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Pancreatic STAT5 activation promotes Kras^{G12D}-induced and inflammation-induced acinar-to-ductal metaplasia and pancreatic cancer

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ABSTRACT

Objective Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy because it is often diagnosed at a late-stage. Signal transducer and activator of transcription 5 (STAT5) is a transcription factor implicated in the progression of various cancer types. However, its role in KRAS-driven pancreatic tumourigenesis remains unclear.

Design We performed studies with *LSL-Kras*^{G12D}; *Ptf1a-Cre*^{ERT} (KC^{ERT}) mice or LSL-Kras^{G12D}; *LSL-Trp53*^{R172H}; *Pdx1-Cre* (KPC) mice crossed with conditional disruption of STAT5 or completed deficiency interleukin (IL)-22. Pancreatitis was induced in mice by administration of cerulein. Pharmacological inhibition of STAT5 on PDAC prevention was studied in the orthotopic transplantation and patient-derived xenografts PDAC model, and KPC mice.

Results The expression and phosphorylation of STAT5 were higher in human PDAC samples than control samples and high levels of STAT5 in tumour cells were associated with a poorer prognosis. The loss of STAT5 in pancreatic cells substantially reduces the KRAS mutation and pancreatitis-derived acinar-to-ductal metaplasia (ADM) and PDAC lesions. Mechanistically, we discovered that STAT5 binds directly to the promoters of ADM mediators, hepatocyte nuclear factor (HNF) 1β and HNF4α. Furthermore, STAT5 plays a crucial role in maintaining energy metabolism in tumour cells during PDAC progression. IL-22 signalling induced by chronic inflammation enhances KRAS-mutant-mediated STAT5 phosphorylation. Deficiency of IL-22 signalling slowed the progression of PDAC and ablated STAT5 activation.

Conclusion Collectively, our findings identified pancreatic STAT5 activation as a key downstream effector of oncogenic KRAS signalling that is critical for ADM initiation and PDAC progression, highlighting its potential therapeutic vulnerability.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Somatic KRAS mutations and chronic pancreatitis are risk factors for acinar-to-ductal metaplasia (ADM) and pancreatic ductal adenocarcinoma (PDAC). Identifying the downstream targets of inflammation and oncogenic KRAS may reveal new therapeutic strategies for preventing inflammation-linked and KrasG12D-linked pancreatic carcinogenesis.

WHAT THIS STUDY ADDS

⇒ We investigated the role of pancreatic tissue-derived signal transducer and activator of transcription 5 (STAT5) signalling. STAT5 is an important downstream signal of inflammation signal interleukin-22 and KRAS mutation in pancreatic cells and promotes the progression of pancreatic cancer by mediating cellular energy metabolism and ADM formation.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Pancreatic STAT5 signal promotes cell energy metabolism and pancreatic cancer progression, which is a potential therapeutic target for pancreatic cancer.

INTRODUCTION

The tumourigenesis of pancreatic ductal adenocarcinoma (PDAC) is believed to progress stepwise from preneoplastic mucinous lesions to invasive adenocarcinoma.[1] Despite effective combination therapies and new diagnostic methods, PDAC remains the second leading cause of cancer-related deaths, largely because it is often diagnosed at an advanced stage, precluding surgery.[1 2] Therefore, elucidating the mechanisms underlying early pancreatic tumourigenesis is of paramount importance to identify screening biomarkers and develop preventive interventions.

Acinar-to-ductal metaplasia (ADM), a process of pancreatic regeneration after an injury that is reversible once the injury is resolved and exhibits tumourigenic potential when irreversible in response to KRAS activity, is the initial morphological change in pancreatic intraepithelial neoplasia (PanIN)-derived PDA formation.[3–6] In addition to somatic KRAS mutations, chronic pancreatitis is a risk factor for ADM and PDAC.[7–10] The mutant KRAS^{G12D} in the mouse pancreas is sufficient to initiate ADM and lock metaplastic cells into a ductal state; however, this occurs with low penetrance and long latency. Therefore, the effective induction and transformation of pancreatic acinar cells requires secondary

events such as inflammatory cytokines or growth factor signalling.[3 7] These chronic transcriptomic alterations, such as signal transducer and activator of transcription (STAT)3, nuclear factor (NF)-κB and transforming growth factor (TGF)-β signalling, may mediate persistent epigenetic and phenotypic modifications in acinar cells, particularly in the context of additional genetic insults like KRAS activation.[11–13] Most clinical trials targeting KRAS activation in patients with pancreatic cancer have demonstrated failure or conflicting results in recent years, mainly due to the presence of a large number of activated KRAS mutations and the special structural characteristics of KRAS in pancreatic cancer.[3 14 15] Therefore, identifying the drivers of this maladaptive acinar stress response and the downstream targets of oncogenic KRAS that promote ADM progression may reveal new therapeutic strategies for preventing inflammation-linked and $Kras^{G12D}$-linked pancreatic carcinogenesis.

Emerging pancreatic cancer therapies target energy metabolism,[16] because the growth of pancreatic tumour cells is strongly dependent on oxidative phosphorylation (OXPHOS) and glycolysis.[17 18] Mitochondrial hyperpolarisation negatively impacts acinar cell function in a cerulein-induced pancreatitis model.[19] In vitro activation of KRAS mutant isoforms in acinar cells affects glycolysis and mitochondrial oxidation, leading to stress and epidermal growth factor receptor (EGFR) signalling upregulation, thereby promoting the formation of precancerous lesions.[20] Pancreatic cancer cell lines exhibited elevated fatty acid β-oxidation (FAO) relative to OXPHOS.[21] Although improved energy metabolism is closely associated with pancreatic pathology, the regulatory signals of energy metabolism during the ADM and PanIN stages remain unclear, warranting further research to understand the complexity of metabolic processes during the ADM phenotype transition and PanIN stage.

STAT proteins play critical roles in carcinogenesis.[22] Aberrant STAT5 signalling, mostly due to constitutive activation, has been observed in various cancers.[23] Aberrant STAT5 signalling promotes the expression of target genes, such as cyclin D, and Bcl-2, that increases cell proliferation, survival, metastasis and resistance to anticancer therapies. STAT5 plays a crucial role in the development of the immune system and regulates immune responses of T cells and myeloid cells during tissue injury or inflammation.[24] Clinical studies and experiments on pancreatic cancer cell lines have indicated that STAT5 expression in pancreatic cancer cells promotes cancer cell proliferation and mediates chemoresistance,[25 26] suggesting its potential as a therapeutic target for pancreatic cancer. However, the function of STAT5 in KRAS mutation, inflammation-mediated ADM and PDAC metabolism has not been reported.

In the present study, we genetically deleted STAT5 in the pancreatic cells of murine pancreatic cancer models. Our findings demonstrate that STAT5 in pancreatic cells promotes energy metabolism by downstream KRAS signals and injury to boost the transcriptional activities required for ADM and subsequent tumour initiation. This finding is suggestive of an unsuspected pathway crucial for KRAS mutation-driven PDAC progression by promoting energy metabolism in PDAC cells.

MATERIALS AND METHODS

Mice

Pdx1-Cre (C), *Ptf1-Cre*^{ERT} (C^{ERT}), *Stat5a/b*-Floxed (*Stat5a/b* $^{FL/FL}$) (S), *LSL-Kras*$^{G12D/+}$ (K), *LSL-Trp53*^{R172H}(P), *Il22*$^{-/-}$, *Il22ra1*-floxed (*Il22ra*$^{FL/FL}$) mice were purchased from Jackson Laboratory. were purchased from GemPharmatech (Nanjing, China). *LSL-Kras*$^{G12D/+}$*; Pdx-1-Cre* (KC) mice and *LSL-Kras*$^{G12D/+}$*; Ptf1-Cre*^{ERT} (KC^{ERT}) mice were generated by crossbreeding *LSL-Kras*$^{G12D/+}$ mice with *Pdx-1-Cre* mice or *Ptf1-Cre*^{ERT} mice. *LSL-Kras*$^{G12D/+}$*; LSL-Trp53*^{R172H}*; Pdx-1-Cre* (KPC) mice were generated by crossbreeding *LSL-Kras*$^{G12D/+}$, *LSL-Trp53*^{R172H} *and Pdx-1-Cre* mice. KCS mice, KC; *Il22ra*$^{FL/FL}$ mice and KC; *Il22*$^{-/-}$ mice were generated by crossing KC transgenic mice with *Stat5a/b*$^{FL/FL}$, *Il22*$^{-/-}$ mice or *Il22ra1* $^{FL/FL}$ mice. KCS^{ERT} mice were generated by *Stat5a/b* $^{FL/FL}$ mice with KC^{ERT} mice. KPCS and KPC; *Il22*$^{-/-}$ mice were generated by crossbreeding KPC mice with *Stat5a/b* $^{FL/FL}$ mice or *Il22*$^{-/-}$ *mice*. C57BL/6J and nude mice were purchased from the Chinese Academy of Sciences (Shanghai, China). The NSG mice were purchased from GemPharmatech (Nanjing, China).

