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Oncogenic KRAS Reduces Expression of FGF21 in Acinar Cells to Promote Pancreatic Tumorigenesis in Mice on a High-Fat Diet

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W.L. conceived, designed, and supervised the whole project, and prepared the manuscript; C.D.L., J.L.A., and V.W.Y. provided the research platform, supervised the project, and prepared the manuscript. Y.Luo, Y.Y., and M.L. performed the experiments and drafted the manuscript. Y. Luo also conceived and designed the experiments, and contributed reagents. D.W., Y.B., J.J., S.L., Y. Liu., S.X., A.K.S., G.W., and C.K.L. performed immunohistochemistry analyses, qRT-PCR, Western blot analyses, data quantification, and assisted in breeding and genotyping the mice. D.W. and Y.B. performed acinar cell extraction and 3D explant cultures, F.W., R.C., X.W., A.B.B., H.H., G.L., C.L.P., X.L., B.J., R.A.W., W.P., Z.L., E.L., X. Li., T.J., and S.B. contributed the research materials, reagents, analysis tools, and discussion. M.W., K.R.S., Y.Y., and S.L. analyzed H&E images. All authors read and critically reviewed the manuscript.

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Abstract

Background & Aims: Obesity is a risk factor for pancreatic cancer. In mice, a high-fat diet (HFD) and expression of oncogenic KRAS lead to development of invasive pancreatic ductal adenocarcinoma (PDAC) by unknown mechanisms. We investigated how oncogenic KRAS regulates the expression of fibroblast growth factor 21 (FGF21), a metabolic regulator that prevents obesity, and the effects of recombinant human FGF21 (rhFGF21) on pancreatic tumorigenesis.

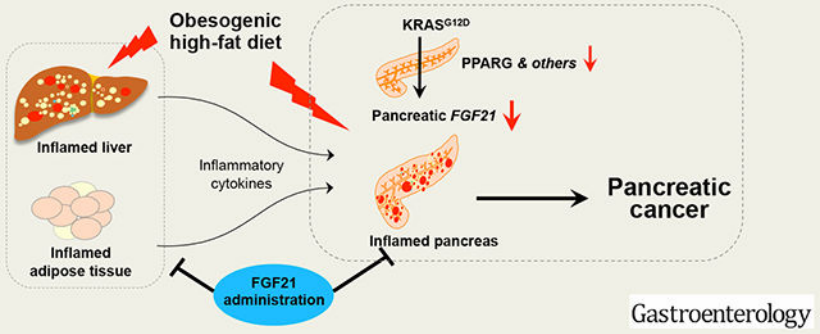
Methods: We performed immunohistochemical analyses of FGF21 levels in human pancreatic tissue arrays, comprising 59 PDAC specimens and 45 non-tumor tissues. We also studied mice with tamoxifen-inducible expression of oncogenic KRAS in acinar cells ($Kras^{G12D/+}$ mice) and $fElas^{CreERT}$ mice (controls). $Kras^{G12D/+}$ mice were placed on a HFD or regular chow diet (control) and given injections of rhFGF21 or vehicle; pancreata were collected and analyzed by histology, immunoblots, quantitative PCR, and immunohistochemistry. We measured markers of inflammation in the pancreas, liver, and adipose tissue. Activity of RAS was measured based on the amount of bound GTP.

Results: Pancreatic tissues of mice expressed high levels of FGF21 compared with liver. FGF21 and its receptor proteins were expressed by acinar cells. Acinar cells that expressed $Kras^{G12D/+}$ had significantly lower expression of *Fgf21* mRNA, compared with acinar cells from control mice, partly due to downregulation of PPARG expression—a transcription factor that activates *Fgf21* transcription. Pancreata from $Kras^{G12D/+}$ mice on a control diet and given injections of rhFGF21 had reduced pancreatic inflammation, infiltration by immune cells, and acinar-to-ductal metaplasia compared with mice given injections of vehicle. HFD-fed $Kras^{G12D/+}$mice given injections of vehicle accumulated abdominal fat, developed extensive inflammation, pancreatic cysts, and high-grade pancreatic intraepithelial neoplasias (PanINs); half the mice developed PDAC with liver metastases. HFD-fed $Kras^{G12D/+}$ mice given injections of rhFGF21 had reduced accumulation of abdominal fat and pancreatic triglycerides, fewer pancreatic cysts, reduced systemic and pancreatic markers of inflammation, fewer PanINs, and longer survival— only about 12% of mice developed PDACs and none of the mice had metastases. Pancreata from HFD-fed $Kras^{G12D/+}$ mice given injections of rhFGF21 had lower levels of active RAS than from mice given vehicle.

