.
significantly affected by Sin3B deletion (Figure 2E and Supplemental Figure 2B). Together, these results indicate that ADM initiation and efficiency are not affected by Sin3B inactivation in vitro. However, because we found that the ADM process is delayed in vivo, we hypothesized that SIN3B could promote pancreatic cancer progression in a non–cell autonomous manner.

**Sin3B deletion impairs oncogene KRAS-induced senescence in vivo.** Recent work has established that senescent cells alter their microenvironment by secreting inflammatory factors, growth factors, and remodeling factors in a process called SASP. SASP triggers senescence in neighboring cells and mobilizes immune cells, but can also induce inflammation and thus promote cancer progression (18, 42, 43). This phenomenon is particularly relevant in pancreatic cancer, where inflammation is a well-established factor in tumor progression (23, 26). Based on our recent discovery that SIN3B is required for oncogene-induced senescence in mouse embryonic fibroblasts (34), we investigated whether Sin3B deletion affects the senescence process in pancreatic lesions. Consistent with a direct role of SIN3B in oncogenic KRAS-induced senescence in vivo, Sin3Bp–/– KrasG12D pancreata displayed significantly reduced expression levels of senescence markers, including Dec1, p15INK4B, p21, and p53 as compared with those observed in Sin3Bp+/– KrasG12D littermates (Figure 3A). Accordingly, senescence-associated β-gal (SA–β-gal) positivity (44) was markedly reduced in Sin3B-deficient pancreata (Figure 3B). Recent studies suggested that SA–β-gal positivity alone cannot be used to definitely specify senescent cells in the pancreas (11). We further probed, by IHC analysis, the presence of phosphorylated HP1γ (p-HP1γ) and DEC1,
which identify senescent cells in PanINs (11). As expected, we detected p-HP1γ-positive ductal cells in Sin3Bp+/– KRaspG12D lesions (Figure 3C). In contrast, p-HP1γ was undetectable in any of the rare lesions we could monitor in Sin3Bp–/– KRaspG12D mice (Figure 3C).

We validated these results in 24-week-old Sin3B p+/– KRaspG12D and Sin3Bp–/– KRas pG12D pancreata (Supplemental Figure 3A). To the same extent, DEC1 was not detectable in the Sin3B p–/– KRaspG12D lesions at 8 weeks compared with the same grade lesions in Sin3Bp+/– KRaspG12D pancreata (Figure 3D). To confirm that the dramatic reduction in senescent ductal cells observed in Sin3Bp–/– KRaspG12D pancreata is not merely due to the difference in the stage of the pancreatic lesions, we quantified the percentage of DEC1-positive ductal cells in lesions of each grade (Figure 3E). DEC1 expression was present significantly less in the residual metaplastic lesions and PanIN at 6 to 8 weeks in the Sin3Bp–/– KRaspG12D compared with Sin3Bp+/– KRaspG12D pancreata. Similarly, cells composing PanINs in 24-week-old Sin3B-deleted mice expressed significantly less DEC1 than those found in their Sin3B-expressing counterparts (Figure 3E). Importantly, the selected SIN3B-expressing cells that formed tumors in the Sin3Bp–/– KRaspG12D mice also expressed DEC1 (Supplemental Figure 3), reinforcing the correlation among SIN3B expression, senescence, and tumor progression.