Human specimens

Freshly resected cancer and non-cancer tissues were obtained from patients diagnosed with PDAC between 2018 and 2023 at the Renji Hospital. Pancreatic tissues more than 2 cm away from the tumour margin were designated as non-cancer tissues. The PDAC tissue microarray was constructed as previously described, in which all samples were obtained from patients with PDAC who had undergone tumour resection between 2008 and 2018 at the Renji Hospital. Online supplemental table 1 summarises the clinical features of the patients. None of the patients with PDAC who underwent complete tumour resection had previously received adjuvant therapy. Informed consent was obtained from each patient, and the study was conducted in accordance with the ethical guidelines.

Additional methods and details are provided in online supplemental methods.

RESULTS

Pancreatic STAT5 activation is associated with adverse outcomes of pancreatic cancer

To investigate the role of STAT5 in PDAC, we conducted a retrospective analysis of samples from patients with PDAC. *STAT5A*/*STAT5B* messenger RNA and protein levels were substantially upregulated in the tumour tissues of patients with PDAC compared with those in normal pancreatic tissues (figure 1A,B). Real-time quantitative PCR (RT-PCR) results revealed that the expression of STAT5 within the pancreatic tissue was mainly $EPCAM^+$ tumour cells and $CD45^+$ immune cells, which was further confirmed by single-cell RNA sequencing analysis (figure 1C and online supplemental figure 1A,B). The primers used were listed in online supplemental table 2. Immunohistochemical (IHC) analysis suggested that STAT5 phosphorylated (p-STAT5) in both tumour and stromal cells (figure 1D,E). To further analyse the impact of STAT5 on pancreatic cancer, we distinguished tumour and stromal regions based on tumour morphology, and then grouped patients according to the level of p-STAT5 on tumour cell (TC) and stromal cell (SC), respectively. $TC^{p\text{-}STAT5\text{-}High}$ patients had lower survival than $TC^{p\text{-}STAT5\text{-}Low}$ patients (median survival: 482 days vs 767 days, HR=0.558, p=0.007), while $SC^{p\text{-}STAT5\text{-}High}$ patients had no significant difference in survival compared with $SC^{p\text{-}STAT5\text{-}Low}$ patients (median survival: 486 days vs 671 days, p>0.05), suggesting higher level of p-STAT5 in TCs was associated with a poorer prognosis among patients with PDAC (figure 1F and online supplemental figure 1C). In addition, patients showing $SC^{p\text{-}STAT5\text{-}Low}$ $TC^{p\text{-}STAT5\text{-}Low}$ had better outcome than high levels of both (median survival: 942 days vs 473 days, HR=0.44, p=0.004) (online supplemental figure 1D).

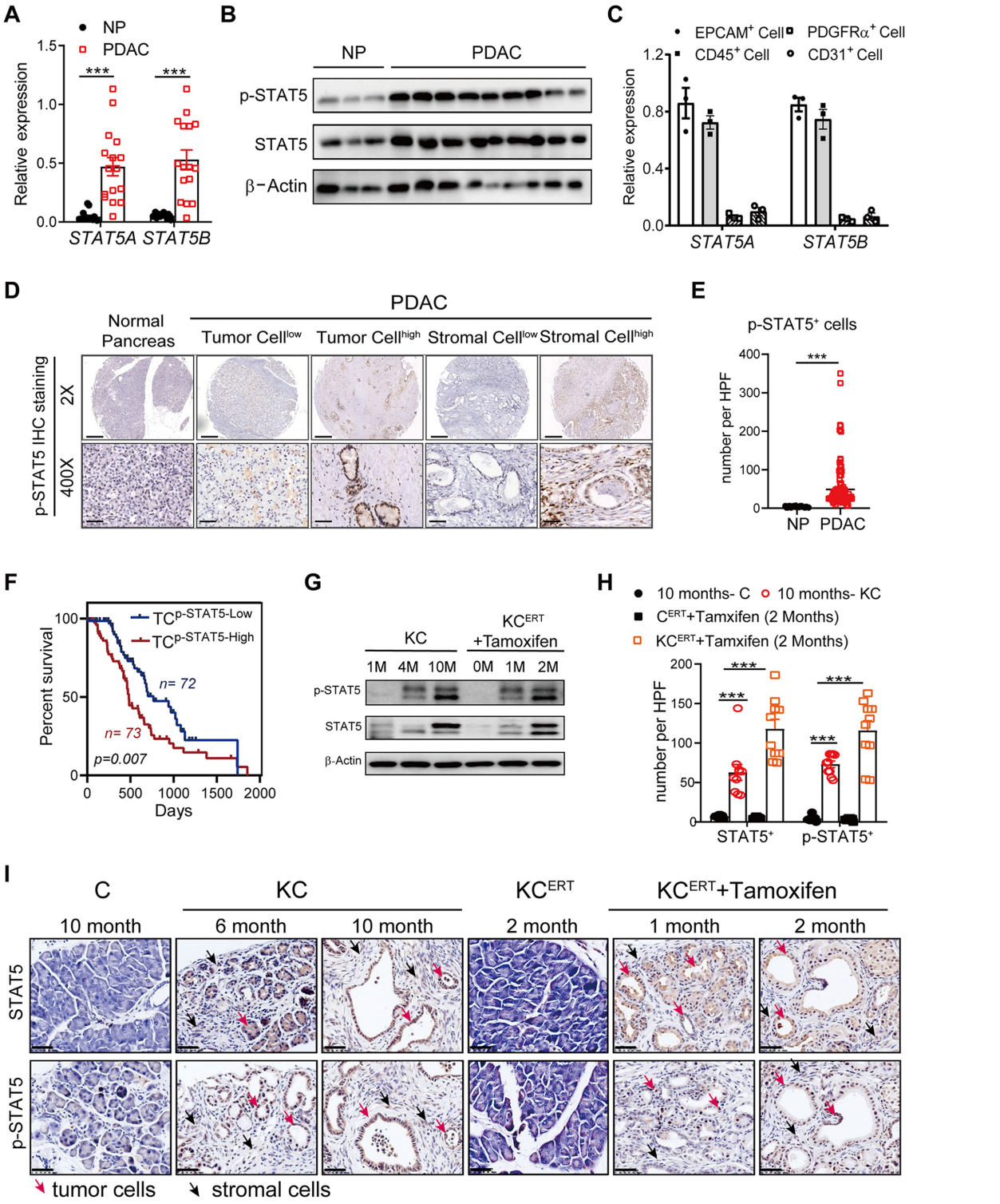

Figure 1 Activated pancreatic signal transducer and activator of transcription 5 (STAT5) in pancreatic cancer and correlates with adverse patient outcomes. (A) STAT5A/STAT5B mRNA in matched non-cancer and cancer tissues of patients with pancreatic ductal adenocarcinoma (PDAC) (N=16). (B) Western blot showing STAT5 and STAT5 phosphorylation (p-STAT5) protein levels in non-cancer and cancer tissues of patients with PDAC (NP=normal pancreas). (C) STAT5A/STAT5B mRNA in isolated tumour cells, fibroblasts, immune cells and endothelial cells in the PDAC cancer tissues (N=3). (D and E) Representative immunohistochemistry (IHC) p-STAT5-positive staining images (D) and the numbers of p-STAT5^{+} cells in three randomly chosen 400× HPFs of normal pancreases (N=21) or PDAC cancer tissues (N=120) (E). Scale bar: up 1 mm, 2×; down 50 µm, 400× magnification. (F) Kaplan-Meier survival analysis of human PDAC tissue microarray based on p-STAT5^{+} level on tumour cells (N=145). (G–I) Representative western blot (G) cell numbers per HPF (H) and IHC staining (I) of STAT5 and p-STAT5 protein levels in *Pdx1*-Cre mice (C) Kras$^{G12D/+}$; *Pdx1*-Cre mice (KC), or *Ptf1a*-Cre^{ERT} mice (C^{ERT}), Kras$^{G12D/+}$; *Ptf1a* -Cre^{ERT} mice (KC^{ERT}) with tamoxifen induction. For H and I, C, KC mice were used at 6, and 10 months after birth. C^{ERT} and KC^{ER} were used at 1, and 2 months after tamoxifen induction. N=10, Scale bar 50 µm, 400× magnification. Results are representatives of at least three independent experiments, data shown as mean±SEM, ***p<0.001, **p<0.01, *p<0.1, ns, no significance. HPF, high-power field; mRNA, messenger RNA; TC, tumour cell.