Conclusions: Normal acinar cells from mice and humans express high levels of FGF21. In mice, acinar expression of oncogenic KRAS significantly reduces FGF21 expression. When these mice are placed on a HFD, they develop extensive inflammation, pancreatic cysts, PanINs, and

PDACs, which are reduced by injection of FGF21. FGF21 also reduces the GTP binding capacity of RAS. FGF21 might be used in prevention or treatment of pancreatic cancer.

Graphical Abstract



Lay Summary

A high-fat diet promotes development of pancreatic cancer. We found that acinar cells that express oncogenic KRAS reduce expression of fibroblast growth factor 21. In mice on a high-fat diet, injection of fibroblast growth factor 21 reduces pancreatic inflammation and tumor development.

Keywords

gene regulation; FGFR1; KLB; signaling

Introduction

Pancreatic cancer is a fatal disease and is predicted to become the second leading cause of cancer-related deaths within a decade[1]. It is widely observed that mutations of *KRAS* (e.g., $KRAS^{G12D}$) are an essential initiating event for pancreatic cancer[2]. Previous studies have shown that mutant *Kras*, when expressed in pancreatic progenitor cells from the embryonic stage, is sufficient to drive PDAC in the $Pdx\text{-}1^{Cre}$;$Kras^{LSL\text{-}G12D/+}$ (KC) mice[3]. However, human PDAC likely originates from somatic mutations in *KRAS* in acinar cells during adulthood, suggesting that mice expressing mutant *KRAS* in acinar cells after birth may be more clinically relevant. Notably, mutant *Kras* expressed at an endogenous level in adult acinar cells does not, on its own, lead to PDAC[4, 5], suggesting that an additional challenge is required.

Obesity is a modifiable risk factor for PDAC, and HFD is a major dietary component that drives obesity. Recent studies have shown that when fed a HFD, mice expressing an endogenous level of $Kras^{G12D/+}$ in acinar cells developed marked inflammation, PanIN lesions, and invasive PDAC leading to lethality with high penetrance[6]. However, the mechanism underlying the cooperation between HFD and oncogenic KRAS remains a major knowledge gap.

The endocrine FGF21 has emerged as a novel metabolic regulator for the maintenance of lipid, glucose, and energy homeostasis[7, 8]. Studies have shown that the liver is one of the major sources of endocrine FGF21 under stress conditions[9–11]. By serving as an endocrine

factor, FGF21 promotes the clearance of systemic lipids and glucose, while enhancing insulin sensitivity, fatty acid oxidation, and energy expenditure without apparent effects on cell proliferation and growth[7, 12]. Therefore, FGF21 has the pharmacological potential to treat obesity, diabetes, and meta-inflammation[7, 12–14]. The effects of endocrine FGF21 are mediated by binding to the transmembrane binary complex of fibroblast growth factors receptor 1 (FGFR1) and β-Klotho (KLB)[15–17]. In the pancreas, under wild-type KRAS conditions, FGF21 acts directly on acinar cells to protect the pancreas from pancreatitis and proteotoxic stress[18, 19]; however, the interaction between pancreatic FGF21 and oncogenic KRAS in pancreatic tumorigenesis has not been previously shown.

In the current study, by using mice with conditional knock-in of $Kras^{LSL\text{-}G12D/+}$ under the control of a TM-inducible $fElas^{CreERT}$ driver as described[6, 20, 21], we report that FGF21, its target receptor FGFR1, and its co-receptor KLB were abundantly expressed in normal acinar cells, suggesting that acinar cells are both a source and a target of FGF21. The expression of pancreatic *FGF21* was dramatically downregulated, partly through a PPARG-dependent mechanism, upon an endogenous level of $Kras^{G12D/+}$ expression. Remarkably, under chronic HFD challenge, administration of rhFGF21, which compensates for the loss of FGF21 in acinar cells, significantly suppressed both pancreatic and systemic inflammation, inhibited RAS activation, PanIN lesions, tumor incidence, and liver metastasis, as well as extended the survival of $Kras^{G12D/+}$ mice in comparison to those without rhFGF21 treatment. Our results reveal that pancreatic FGF21 is downregulated by $KRAS^{G12D}$, which renders the pancreas vulnerable to obesogenic HFD challenge, leading to exacerbated inflammation and invasive PDAC, while pharmacological FGF21 counteracts the damaging effects of HFD and mitigates this vulnerability.