Figure 5
SIN3B is required for cell-autonomous Il1α expression. (A) Pathway enrichment analysis using DAVID gene ontology for genes affected transcriptionally by Sin3B deletion. Pathway enrichment shows genes transcriptionally repressed (top) or induced (bottom) after Sin3B deletion in KrasG12D pancreas. GO analyses were made on genes that presented at least a 1.5-fold significant expression change in Sin3Bp–/– KrasG12D compared with Sin3Bp+/– KrasG12D pancreas (P < 0.05). Bars represent fold enrichment of the pathway in order of significance (P values) listed on the right of the bars. Functional categorizations of differentially expressed genes upon knockout of Sin3B were analyzed by Gene Ontology Biological Process (GO_BP) with the DAVID. (B) Heat map representation of enriched expression for cytokines, NF-κB, and IL-1α pathways in Sin3Bp+/– KrasG12D and Sin3Bp–/– KrasG12D pancreata and PDEC lines. Heat map represents top enriched genes in Sin3Bp–/– KrasG12D compared with Sin3Bp+/– KrasG12D pancreata. NES, normalized enrichment score (red, high expression; blue, low expression). (C) Quantitative PCR for Sin3B and Il1α mRNA expression in PDEC cultures obtained from Sin3Bp+/– KrasG12D (black bars) and Sin3Bp–/– KrasG12D (gray bars) pancreata. Sin3Bp–/– KrasG12D mRNA expression levels are relative to the Sin3Bp+/– KrasG12D expression levels. (D) Quantitative PCR for Il1α mRNA expression in acinar 3D cultures obtained from Sin3Bp+/– KrasG12D and Sin3Bp–/– KrasG12D mice 1 day, 3 days, and 5 days after plating in 3D culture. Black bars represent Sin3Bp+/– KrasG12D cells, and gray bars Sin3Bp–/– KrasG12D cells. Sin3Bp–/– KrasG12D mRNA expression levels are relative to the Sin3Bp+/– KrasG12D expression levels. *P < 0.01; **P < 0.05.
Loss of Sin3B mitigates oncogenic KRAS-driven inflammatory response in vivo. Since we observed markedly fewer senescent cells in the Sin3B-deleted pancreas and recent reports demonstrated that senescence-associated inflammation can be a protumorigenic event (18, 45), we investigated whether the inflammatory response was affected by Sin3B deletion. Sin3B<sup>p-/−</sup> KRaspG12D pancreata at 6 weeks of age presented extensive Masson trichrome staining, marking desmoplastic tissue (Figure 4A). In contrast, Sin3B<sup>p-/−</sup> KRaspG12D pancreata only exhibited localized desmoplasia associated with rare PanINs at up to 24 weeks (Figure 4A). Likewise, immune infiltration (CD4<sup>+</sup>, CD68<sup>+</sup>, and F4/80-positive cells) was limited and highly localized in Sin3B-deficient animals compared with controls (Figure 4, B and D), when analyzed before 24 weeks of age. Inflammation in evolving PanINs is associated with a positive feedback loop of cytokine secretion involving pancreatic cells, immune cells, and cancer-associated fibroblasts (CAF) (25, 26). Neoplastic cells mediate this process by secreting inflammatory cytokines, including IL-1α and IL-6, which are induced upon oncogenic KRAS expression through activation of the ERK1/2, STAT3, and NF-κB pathways (27). Consistently, cells from Sin3B<sup>p-/−</sup> KRaspG12D PanINs presented strong nuclear signals for activated STAT3 (p-STAT3), ERK1/2 (p-ERK1/2), and p65 (active P65), whereas staining was virtually absent in the corresponding Sin3B-deleted cells (Supplemental Figure 4A). Western blots on pancreata whole-cell extracts confirmed the strong decrease in STAT3 and ERK1/2 activation upon Sin3B deletion (Supplemental Figure 4B). These effects were associated with a strong decrease in the abundance of Il6 and Il1α transcripts in the Sin3B-deleted pancreas compared with their Sin3B-expressing littermates at 6 to 8 weeks and at up to 24 weeks for Il1α (Figure 4C and Supplemental Figure 4C). Thus, the delayed progression of PanINs caused by Sin3B deletion is associated with a pronounced impairment of the inflammatory response.
SIN3B is required for cell-autonomous IL1a expression. We next sought to determine whether SIN3B regulated KRAS-induced IL1a and IL6 expression in a cell-autonomous manner. To this end, gene expression was profiled in Sin3Bp+/– KRasG12D and Sin3Bp–/– KRasG12D pancreata and compared with that of cultured primary pancreatic duct epithelial cells (PDEC) of the same genotype. Gene Ontology (GO) and pathway analysis using the Database for Annotation, Visualization and Integrated Discovery (DAVID) functional annotation tool revealed that the immune response was significantly perturbed in the Sin3Bp+/– KRasG12D pancreata compared with their Sin3Bp+- expressing counterparts (Figure 5A and Supplemental Figure 5A). In contrast to whole pancreata, few Sin3Bp+-dependent changes were identified in primary PDEC cultures (Figure 5B and data not shown). For example, IL6 levels or the NF-κB and the IL-1R pathways were mostly downregulated in Sin3Bp+/– KRasG12D pancreata compared with Sin3Bp+- KRasG12D pancreata, but were not affected by the loss of Sin3B in PDEC cell lines (Figure 5B). Strikingly, IL1a stood out as one of the rare cytokines affected by Sin3B inactivation in both pancreata and PDEC lines (Figure 5B). Further validating these observations, a significant reduction in IL1a expression, but not IL6, was detected in 2 additional Sin3Bp+/– KRasG12D PDEC cell lines compared with their Sin3Bp+- KRasG12D PDEC counterparts (Figure 5C and Supplemental Figure 5B). We extended these findings in acinar cultures isolated from Sin3Bp+/– KRasG12D and Sin3Bp–/– KRasG12D mice, which revealed a specific reduction in IL1a transcript levels in the Sin3B-deleted primary cells undergoing ADM (Figure 5D and Supplemental Figure 5C). Thus, SIN3B functions in a cell-autonomous manner to promote oncogenic KRAS-driven IL1a expression in pancreatic cells.