Two spontaneous PDAC mouse models of pancreatic cell-specific expression of *Pdx1-Cre* (C), LSL-*Kras*$^{G12D/+}$; *Pdx1-Cre* (KC), *Ptf1α-Cre*^{ERT} (C^{ERT}) and LSL-*Kras*$^{G12D/+}$; and *Ptf1α-Cre*^{ERT} (KC^{ERT}) were employed to further corroborate the expression of STAT5 in TCs. The levels of STAT5 expression and phosphorylation exhibited a significant increase in the pancreatic tissue in both mouse models as PDAC progressed (figure 1G–I). Furthermore, this increase was observed in both stromal and TCs (figure 1I). Altogether, these data demonstrated that STAT5 is highly expressed and activated in both malignant and non-malignant cell populations in pancreatic tumours. Increased pSTAT5 level specifically in TCs is correlated with adverse outcomes in human PDAC.

Genetic ablation of STAT5 in pancreatic cells reduces oncogenic KRAS-induced PDAC progression and ADM formation

We supported the idea that the expression of STAT5 on TCs may contribute to the progression of pancreatic cancer. To validate this, the *Kras*$^{G12D/+}$ *Ptf1a-Cre*^{ERT} mice and *Stat5a/b*$^{FL/FL}$ mice were crossed to generate the *STAT5a/b*$^{FL/FL}$; *Kras*$^{G12D/+}$; and *Ptf1α-Cre*^{ERT} (KCS^{ERT}) strains, subsequently knocking out STAT5 on pancreatic cells with tamoxifen to verify the role of pancreatic STAT5 in the progression of pancreatic cancer (figure 2A,B). IHC and western blotting results showed that the protein level of STAT5 decreased in pancreatic tissues of KCS mice (online supplemental figure 2A,B). Deletion of STAT5 in pancreatic acinar cells of mutant Kras^{G12D} increased the survival rate (figure 2C). Compared with KC^{ERT} mice, the KCS^{ERT} mice exhibited ADM reversal on H&E staining and Alcian blue staining of pancreatic tissues at 1 and 2 months after tamoxifen administration (figure 2D–F). Masson staining and histological analysis revealed decreased fibrosis and PanIN in KCS^{ERT} mice than did the KC^{ERT} mice (figure 2D,G,H). In addition, the lack of STAT5 downregulated EGFR expression in the pancreatic tissues of KC^{ERT} mice at 1 month after tamoxifen administration (online supplemental figure 2C). The pancreatic deletion of STAT5 in KCS mice consistently delayed tumour progression and improved survival rates than did KC mice after tamoxifen administration (online supplemental figure 2D–G). CK19 and amylase (Amy) immunofluorescence staining were used to detect local ADM in the pancreatic tissues after tamoxifen administration at 1 month. Consistent with the H&E staining results, co-staining with CK19 and Amy showed that a lack of STAT5 reduced ADM formation (figure 2I). Because ADM is vital for pancreatic cancer progression, the role of STAT5 in the progression of pancreatic ADM was further analysed. The pancreatic acinar cells were isolated from *Kras*$^{G12D/+}$ (K) and *STAT5a/b*$^{FL/FL}$; *Kras*$^{G12D/+}$ (KS) mice and induced with Cre in vitro to detect ADM transformation (figure 2J). The absence of STAT5 significantly reduced the formation of ductal cells from the acinar cells (figure 2K). Further RNA sequencing showed that the lack of STAT5 downregulated the expression of multiple ADM-related genes, such as *Krt19, Krt7 and Mmp11* (figure 2L,M). These results suggest that the lack of STAT5 inhibits KRAS mutation-induced ADM formation.

STAT5 deficiency in pancreatic cells reduces inflammation-mediated ADM formation

In addition to the Kras^{G12D} mutation, inflammation is another major risk factor associated with pancreatic ADM formation. To investigate the role of STAT5 in pancreatitis-induced ADM, mice were administered tamoxifen and then treated with cerulein to induce inflammation (figure 3A). *Stat5a/5b* expression was induced by cerulein treatment (figure 3B). The loss of pancreatic STAT5a/b had little effect on CD45^{+} cell infiltration in pancreatitis, suggesting that STAT5 did not affect cerulein-induced inflammation (online supplemental figure 3). H&E and Alcian blue staining of pancreata from *Ptf1a-Cre*^{ERT} (C^{ERT}) control mice 3 days after cerulein treatment revealed a distinct area of ADM, characterised by the presence of duct-like structures (figure 3C,D). The extent of ADM was greater in the pancreata of *Kras*$^{G12D/+}$; *Ptf1a-Cre*^{ERT} mice after cerulein treatment than in the control group (figure 3C,D). In contrast, CS^{ERT} and KCS^{ERT} mice at the 3-day time point exhibited significantly smaller areas of ADM lesions and fewer Alcian blue-positive cells. By day 21, the pancreata of the C^{ERT} and CS^{ERT} mice had a normal histology. However, KC^{ERT} mice exhibited enlarged regions containing ADM and PanIN lesions, whereas the pancreata of the KCS^{ERT} mice displayed significant regression of ductal lesions (figure 3C,D). Co-immunofluorescence of Amy and CK-19 and quantification of CK19-positive cells validated the previous findings (figure 3E,F). These findings illustrate the significance of STAT5 in the development of ADM in the presence of mutant Kras^{G12D}, which inhibits pancreatitis-induced ADM formation in vivo.

STAT5 deficiency delays KPC mice and orthotopic pancreatic tumour growth

Following the initiation of pancreatic cancer by ADM, the proliferation and transformation of neoplastic cells are imperative for its progression. To investigate the role of STAT5 in PDAC progression, *Kras*$^{G12D/+}$; *P53*$^{Mut/+}$; and *Pdx1-Cre* (KPC) mice were used. Compared with KPC mice, *Kras*$^{G12D/+}$; *P53*$^{Mut/+}$; *Pdx1-Cre; STAT5a/b*$^{FL/FL}$ (KPCS) mice pancreatic tissue collected at 3 and 5 months exhibited a significant delay in pancreatic cancer progression. suggesting a potential promoting role of STAT5 in PDAC stages (figure 4A–C). Consistently, KPCS mice had a significantly better survival rate compared with KPC mice (online supplemental figure 4A). Knockdown of STAT5 expression in the PDAC cell lines significantly suppressed the growth of the pancreatic tumour in both immunocompetent C57BL/6J wild-type mice and immunodeficient nude mice, suggesting that STAT5 deletion delayed PDAC progression (figure 4D,E, online supplemental figure 4B,C). Moreover, treatment with a STAT5 inhibitor resulted in a notable delay in orthotopic growth in pancreatic cancer cell lines with KRAS mutation, patient-derived xenografts and the tumour progression of KPC mice (figure 4F–L and online supplemental figure 4D,E). In addition, STAT5 inhibition enhanced the antitumour effects of gemcitabine (figure 4H,I). The combination of STAT5 inhibitors and gemcitabine significantly improved the survival rate of the patient-derived xenografts-constructed PDAC mice (figure 4J). Overall, these results elucidated that STAT5 plays a facilitative role in PDAC progression.

STAT5 deficiency impairs pancreatic tumour energy metabolism

RNA sequencing results were further analysed to explore the role of STAT5 in pancreatic malignancy. Downregulation of multiple metabolic-associated signalling pathways was observed on STAT5 knockout, of which metabolic processes, including lipid, glutathione and steroid metabolic processes, which are important upstream signals for cell energy metabolism exhibited a significantly upregulated (figure 5A,B, online supplemental tables 3 and 4). Glucose and fatty acids are the two major