Materials and Methods

Mouse strains

CreERT-LoxP recombination approaches were used to control gene expression in pancreatic acinar cells. $Kras^{LSL\text{-}G12D/+}$ mice, which possess the conditional knock-in of mutant $Kras^{G12D}$, were obtained as described[6]. $fElas^{CreERT}$ mice, which express TM-regulated Cre recombinase under a full-length *Elastase* promoter specifically in acinar cells, were described previously[22]. $Kras^{LSL\text{-}G12D/+}$ mice and $fElas^{CreERT}$ mice were cross-bred to generate $fElas^{CreERT};Kras^{LSL\text{-}G12D/+}$ double-transgenic mice (called $Kras^{G12D/+}$ after TM)[6, 23]. Alternatively, $Ptf1a^{CreERT}$ mice[24] were crossed with $Kras^{LSL\text{-}G12D/+}$ mice to generate $Ptf1a^{CreERT};Kras^{LSL\text{-}G12D/+}$ mice (called $Ptf1a^{CreERT};Kras^{G12D/+}$ after TM)[25]. $Trp53^{LSL\text{-}R172H}$ mice[26] were crossed with $fElas^{CreERT}$ mice to generate $fElas^{CreERT};Trp53^{LSL\text{-}R172H/+}$ (called $Trp53^{R172H/+}$ after TM) mice. All animal experiments were reviewed and approved by Stony Brook University Institutional IACUC and the University of Texas, MD Anderson Cancer Center IACUC.

Additional experimental details are provided in the Supplementary Information.

Results

Pancreatic acinar cells are both a potential source and a target of FGF21

Previous studies have shown that HFD and KRAS^{G12D} cooperate to promote PDAC with high penetrance[6], suggesting that acinar cells containing mutant KRAS are susceptible to chronic HFD challenge. FGF21 is an important regulator of lipid metabolism and elicits anti-obesity effects[8, 12] while also protecting the pancreas from pancreatitis and metabolic stress[18, 19]. In order to understand the role of FGF21 in the pancreas and pancreatic tumorigenesis, we employed the established *fElas*^{CreERT} and *fElas*^{CreERT};*Kras*$^{LSL\text{-}G12D/+}$ mice[6, 23]. We first compared the pancreatic and hepatic *Fgf21* expression in young adult *fElas*^{CreERT} control mice one week post-TM. Pancreatic *Fgf21* expression was significantly higher (>80 times) than that of hepatic tissue, which is known as the primary source of endocrine FGF21 under stress conditions (Figure 1A). This result concurs with a previous report showing that *Fgf21* is highly expressed in the normal pancreas[27]. Immunohistochemistry (IHC) and Western blot further confirmed the presence of FGF21 in acinar cells (Figure 1B–C). Correspondingly, the expression of pancreatic FGF21 receptor, *Fgfrl*, was significantly higher (>10 times) than that of the liver (Figure 1D–E). The expression of FGF21 co-receptor, *Klb*, was comparable to that of the liver (Figure 1F), which is known to express a high level of *Klb*[28, 29]. Western blot (Figure 1G) and IHC (Figure 1H, open arrow) studies further confirmed the abundant presence of KLB in acinar cells. Comparison of *Fgf21, Fgfr1*, and *Klb* expression across the liver, pancreas, and adipose tissues in 7-month-old *fElas*^{CreERT} mice after five months of TM induction revealed that pancreatic *Fgf21* expression remained the highest (Figure 1I). Both the pancreas and adipose tissue expressed significantly higher levels of *Fgfr1* than the liver (Figure 1J). *Klb* expression levels were comparable across all tissues (Figure 1K). Pancreatic *Fgf21* expression was stable regardless of the age and the duration post TM treatment (Supplementary Figure 1A). All these data reveal that FGF21, FGFR1, and KLB are highly expressed in normal acinar cells, suggesting that pancreatic acinar cells are both a source and a target of FGF21.