IL1a expression correlates with senescence in the pancreas. Our findings thus far indicate that Sin3B deletion impairs senescence and IL1a induction and leads to a delay in PanIN initiation in the pancreas. IL1a has recently been shown to serve as an upstream regulator of SASP production (45). We next investigated whether the reduction of IL1a expression and the delay in lesion progression observed after Sin3B deletion were dependent on the senescence process. To do so, we generated Sin3B+/– KRasG12D Ink4a/Arffluc/+ and Sin3Bp+/– KRasG12D Ink4a/Arffluc/+ mice (hereafter referred to as Sin3Bp+/– KRasG12D Ink4afluc/+ and Sin3Bp–/– KRasG12D Ink4afluc/+), since deletion of the Ink4a/Arf locus results in the bypass of senescence (46, 47). As expected, Ink4a-deleted mice developed PDAC with a low latency, consistent with impaired proliferation control (Supplemental Figure 6, A and B). Both Sin3Bp+/– KRasG12D Ink4afluc/+ and Sin3Bp–/– KRasG12D Ink4afluc/+ mice exhibited advanced pancreatic cancer and diffuse CK19 expression (Supplemental Figure 6, B and C). Indeed, upon concomitant deletion of the Ink4a/Arf locus and KRAS activation, Sin3B deletion failed to confer any protection against cancer incidence, suggesting that in a context in which senescence is disabled, SIN3B does not affect cancer progression. Absence of SA-β-gal staining and lack of p15INK4B and Dec1 expression confirmed the bypass of senescence in the KRasG12D Ink4afluc/+ pancreas, regardless of the Sin3B status (Supplemental Figure 6, D and E). Interestingly, despite extensive immune cell infiltration, the bypass of senescence in KRasG12D Ink4afluc/+ pancreas correlated with the lack of IL6 and IL1a expression (Supplemental Figure 6, F and G). Thus, in mouse pancrea, the expression of IL1a is positively correlated with senescence. Moreover, the diminished tumor progression caused by Sin3B deletion is paradoxically associated with its ability to promote senescence, suggesting a protumorogenic role for SIN3B-associated senescence in PDAC progression.

Discussion

Senescence has commonly been considered a tumor-suppressive mechanism (48). Our recent discovery that the chromatin modifier SIN3B mediates RAS-induced senescence in mouse fibroblasts coupled with our observation that SIN3B is upregulated in RAS-driven PanINs initially hinted toward a tumor-suppressive role for SIN3B in the pancreas (34). We demonstrate here that genetic inactivation of the chromatin-associated SIN3B protein impairs the occurrence of oncogene-induced senescence in vivo and results in a cell-autonomous defect in KRAS-driven production of the proinflammatory IL-1α cytokine. While we cannot exclude the possibility that inflammation acts independently of senescence to promote pancreatic cancer progression, these results point, for what we believe is the first time, to a protumorigenic effect of senescence in PDAC progression in vivo.