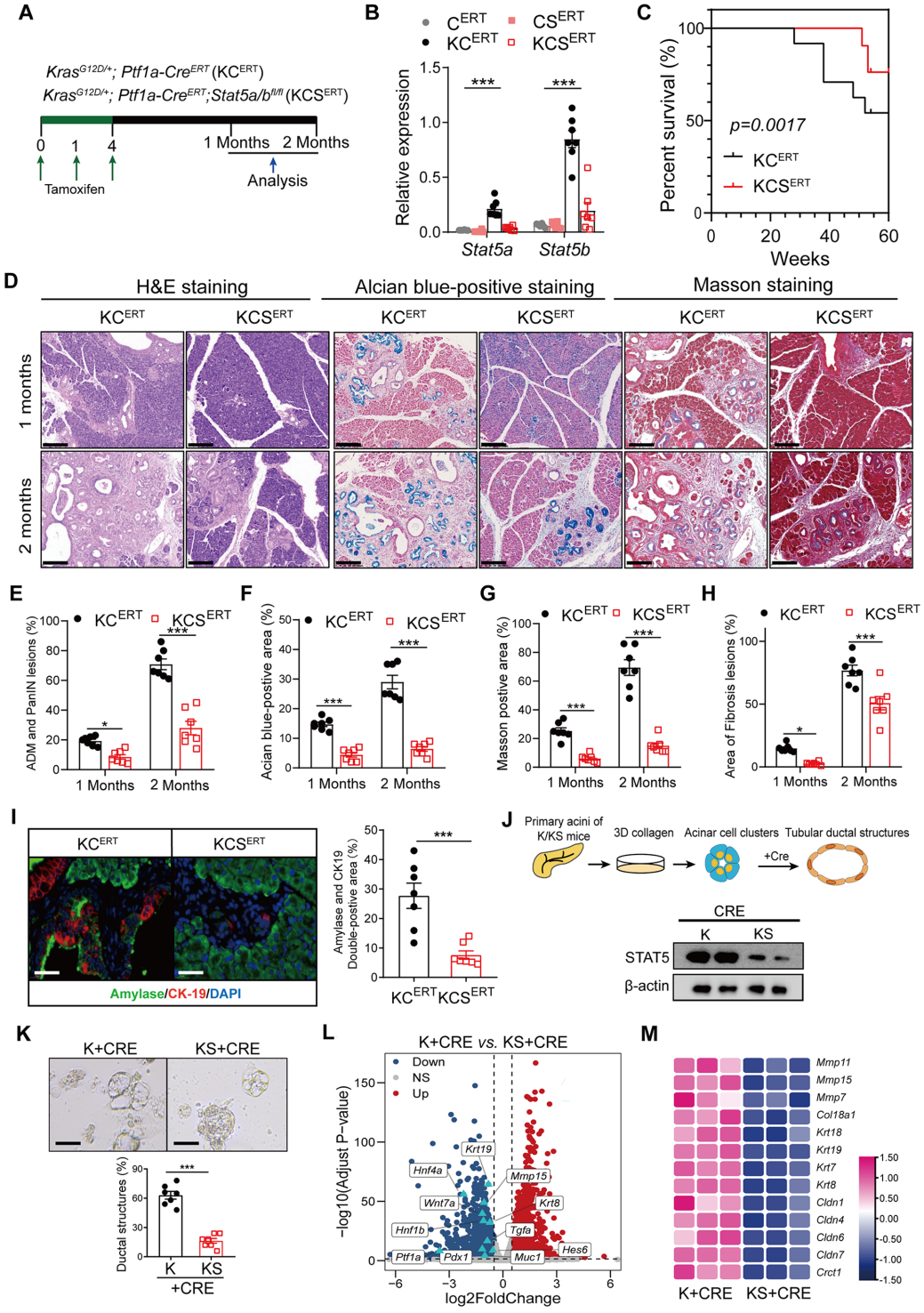

Figure 2 Genetic ablation of pancreatic STAT5 reduces oncogenic KRAS-induced pancreatic ductal adenocarcinoma progression and acinar-to-ductal metaplasia (ADM) formation. (A) Schematic diagram of the study design/strategy. (B) STAT5A/STAT5B messenger RNA in KC^{ERT}, C^{ERT}, *Stat5*$^{FL/FL}$; *Ptf1a*-Cre^{ERT} mice (CS^{ERT}), *Stat5*$^{FL/FL}$; Kras$^{G12D/+}$; *Ptf1a*-Cre^{ERT} mice (KCS^{ERT}) with tamoxifen induction (N=7 per group). (C) Kaplan-Meier curves of overall survival of KC^{ERT} and KCS^{ERT} mice. (D–H) Representative images of H&E staining, Alcian blue-positive staining and Masson staining (D) Percentages of the area of ADM and pancreatic intraepithelial neoplasia (PanIN) (E) Alcian blue positive area (F) Masson positive area (G) area of fibrosis lesions (H) in KC^{ERT} and KCS^{ERT} mice post-tamoxifen induction (scale bar: 200 µm, 100× magnification). (I) Representative images and quantity of amylase (green), CK-19 (red) and DAPI (blue) staining in pancreatic sections of KC^{ERT} and KCS^{ERT} mice post-tamoxifen induction. (Scale bar: 50 µm, 400× magnification) (N=7 per group). (J–M) Schematic diagram of the study design and representative western blotting of STAT5 protein levels (J) brightfield images and quantification of tubular ductal structures (K) volcano plot of RNA sequencing data. (L) Heatmap feature of ADM associated genes (M) of explanted acinar cells collected after 5 days from 5-week-old *Kras*$^{G12D/+}$ or *Kras*$^{G12D/+}$; *Stat5*$^{FL/FL}$(KS) mice infected with adenoviruses encoding with or without Cre (–Cre or +Cre). Scale bar, 100 µm, 200× magnification. N=7 for J, N=3 for J–M. Results are representatives of at least three independent experiments, data shown as mean±SEM, ***p<0.001, **p<0.01, *p<0.1, ns, no significance. STAT5, signal transducer and activator of transcription 5.

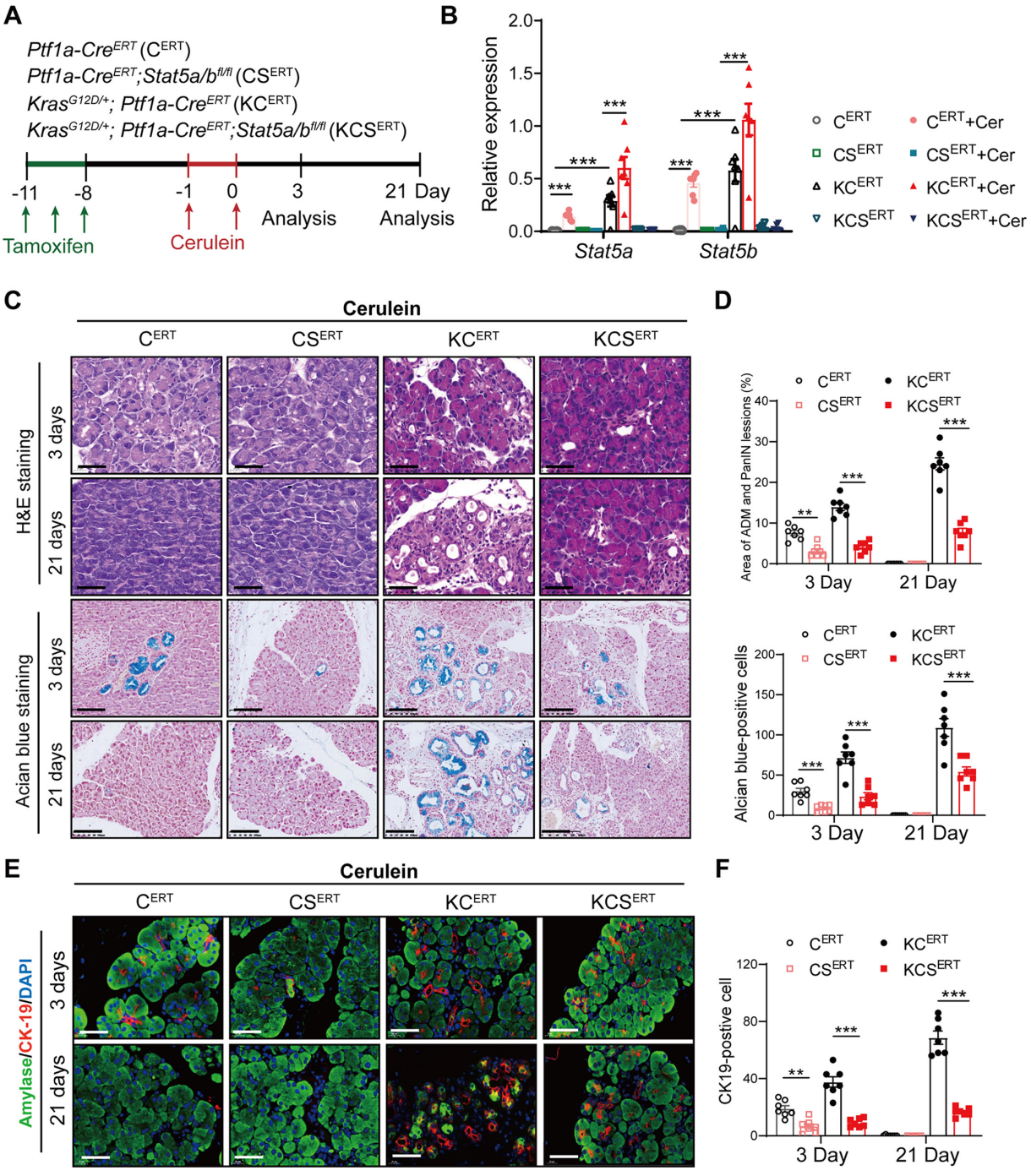

Figure 3 Pancreatic STAT5 deficiency reduces inflammation-mediated ADM formation. (A) Schematic diagram of the study design/strategy. (B) *Stat5a/5b* messenger RNA in the pancreata of C^{ERT}, KC^{ERT}, CS^{ERT} and KCS^{ERT} mice with or without cerulein-treated (N=8 per group). (C–F) Representative images of H&E staining, Alcian blue-positive staining. (C) Percentages of the area of the ADM and pancreatic intraepithelial neoplasia, Alcian blue-positive area. (D) Representative images (E) and quantity (F) of amylase (green), CK-19 (red) and DAPI (blue) staining of the pancreas from C^{ERT}, CS^{ERT}, KC^{ERT}, KCS^{ERT} at 3 and 21 days after cerulein treatment (scale bar: 50 µm, 400× magnification for H&E staining) and (E). Scale bar: 100 µm, 200× magnification for Alcian blue staining. N=7 per group. Results are representatives of at least three independent experiments, data shown as mean±SEM, ***p<0.001, **p<0.01, *p<0.1, ns, no significance. ADM, acinar-to-ductal metaplasia; STAT5, signal transducer and activator of transcription 5.