FGF21 is a downstream target of oncogenic KRAS^{G12D}

To test if FGF21 plays a role in human pancreatic cancer, we performed IHC analysis of FGF21 in human pancreatic tissue arrays and found that the PDAC specimens (n=59) had substantially lower levels of FGF21 than normal human pancreatic tissues (n=45), which exhibited strong FGF21 positive staining in acinar cells (Figure 2A–B). FGF21 was also stained positively in normal ducts of both the normal human pancreas and PDAC specimens (Supplementary Figure 2A, *inset*). In addition, from the human GEO database (https://www.ncbi.nlm.nih.gov/geoprofiles/78912317), *FGF21* expression in human PDAC tissues dropped significantly in comparison to the paired normal pancreata (n=39 pairs) (Figure 2C). Furthermore, *FGF21* expression in human pancreatic cancer cell lines carrying *KRAS* mutations decreased significantly in comparison to that of normal human pancreatic ductal epithelial (HPDE) cells (Figure 2D), suggesting an inverse association between pancreatic FGF21 and oncogenic KRAS.

Given that PDAC likely originates from acinar cells with somatic mutations in *KRAS* occurring after birth, we next analyzed the potential changes of FGF21 in the *fElas*^{CreERT};*Kras*$^{LSL\text{-}G12D/+}$ mice that express an endogenous level of *Kras*$^{G12D/+}$ in nearly 100% acinar cells upon TM induction[6]. A striking decrease of >100 fold in *Fgf21* expression was observed in mice five months post-TM relative to the age-matched *fElas*^{CreERT} mice (Figure 2E). IHC analysis confirmed the substantially lower levels of FGF21 in the pancreatic tissues of *Kras*$^{G12D/+}$ mice compared to that of *fElas*^{CreERT} mice (Supplementary Figure 2B). To determine whether the drastic reduction of pancreatic *Fgf21* is an early event related to *Kras*$^{G12D/+}$ expression, we first compared *Fgf21* expression in the 40-day-old *fElas*^{CreERT} control mice one week post-TM to that without TM. No significant differences were observed between the two groups, suggesting that neither TM nor Cre recombinase affected *Fgf21* expression (Supplementary Figure 2C). We then compared *Fgf21* expression one week post-TM induction of *Kras*$^{G12D/+}$ to that of the age-matched (40 days old) mice without TM and found that *Fgf21* expression was significantly reduced upon *Kras*$^{G12D/+}$ induction (Figure 2F), suggesting that the downregulation of *Fgf21* is specific to *Kras*$^{G12D/+}$. Notably, this occurred when no obvious alterations of pancreatic histology were observed (Figure 2G).

To further confirm our observation that KRAS^{G12D} specifically downregulates *Fgf21* expression, we employed another mouse strain, the *Ptf1a*^{CreERT};*Kras*$^{LSL\text{-}G12D/+}$ mice[25]. qRT-PCR data showed that *Fgf21* expression was significantly reduced in these mice two weeks post-TM in comparison to the age-matched (two months of age) mice without TM induction (Figure 2H). No changes to the pancreatic histology were observed in the TM-induction group (Supplementary Figure 2D). Consistent with a role in tumorigenesis, the p53 mutations occur in 50–75% of human PDAC following an initiating mutation in *KRAS*. To determine if mutant p53 could also inhibit pancreatic *Fgf21*, we employed the *fElas*^{CreERT};*Trp53*$^{LSL\text{-}R172H/+}$ mouse strain. Five months post-TM, no significant differences in pancreatic *Fgf21* expression were observed between the age-matched *Trp53*$^{R172H/+}$ and *fElas*^{CreERT} mice (Supplementary Figure 2E), indicating that *Kras*$^{G12D/+}$, but not *Trp53*$^{R172H/+}$, specifically induces the marked downregulation of *Fgf21*. Furthermore, we compared *Fgfr1* and *Klb* expression in the age-matched *fElas*^{CreERT} and *Kras*$^{G12D/+}$ mice one week post-TM. No significant changes for both genes were noticed (Supplementary Figure 2F–G).