In agreement with our observations, a recent study indicated that the senescence secretome promotes liver cancer progression (21). However, the molecular factors that link oncogene activation to SASP expression remain largely elusive. IL-1α production has been described as the initiating event in the establishment of the SASP in cultured cells (49). In addition, a recent study indicated that disabling the IL-1R/IL-1α pathway partially prevents oncogene-induced senescence and paracrine senescence (50). Finally, recent studies demonstrate a critical function for IL-1α in the initiation of the proinflammatory phenotype in the pancreas (26, 28–30). It is tempting to speculate that upon oncogene activation, SIN3B, through a cell-autonomous upregulation of IL-1α, drives SASP production and promotes a proinflammatory tumor microenvironment. Therefore, our study places the chromatin-associated SIN3B protein at the nexus of senescence and inflammation and further supports a central role for IL-1α in initiating proinflammatory cytokine production in PDAC. It will be of interest to assess whether SIN3B and the associated SASP...
mediate the protumorigenic inflammatory microenvironment
seen in other cancers and will therefore represent a new strategy
for the treatment of diverse inflammatory cancers.

Consistent with the results presented here in pancreatic cells,
we also observed a strong reduction in oncogenic RAS-driven Il1a
expression in Sin3b-null mouse embryonic fibroblasts, sug-
suggesting that SIN3B-dependent regulation of Il1a expression is not
tissue specific (G. David, unpublished observation). Given the
known repressive activity of SIN3B and its associated complex,
these results suggest that the effect of SIN3B on IL-1α production
is likely indirect. However, our transcriptomic analysis failed to
identify potential intermediate factors that positively regulate Il1a
levels, while being repressed by SIN3B. An alternative explana-
tion for this positive regulation of IL-1α by SIN3B may stem from our
recent observation that SIN3B participates in the release of poly-
merase pausing at active promoters, thus modulating the levels of
actively transcribed genes (51).

Intriguingly, all pancreata with PDAC were positive for SIN3B
expression regardless of the Sin3b status. Kawaguchi and col-
leagues previously described the p48 locus as being transcription-
ally silent in approximately 5% of pancreatic ductal cells (36).
Coupled with our observation of a strong counterselection of
Sin3b-deleted pancreatic cells during PDAC progression, it seems
likely that residual Sin3b-positive cells had an advantage over
Sin3b-null cells, further supporting the protumorigenic role of
SIN3B in our PDAC mouse model.

Nevertheless, in an Ink4a/Arf-deleted mouse model, absence
of SIN3B did not confer a survival advantage and Sin3b-deleted
pancreata developed PDAC to the same extent as control ani-
imals demonstrating the requirement of senescence for SIN3B to
promote PDAC progression. Loss of the Ink4a/Arf locus occurs
in more than 80% of sporadic human PDAC (1). While SIN3B
inhibition may not be a viable therapeutic approach for these
patients, our results identify SIN3B inhibition as a potential new
avenue of treatment for PDAC patients with an intact
Ink4a/Arf locus. Accordingly, HDAC inhibitors are currently being tested
in association with radiotherapy or chemotherapy in phase I and
phase II clinical trials in patients with local, advanced, and meta-
static disease (52–54). Furthermore, IL-1α is a central cytokine
expressed after acute pancreatitis (55) and preliminary data in
our laboratory show that IL-1α positively correlates with ADM
maintenance in a mouse pancreatitis model (M. Riedland and G.
David, unpublished observations). Since repeated episodes of
acute pancreatitis can progress to chronic pancreatitis, a well-
known risk factor for PDAC (56), mitigating the effects of acute
pancreatitis may offer an approach for lowering the frequency of
PDAC in the population. In this regard, inhibiting IL-1α pro-
duction in the pancreas via the use of SIN3B/HDAC1/2 complex
inhibitors could serve as a novel targeted approach for prevent-
ing inflammation and facilitating regeneration of the pancreas
following acute pancreatitis in human patients. Overall, our
study solidifies the relationship between chromatin modifiers
and cancer progression, while suggesting a protumorigenic func-
tion of senescence in vivo.