fuels that meet energy requirements during pancreatic cancer proliferation and malignant transformation.[27] Therefore, we explored whether STAT5 is involved in pancreatic proliferation by promoting energy metabolism, especially in lipid metabolism-associated FAO and OXPHOS. The FAO inhibitor etomoxir and OXPHOS inhibitor oligomycin A were initially used to screen pancreatic cancer cell lines dependent on FAO and OXPHOS metabolism. The results showed that the proliferation of mouse pancreatic TC lines KPC119 and Panc-02 and human pancreatic TC lines Capan-2, PANC-1 and CFPAC-1 was dependent on FAO and OXPHOS (figure 5C,D). Oxygen consumption in pancreatic tumour tissues and pancreatic TCs lacking STAT5 were analysed, and a notable reduction in the oxygen consumption rate (OCR) was observed in the pancreatic tissue of KCS

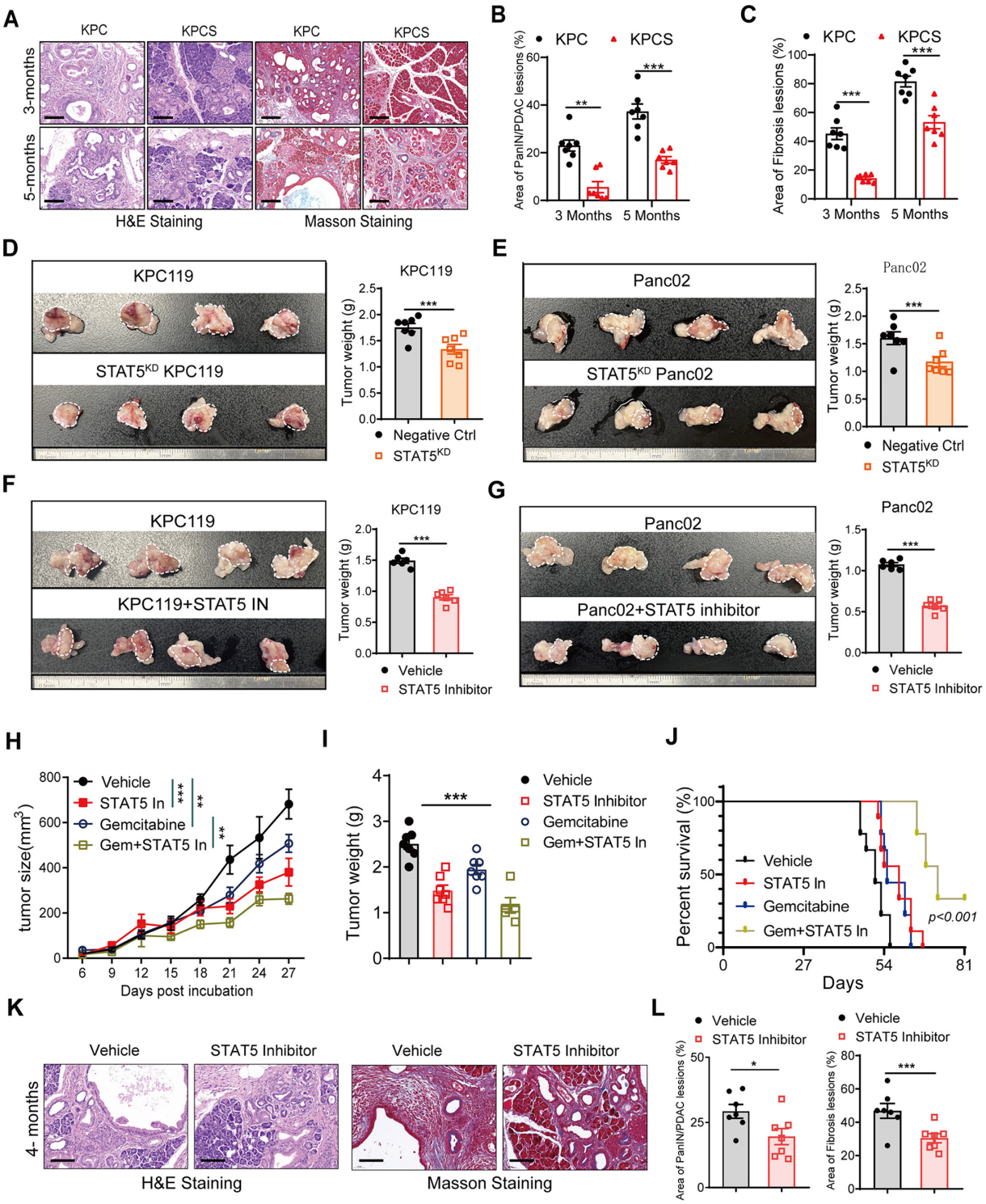

Figure 4 STAT5 deficiency delays KPC mice and orthotopic pancreatic tumour growth. (A–C) Representative images of H&E and Masson staining (A) percentages of the area of PanIN/PDAC (B) and fibrosis lesions (C) of KPC and KPCS mice at 3 and 5 months. Scale bar: 100 µm, 200× magnification. (D and E) Representative images of pancreas and tumour weight from orthotopic PDAC model constructed by KPC119 and *Stat5*^{KD} KPC119 (D) or by Panc02 and *Stat5*^{KD} Panc02 cells (E). (F and G) Representative images of pancreas and tumour weight from an orthotopic PDAC model constructed by KPC119 (F) or Panc02 (G) cell lines with or without STAT5 inhibitor administration. (H–J) Tumour growth curves (H) tumour weight (I) and survival curve (J) of patient-derived xenografts from patients with PDAC. N=7 for H and I, N=9 for J. (K and L) Representative images of H&E staining and Masson staining (K) percentages of the area of PanIN/PDAC and fibrosis lesions (L) of 3 months KPC mice treated with or without STAT5-IN for 1 months. (N=7) Results are representatives of at least two independent experiments, data shown as mean±SEM, ***p<0.001, **p<0.01, *p<0.1, ns, no significance. PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma; STAT5, signal transducer and activator of transcription 5.