Oncogenic KRAS reduces pancreatic *Fgf21* expression partially through downregulating *Pparg*

To understand how oncogenic KRAS may potentially intervene to suppress *FGF21* expression, we analyzed the expression of the transcription factors that are known to possess consensus binding sites on the *Fgf21* promoter region and responsible for *Fgf21* expression[9, 10, 30–32]. Among them, peroxisome proliferator-activated receptor gamma (*Pparg*, which encodes PPAR-gamma or PPARG), peroxisome proliferator-activated receptor alpha (*Ppara*), and retinoic acid receptor-related orphan receptor (*Rora*) were significantly downregulated (Figure 2I, Supplementary Figure 2H–I) in the same pancreatic tissues where *Fgf21* was reduced by Kras^{G12D}. However, other known *Fgf21*-regulating factors, such as *Rarb* and *ChREBP* (Supplementary Figure 2J–K), as well as *SREBP1* and *Lxrb* (data not

shown), were not changed. Treatment of mutant *KRAS*-bearing Panc-1 cells that have a weak *FGF21* expression with a specific PPARG agonist, Rosiglitazone, resulted in a dose-dependent increase in *FGF21* expression (Figure 2J), suggesting that PPARG is a potential mediator of $KRAS^{G12D}$-downregulated expression of pancreatic *FGF21*.

Replenishment of FGF21 inhibits $KRAS^{G12D}$-mediated pancreatic inflammation and PanIN lesions under normal dietary conditions

Studies have demonstrated that under wild-type KRAS conditions, FGF21 signaling was induced in acinar cells upon experimental induction of pancreatitis and that genetic deletion of *Fgf21* exacerbated pancreatic injury[19]. FGF21 acts directly on acinar cells to promote exocrine secretion without increasing protein synthesis, thereby mitigating proteotoxic stress[18], suggesting that acinar cell FGF21 alleviates metabolic stress and associated pathogenic effects in the presence of wild-type KRAS. By contrast, our above data reveal that oncogenic KRAS downregulates acinar cell FGF21.

To determine if FGF21 replenishment plays a role in restricting $Kras^{G12D/+}$-mediated pancreatic inflammation and fibrosis under the normal diet (ND) conditions, we administered rhFGF21 to $Kras^{G12D/+}$ mice for 10 weeks to compensate for the loss of acinar cell FGF21 (Figure 3A). rhFGF21 treatment significantly inhibited pancreatic inflammation and immune cell infiltration as measured by IHC staining of inducible cyclooxygenase-2 (COX-2) and F4/80, respectively, compared to those without rhFGF21 treatment (n=8) (Figure 3B, Supplementary Figure 3A–B). rhFGF21 also significantly reduced pancreatic fibrosis as measured by Sirius Red staining of collagen fibrils (Figure 3B, Supplementary Figure 3C). These data suggest that rhFGF21 supplementation effectively inhibits pancreatic inflammation and fibrosis induced by $KRAS^{G12D}$ under the ND conditions.

To test whether rhFGF21 administration could inhibit $KRAS^{G12D}$-mediated acinar-to-ductal metaplasia (ADM), we evaluated acinar and duct cell markers in the ND-fed $Kras^{G12D/+}$ mice upon rhFGF21 treatment. Co-immunofluorescence (Co-IF) analyses of amylase and CK19, which mark acinar and duct cells, respectively, revealed that rhFGF21 treatment significantly reduced CK19+ staining and preserved amylase+ acinar cells compared to those without rhFGF21 treatment (Figure 3C–E). These data suggest that rhFGF21 suppresses the ADM reprogramming induced by oncogenic KRAS under normal feeding conditions.

To determine whether rhFGF21 directly acts on acinar cells, we isolated acinar cell clusters from 80-day-old $Kras^{G12D/+}$ mice one week post-TM and seeded them in collagen plates for 3-dimensional (3D) explant cultures with daily rhFGF21 treatment. On day one, treatment with rhFGF21 had little effects on the acinar cell clusters; however, by day four, rhFGF21 significantly inhibited ductal cell formation compared to PBS treatment group (Figure 3F, Supplementary Figure 3D), suggesting that rhFGF21 directly acts on acinar cells to inhibit $KRAS^{G12D}$-mediated ADM reprogramming.