Methods
Acinar cell isolation and culture. Primary acinar cell cultures were prepared
by modifying published protocols (41, 57, 58). Cultures were main-
tained on Matrigel (BD Biosciences) using RPMI 1640 Medium (Gibco;
Invitrogen) supplemented with 10% FBS, penicillin G, streptomycin,
0.1 mg/ml soybean trypsin inhibitor (Sigma-Aldrich), 1 μg/ml dexametha-
sone (Sigma-Aldrich), 2% Matrigel (BD Biosciences), and 1% glucose (for
details, see Supplemental Methods).

Animal models. The LSL-KRasG12D mice were gifts from Tyler Jacks (Massachu-
setts Institute of Technology, Cambridge, Massachusetts, USA). Dafna Bar-
Sagi (NYU Langone Medical Center, New York, New York, USA) provided the
p48-Cre mice. The Ink4a−/− mice were gifts from Ron DePinho (MD Anderson
Cancer Center, Houston, Texas, USA) and Nabeel Bardeesy (Massachusetts
General Hospital, Boston, Massachusetts, USA). The LSL-KRasG12D, p48-Cre,
Ink4a−/−, and Sin3b−/− strains have been described previously (2, 35–37).
The strains were mated to obtain mice with the correct genotypes. All
animals were maintained in a mixed C57BL/6-FVB background.

Cell culture. BxPc3 and AsPc1 human pancreatic cancer cell lines were
provided by Dafna Bar-Sagi (NYU Langone Medical Center). Cells were cul-
tured in RPMI, 10% fetal bovine serum, sodium pyruvate, hepes, penicillin,
and streptomycin. The cultures were maintained in 5% CO₂ at 37°C.

Gene expression microarray analysis. Total RNA from Sin3b+/−, Sin3b−/−, and
Sin3b+/−KRasG12D pancreata (2 pancreata for each genotype) was prepared and
subjected to Gene Expression Console and Gene pattern software for the calculation of average expression levels of
each chromosome, with each array normalized with the robust multichip
array (RMA) algorithm. GO analysis was then performed by uploading the
microarray data to DAVID. The complete microarray data set is available
from GEO (GSE54197).

Human pancreatic tissue samples. A pilot TMA of 20 patients with pancre-
atic cancer and 10 control patients was created. From each patient with
pancreatic adenocarcinoma, cores of normal pancreas, pancreatitis, low-
grade PanIN lesion, high-grade PanIN lesion, tumors from most cellular
areas, tumors from most desmoplastic area, and metastatic tumors to the
lymph nodes were submitted. As controls, patients with pancreas resection
for neuroendocrine tumor, solid pseudo papillary neoplasm, serous cyst
adenoma, or metastatic tumors to the pancreas were used. The nature of
the lesions was confirmed by a pathologist for each core. Each TMA con-
sisted of 6 to 7 patients and 2 to 3 controls. The core diameter was 2 mm.
Sections (5 μm) were cut from formalin-fixed paraffin-embedded samples
for the purpose of IHC. For orientation, a core of liver tissue is used.