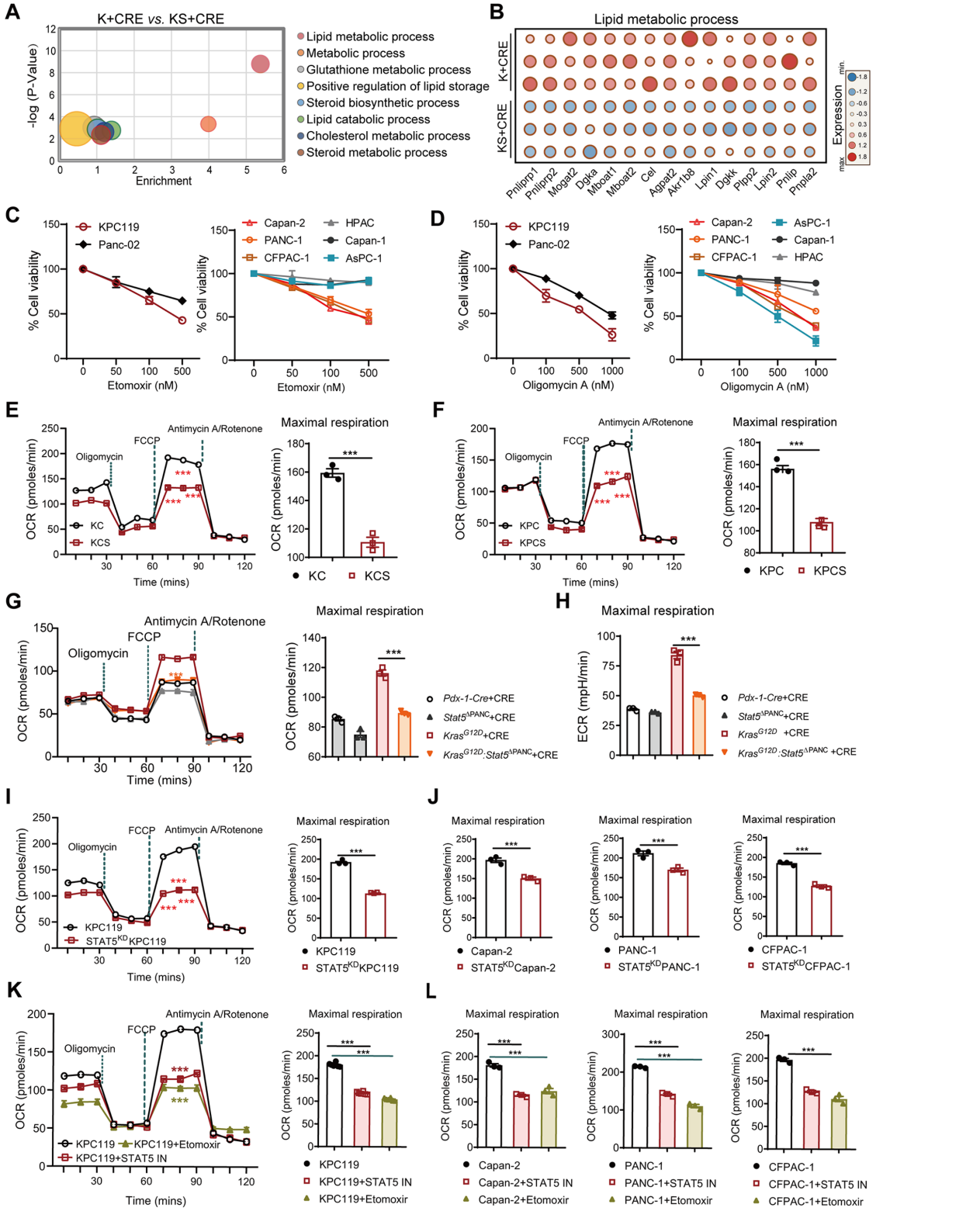

Figure 5 STAT5 deficiency impairs pancreatic tumour energy metabolism. (A) Bubble diagram of GO pathways (A) heatmap feature of lipid-metabolic process-associated genes (B) of explanted acinar cells collected from 3-week-old K or KS mice infected with Cre. (C and D) Cell viability of KRAS-mutant mouse tumour cell lines KPC119, Panc02 and human tumour cell lines Capan-2, PANC-1, CFPAC-1, HPAC, Capan-1, AsPC1 treated with different concentrations of etomoxir (C) or oligomycin A (D). (E and F) Oxygen consumption rate (OCR) of pancreatic explants from KC and KCS mice at 6 months (E) or KPC and KPCS mice at 3 months (F). (G and H) OCR (G) and ECR (H) of explanted acinar cells collected from 5-week-old C, CS, K, or KS mice affected with Cre. (I and J) OCR of KPC119 and *Stat5*^{KD} KPC119 cells (I) or Capan-2 and *Stat5*^{KD} Capan-2, PANC-1 and *Stat5*^{KD} PANC-1, CFPAC-1 and *Stat5*^{KD} CFPAC-1 cells (J). (K and L) OCR of KPC119 cells (K) or Capan-2, PANC-1, CFPAC-1 and (L) cells treated with or without STAT5-IN. Oligomycin (Oligo), FCCP, rotenone and antimycin A were injected as indicated. N=3 per group, results are representatives of at least three independent experiments, data shown as mean±SEM, ***p<0.001, **p<0.01, *p<0.1, ns, no significance. ECR, extracellular acidification rate; GO, gene ontology; STAT5, signal transducer and activator of transcription 5.

mice than in KC mice (figure 5E). In addition, compared with KPC mice, the absence of STAT5 affected the rate of OXPHOS in the pancreatic tissue of KPCS mice (figure 5F). Consistently, the absence of STAT5 impaired the extracellular acidification rate (ECAR) and OCR of acinar explants of KRAS mutation mice ex vivo (figure 5G,H). This was further supported by the fact that OCR levels in pancreatic cancer cells were significantly reduced by STAT5 signalling interference through the use of STAT5 siRNA or STAT5 inhibitors (figure 5I–L). The ECAR of acinar cell explants or TCs was inhibited after STAT5 deletion or inhibition, suggesting that STAT5 affects glycolysis in pancreatic cancer cells (online supplemental figure 5A–G). This observation suggests that STAT5 participates in the survival of pancreatic ductal cells by augmenting the rates of OXPHOS and glycolysis, thereby facilitating pancreatic malignant cell proliferation and survival.

STAT5a/b-mediated ADM formation through direct transcriptional programming

Next, we aimed to clarify how STAT5 deficiency regulates ADM and metabolic shifts. RNA sequencing results showed that the lack of STAT5 downregulated the expression of multiple ADM-related transcription factors related to ductal differentiation, such as *Pdx1, Onecut1, Hnf1a, Hnf1b and Hnf4a* (figure 6A). Furthermore, gene set variation analysis (GSVA) showed that transcriptional activity decreased in STAT5-deficient acinar cells (figure 6B). Consistently, RT-PCR results revealed that STAT5 deficiency decreased the expression of multiple ADM-related transcription factor genes in acinar cells (figure 6C). Therefore, whether the transcriptional activity of STAT5 regulates the deregulation of these transcription factors by KRAS mutation needs to be clarified. Chromatin immunoprecipitation (Ch-IP) results verified that STAT5 could bind to the promoter sequences of *Hnf1b* and *Hnf4a* (figure 6D), but not *Klf5*, *Pdx1*, *Sox9*, *Hnf1a, Hes6* and *Ptf1a* in primary KRAS mutant acinar cells (data not shown). The KRAS-mutant TC lines, KPC119, AsPC-1, PANC-1 and CAPAN2, confirmed the Ch-IP results (figure 6D and online supplemental figure 6A). Hepatocyte nuclear factor (HNF) 4α is involved in energy metabolism,[28 29] and STAT5 expression interference in acinar cell explants isolated from KC mice or pancreatic TCs decreased HNF1β and HNF4α expression (figure 6E and online supplemental figure 6B). In addition, the STAT5 inhibitor effectively downregulated the expression of HNF1β and HNF4α (figure 6F and online supplemental figure 6C). These findings were consistent with IHC staining results, which showed that HNF1β and HNF4α protein levels were downregulated in STAT5-knockout KC^{ERT} mice compared with those in KC^{ERT} mice (figure 6G and online supplemental figure 6D). Furthermore, HNF1β or HNF4α knockout significantly downregulated ductal structures and the expression of ADM-related genes in KRAS-mutant acinar cells (figure 6H,I, and online supplemental figure 6E). Notably, interfering with HNF4α expression but not HNF1β in pancreatic cancer cells decreased OXPHOS and glycolysis (figure 6J). HNF4α overexpression significantly rescued the inhibited OXPHOS and glycolysis in STAT5 KO pancreatic cancer cells (figure 6K). All these findings suggest that STAT5 regulates HNF4α gene expression by binding to their promoters via its transcriptional activity, thereby affecting ADM formation and increased energy metabolism caused by the KRAS mutation.

$Kras^{G12D}$ mutation and IL-22 promoted acinar cell STAT5 activation

Next, we aimed to characterise the upstream activators of STAT5 during inflammation-mediated ADM formation. p-STAT5 levels were significantly higher in KRAS-mutant cells, suggesting that KRAS directly mediated STAT5 activation (figure 7A). Inhibitor of PI3K significantly reduced KRAS mutation-mediated STAT5 activation (figure 7A,B). The effect of inflammatory factors on STAT5 activation in acinar cells was then evaluated, which IL-22, rather than other cytokines, robustly increased the phosphorylation of Janus kinase (JAK)1, JAK2, STAT5 and STAT3 in acinar cell explants, but not JAK3 (figure 7C,D and online supplemental figure 7A). IL-22 receptor expression in the acinar cells was then confirmed (online supplemental figure 7B,C). Notably, KRAS signalling augmented IL-22-dependent STAT5 phosphorylation, indicating a synergistic effect between KRAS mutation and the IL-22 pathways (figure 7C). JAK1/2 inhibitor abrogated KRAS mutant-induced and IL-22-induced STAT5 activation, suggesting that JAK 1/2 was necessary for STAT5 phosphorylation (figure 7D). Furthermore, IL-22-enhanced KRAS-driven ductal structures were mitigated by the STAT5 blockade (figure 7E). Collectively, these findings establish STAT5 as a critical downstream target of IL-22-induced and KRAS signalling-induced ADM formation.

To clarify the role of IL-22 in activating STAT5 during pancreatic cancer progression, tests were performed using KC mice. The ELISA assays demonstrated an increased IL-22 expression in the KC mouse model than in *Pdx1*-Cre mice, with cerulein significantly augmenting this upregulation within the tumour tissues of these mice (figure 7F). RT-PCR revealed that the expression of IL-22 within the pancreatic tissue was mainly $CD45^+$ immune cells. Further delineating the cellular origin of IL-22, the flow cytometry results showed that IL-22 was mainly secreted by $CD4^+$ T cells (figure 7H). We then generated KC: $Il22^{-/-}$ mice, KC: $Il22ra1^{FL/FL}$ mice and IL-22 knockout mice or $Il22ra1^{FL/FL}$ mice crossbred with KC mice to ascertain whether the IL-22 signal was responsible for STAT5 activation in vivo. The absence of IL-22 signalling in pancreatic cells can effectively downregulate ADM formation, the activation of STAT5 and the expression of HNF4α (figure 7I–O). More importantly, the lack of IL-22 delayed the progression of pancreatic cancer in KPC mice (figure 7P,Q). Taken together, these data suggest that IL-22 signalling considerably mediates sustained inflammation-mediated STAT5 activation and promotes pancreatic cancer progression.

DISCUSSION

The role of STAT5 in cancer is intricate and controversial because it has a complex effect on TCs and immune responses, resulting in either oncogenic or tumour-suppressive functions.[30] In the present study, TC's STAT5 expression was increased and associated with poor prognosis in patients with PDAC. We revealed that STAT5 promotes pancreatic tumourigenesis using a series of in vitro and in vivo functional studies. The increase in IL-22 levels caused by inflammatory signalling promotes KRAS-mediated STAT5 activation, which in turn mediates ADM and pancreatic cancer progression by upregulating the expression of ADM-associated transcription factors. Finally, we confirmed the role of STAT5 in maintaining energy metabolism to participate in the further malignant transformation of pancreatic cancer cells and PanIN development.

Although ADM has been established as an important precursor for the initiation and progression of pancreatic cancer, our knowledge of the underlying mechanisms remains unclear.[11] Herein, we elucidated that STAT5 activation can regulate multiple ADM-related transcription factors and participate in ADM progression via its transcriptional activity. Blocking STAT5 signalling

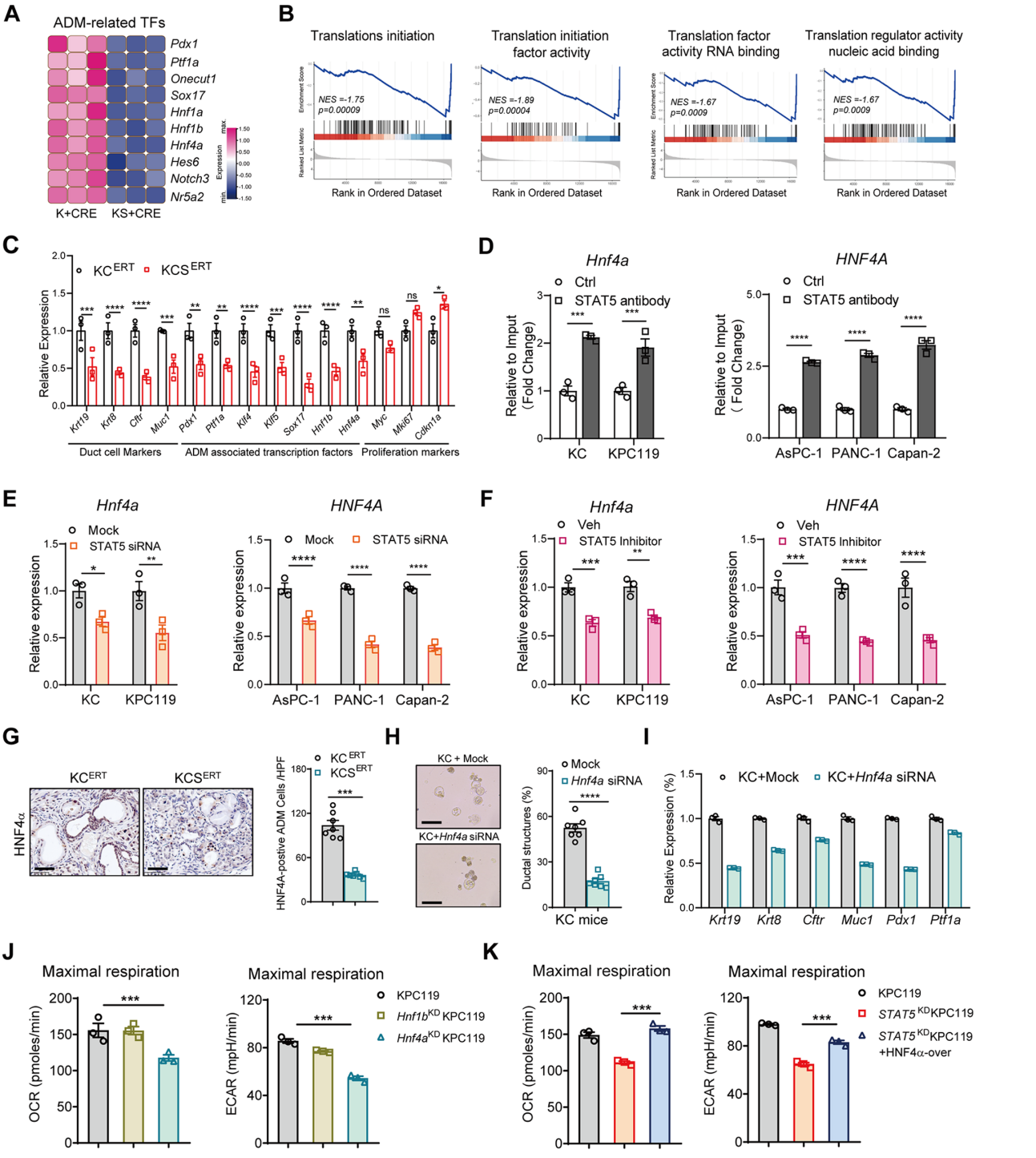

Figure 6 STAT5-mediated ADM formation through direct transcriptional programming. (A and B) Heatmap feature of ADM-related transcription factors (A) and GSVA analysis (B) in pancreata of K and KS mice post Cre induction. (C) Real-time quantitative PCR analysis of ADM formation-associated genes of explanted acinar cells collected from 5-week-old K or KS mice infected with Cre. (D and E) Chromatin immunoprecipitation assay showing STAT5 binding to the *Hnf4a* promoter regions in primary acinar cells collected from 16-week-old KC mice or *Kras*-mutant tumour cell lines KPC119 (D) AsPC1, PANC1 and Capan-2 (E). (F and G) mRNA expression of *Hnf4a* in primary acinar cells (F) and different tumour cell lines (G) infected with *Stat5* siRNA. (H and I) mRNA expression of *Hnf4a* in primary acinar cells (H) and different tumour cell lines (I) treated with STAT5 inhibitor. (J) Representative IHC images of HNF4α (right) and quantification of HNF4α-positive ADM cells (left) in pancreata of KC^{ERT} and KCS^{ERT} mice (scale bar: 50 µm, 400× magnification). (K and L) Brightfield images (K) and relative expression of duct cell markers (L) of explanted acinar cells collected from KC mice infected with *Hnf4a* siRNA (scale bar: 100 µm, 200× magnification). (M) OCR and ECAR of KPC119, $Hnf4a^{KD}$ KPC119 cells and $Hnf1b^{KD}$ KPC119 cells. (N) OCR and ECAR of KPC119, $Stat5^{KD}$ KPC119 cells with or without overexpression HNF4α. N=3 per group. Results are representatives of at least three independent experiments, data shown as mean±SEM, ***p<0.001, **p<0.01, *p<0.1, ns, no significance. ADM, acinar-to-ductal metaplasia; ECAR, extracellular acidification rate; GSVA, gene set variation analysis; HNF, hepatocyte nuclear factor; HPF, high-power field; mRNA, messenger RNA; OCR, oxygen consumption rate; STAT5, signal transducer and activator of transcription 5.