Histology and IHC. Mouse pancreata were fixed overnight in 10% formalin
(Fisher) and processed for paraffin embedding. For histol-
ogy, deparaffinized sections (5 μm) were stained with Gill’s hematoxylin
(Richard-Allan Scientific) and eosin Y (Protocol) followed by an alcohol
dehydratation series and mounting (Permount; Fisher). Trichrome stain-
ing was performed at the NYU School of Medicine Histopathology Core
Facility. For Alcin blue staining, deparaffinized sections (5 μm) were
stained with Alcin blue solution for 30 minutes at room temperature and
counterstained with Gill’s hematoxylin; this was followed by an alcohol
dehydratation series and mounting (Permount; Fisher). For IHC, depara-
ffinized sections (5 μm) were dehydrated and quenched in 1% hydrogen
peroxide/methanol for 15 minutes, and antigen retrieval was performed
in 10 mM sodium citrate and 0.1% Tween-20 (pH 6.0) for 15 minutes in
a microwave oven. Blocking was done in 10% serum, 1% BSA, and 0.1%
Tween-20 for 1 hour at room temperature, followed by incubation with the
primary antibodies diluted in 1% BSA overnight at 4°C. The following
primary antibodies were used: rabbit anti-SIN3B (Santa Cruz A-20;
Novus Biologicals)’ rabbit anti-α-amylase (Sigma-Aldrich); rat anti-CK19
(TromaIII, developed by Rolf Kemler and obtained from Developmental
Studies Hybridoma Bank); rabbit anti-p-STAT3 (Tyr705) (D3A7) (Cell
1. Hezel AF, Kimmelman AC, Stanger BZ, Bardeesy (Developmental Studies Hybridoma Bank) and rabbit anti–α-amylase (Invitrogen), diluted in 1% BSA for 1 hour, and mounted using Vectashield Slides were then incubated with Alexa Fluor–labeled secondary antibodies against primary antibodies diluted in 1% BSA/0.1% Tween-20 overnight at 4°C. Immunofluorescence: Pancreata were removed, fixed in 4% paraformaldehyde overnight, washed in a 10% sucrose solution, and snap-frozen in OCT compound (Tissue-Tek). Frozen sections of 5 μm were air dried, permeabilized with 0.2% Triton X-100 for 20 minutes, and blocked with 10% serum/0.1% Tween-20 for 1 hour. Slides were incubated with primary antibodies diluted in 1% BSA/0.1% Tween-20 overnight at 4°C. Slides were then incubated with Alexa Fluor–labeled secondary antibodies (Invitrogen), diluted in 1% BSA for 1 hour, and mounted using Vectashield mounting medium with DAPI (Vector Laboratories). Slides were examined on a Zeiss Axiosvert 200M microscope. The following antibodies were used: rat anti-α-CK19 (TromAlIII, developed by Rolf Klemmer and obtained from Developmental Studies Hybridoma Bank) and rabbit anti–α-amylase (Sigma-Aldrich). Slides were examined on Zeiss Axioslager A2. The 3D immunofluorescence on ADM spheres was performed as previously described (46). The spheres were counterstained with topoisomerase, and the slides were analyzed with a Zeiss LSM510 microscope.

Isolation and culture of PDEC. Isolation and culture of PDEC were performed as described (46, 57). PDEC were isolated from 5-week-old mice and propagated in Matrigel (BD Biosciences).

RT-PCR and quantitative RT-PCR. Extraction of total RNA from Pancreas and PDECs was performed using RNeasy Mini Kit (QIAGEN). For the pancreas, a piece was snap-frozen and ground, and the frozen powder was added to the RNeasy lyss buffer. Reverse transcription was done using Moloney murine leukemia virus polymerase and oligo(dT) primers. RT-PCR analyses were done using Taq DNA polymerase (5 Prime) and dNTP (Promega). Quantitative RT-PCR analyses were done using the SYBR Green method (for primer sequences see Supplemental Methods), and samples were run on the Bio-Rad iCycler MqS. Expression levels were normalized to GAPDH. Results were reported as relative to the abundance of Sin3B+ or Sin3B− KrasG12D transcripts.

SA–β-gal assay. Frozen sections of pancreatic tissue were fixed with 2% formaldehyde/0.2% glutaraldehyde in PBS for 3 to 5 minutes, washed with PBS, and stained at 37°C for 12 to 16 hours in X-Gal solution (1 mg/ml X-Gal, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, and 1 mM MgCl2 in PBS at pH 6.0). After counterstaining with eosin (Richard-Allan Scientific), slides were subjected to an alcohol dehydration series and mounted with Permount (Fisher) and counterstained with eosin (Protocols). Slides were examined on Zeiss Axioslager A2.

Statistics. Data were analyzed by Student’s t test (unpaired, 2-tailed) and results were considered significant at P < 0.05. Results are presented as mean ± SEM. Survival curves were plotted by the Kaplan-Meier method and compared by the log-rank test.

Study approval. All human tissues were collected using a protocol approved by the NYU School of Medicine Institutional Review Board and were obtained after informed consent. All animal procedures were approved by the NYU School of Medicine Institutional Animal Care and Use Committee.

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