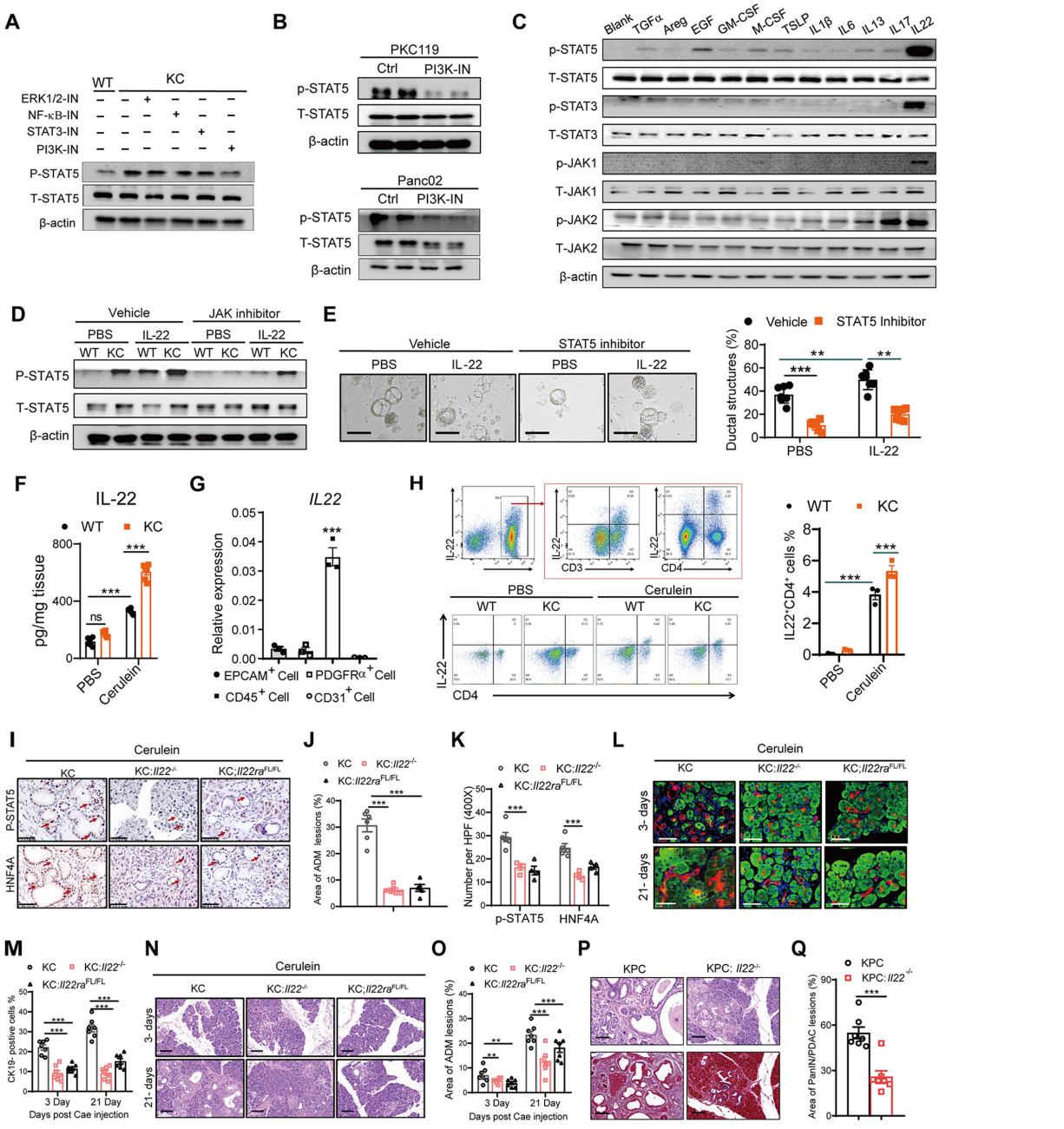

Figure 7 *Kras*^{G12D} mutation and IL-22 promoted pancreatic STAT5 activation. (A and B) Representative western blot showing STAT5 and p-STAT5 protein levels in pancreata of WT and KC mice (A) KPC119 and Panc-02 cell lines (B) treated with or without various inhibitors. (C) Representative western blot showing STAT5, p-STAT5, STAT3, p-STAT3, janus kinase (JAK) 1, p-JAK1, JAK2 and p-JAK2 protein levels in different inflammatory factors-treated acinar cells from KC mice. (D) Representative western blot showing STAT5 and p-STAT5 protein levels in JAKs inhibitor pretreated pancreata of WT and KC mice stimulated without or with IL-22. (E) Brightfield images of explanted acinar cells and quantification of tubular ductal structures collected from KC mice treated without or with IL-22 and/or STAT5 inhibitor (scale bar: 50 µm, 400× magnification) (n=6 per group). (F) IL-22 protein levels were measured by ELISA pancreata of WT and KC mice treated without or with cerulein (n=6 per group). (G) *IL22* messenger RNA in isolated tumour cells, fibroblasts, immune cells and endothelial cells from the PDAC cancer tissues (n=3). (H) Representative flow cytometric figures and percentages of IL-22^{+}CD4^{+} cells in the pancreata of WT and KC mice treated without or with cerulein (n=3 per group). (I–O) Representative immunohistochemical images (I) quantitation of ADM structures (J) and numbers of p-STAT5 and HNF4α cells counted per HPF (K) immunofluorescence staining of amylase (green), CK19 (red) and DAPI (blue) (L) and quantitation of CK19-positive cells (M) representative images of H&E staining (N) and percentages of the area of ADM lesions (O) in pancreata of KC, KC; *Il22*$^{-/-}$ and KC; *Il22ra*$^{FL/FL}$ mice after cerulein treatment. Scale bar, 50 µm, 400× magnification for I and L, scale bar, 100 µm, 200× magnification for N. (P and Q) Representative images of H&E and Masson staining (P) and percentages of the area of pancreatic intraepithelial neoplasia/PDAC (Q) of KPC and KPC; *Il22*$^{-/-}$ mice at 3 months. Scale bar: 100 µm, 200× magnification. N=7 per group for I–Q. Results are representatives of at least three independent experiments, data shown as mean±SEM, ***p<0.001, **p<0.01, *p<0.1, ns, no significance. ADM, acinar-to-ductal metaplasia; Areg, amphiregulin; EGF, epidermal growth factor ; GM-CSF, granulocyte-macrophage colony stimulating factor; HNF, hepatocyte nuclear factor; HPF, high-power field; IL, interleukin; M-CSF, macrophage colony stimulating factor; PBS, phosphate-buffered saline; PDAC, pancreatic ductal adenocarcinoma; p-STAT, phosphorylated STAT; STAT, signal transducer and activator of transcription; TGF, transforming growth factor; TSLP, thymic stromal lymphopoietin.

potentially downstreams KRAS and facilitates oncogenic KRAS mutation-driven PDAC initiation and development. Moreover, STAT5 activation was necessary for tumour progression in the PanIN and PDAC stages, and STAT5 was persistently activated in KRAS-mutated pancreatic TC lines. Conversely, STAT5 deficiency exhibited a significantly delayed pancreatic cancer progression in KPC mice. The STAT5 inhibitor (STAT5-IN), which is supported to directly bind the STAT5 transcriptional region and does not affect STAT3 activation,[31 32] delayed PDAC progression constructed using pancreatic TC lines and human patient-derived xenograft (PDX) tumours. In addition, it should be noted that STAT5 inhibitors may also affect the activation of STAT5 in the SCs in PDAC microenvironment. Although STAT5 is considered to play an important role in T-cell activation,[33] a tumour suppression effect was observed when STAT5 inhibitors were administered to immunocompetent mice. This could be explained by the fact that pancreatic cancer is a type of tumour with a low T-cell response.[34]

Several downstream pathways of KRAS mediate ADM, and the importance of inflammation in this process has been increasingly recognised.[11] We found that STAT5 upregulation during ADM was not restricted to $KRAS^{G12D}$ cells, as STAT5 activation also occurred in normal acini when pancreatitis was induced. Our in vivo and in vitro studies showed that STAT5 can be activated by the KRAS mutation, which is further promoted by inflammatory signals, especially IL-22. This suggests that, in addition to KRAS signalling, IL-22 induced by chronic inflammation is required to maintain STAT5 activation, which leads to the carcinogenesis of ADM in pancreatic cancer. In acute pancreatitis, IL-22 promotes pancreatic tissue repair by stimulating cell proliferation and inhibiting cell apoptosis.[35] Furthermore, IL-22 regulates the proliferation of pancreatic stem cells and promotes the proliferation and invasion of pancreatic cancer cells by activating signalling pathways such as STAT3 and ERK1/2.[36 37] We demonstrated that IL-22 participates in the inflammation-induced progression of pancreatic ADM through the STAT5 signalling pathway, highlighting its significant role in ADM transformation and pancreatic cancer progression.

Pancreatic cells require energy to perform processes such as the transformation of acinar cells into malignant cells and the proliferation of malignant cells.[38] Our results suggest that STAT5 activation not only participates in the transformation process of ADM but also plays an important role in maintaining TC survival by controlling OXPHOS and glycolysis involved in energy metabolism. STAT5 loss contributes to the downregulation of energy, accompanied by a deceleration of pancreatic ADM progression and inhibition of pancreatic cancer proliferation. Further mechanistic studies revealed that STAT5 regulates the expression of the HNF family of proteins. Importantly, we discovered that STAT5 can directly control the expression of HNF4α. Blocking the HNF4α signal downregulates the pancreatic cell energy metabolism. The HNF family is involved in energy metabolism, with HNF4α playing a role in FAO through various mechanisms, regulating cellular mitochondrial energy metabolism and maintaining energy metabolism functions in the pancreas, liver and intestine.[28 29] Our results and those of previous studies suggest that inhibiting OXPHOS or glycolysis in the pancreatic tissue can effectively block pancreatic cancer progression.[39 40] Blocking the HNF4α signal can restrict pancreatic OXPHOS and glycolysis, indicating that STAT5 can influence energy metabolism by regulating HNF4α, thereby participating in ADM transformation and pancreatic cancer cell proliferation.

In summary, our study highlights the central role of STAT5 in the intrinsic regulatory network that promotes ADM formation in the presence of carcinogenic KRAS and chronic inflammation. Treatment targeting STAT5 can inhibit the transformation of ADM and energy metabolism in TCs, indicating a pro-tumour role for STAT5 in PDAC.

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Contributors YL, XY and QX designed research. YL, SP, YW, ZW, YG, WF and YY performed research. QX, JW, LL, WW, XJM and XY contributed to clinical research. SM and QX contributed reagents. YL, SP, JW, ZW and YW analysed data. YL and XY wrote the paper. YL is the guarantor.

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