As ADM is regarded as the precursor to the development of PanIN lesions, we evaluated the effects of FGF21 administration on $KRAS^{G12D}$-mediated PanIN lesions using Alcian blue staining for acidic mucins and IHC staining for Mucin 5AC (MUC5). rhFGF21 injections

caused a noticeable reduction in both acidic mucins and MUC5 compared to those without FGF21 treatment (Figure 3G, Supplementary Figure 3E–F). Using IHC analysis, we observed that rhFGF21 suppressed ductal cell proliferation as measured by the decreased levels of Ki-67 and cyclin D1 (Figure 3H–J). Altogether, our data indicate that the exogenous supplementation of FGF21 to *Kras*$^{G12D/+}$ mice that have a dramatic reduction of FGF21 in acinar cells results in significant beneficial effects on the inhibition of pancreatic inflammation, ADM, and PanIN lesions under the ND conditions.

Pharmacological administration of FGF21 inhibits HFD and KRAS^{G12D} induced PanIN lesions and PDAC

Inspired by the above data, we proceeded to determine if FGF21 plays a suppressive role in HFD and KRAS mediated invasive PDAC by treating the *Kras*$^{G12D/+}$ mice concurrently with HFD and rhFGF21 for 10 weeks (Figure 4A). The HFD-fed *Kras*$^{G12D/+}$ mice accumulated abdominal fat and developed multiple pancreatic cysts (Figure 4B). In contrast, treatment with rhFGF21 alleviated abdominal fat accumulation and pancreatic cysts (Figure 4B). Consistently, rhFGF21 administration reduced the accumulation of pancreatic triglycerides in comparison to those without rhFGF21 treatment (Supplementary Figure 4A). Histological analysis revealed that the HFD-fed *Kras*$^{G12D/+}$ mice displayed high-grade PanIN lesions and PDAC with 50% penetrance (Figure 4C). In marked contrast, with rhFGF21 treatment, most of the mice (87.5%) exhibited well-preserved parenchyma and acinar cell content with only early stage PanIN lesions (Figure 4C–D). Only one out of eight mice treated with rhFGF21 developed PDAC.

Assessment of damage to pancreatic tissues revealed that chronic HFD consumption led to a significant decrease of amylase, which marks acinar cell content, while rhFGF21 treatment restored the lost amylase content (Figure 4E, Supplementary Figure 4B). Alpha-smooth muscle actin (α-SMA), which marks the levels of pancreatic stellate cell activation, was strongly elevated in the HFD-fed *Kras*$^{G12D/+}$ mice. However, this elevation was significantly suppressed by chronic treatment with rhFGF21 (Figure 4E, Supplementary Figure 4C). The Co-IF analysis further revealed that rhFGF21 greatly reduced CK19+ staining and preserved acinar cells (amylase+) compared to the HFD-fed *Kras*$^{G12D/+}$ mice without rhFGF21 treatment (Figure 4F, Supplementary Figure 4D–E). A similar pattern to that observed for amylase was found for MIST1, a transcription factor essential for maintaining an adult acinar cell phenotype (Figure 4F, Supplementary Figure 4F). These data suggest that rhFGF21 preserved acinar cells and inhibited stromal activation induced by HFD in *Kras*$^{G12D/+}$ mice. Furthermore, we observed prominent positive stains of acidic mucins and MUC5 in the pancreata of the HFD-fed *Kras*$^{G12D/+}$ mice; however, rhFGF21 treatment significantly inhibited these PanIN markers (Figure 4G, Supplementary Figure 4G–H). Our data suggest that the replenishment of FGF21 suppresses HFD and KRAS^{G12D} induced rapid PanIN lesions and PDAC.

To determine whether the significant downregulation of pancreatic FGF21 by KRAS^{G12D} can lead to a reduction of serum FGF21 levels, which may induce systemic metabolic alterations, we measured serum FGF21 levels pre-TM, one week and 10 weeks post-TM induction of *Kras*$^{G12D/+}$. No significant differences in serum FGF21 levels were observed

(Supplementary Figure 4I–J), indicating that alteration of pancreatic FGF21 by KRAS^{G12D} does not influence circulating FGF21 levels.

Pharmacological FGF21 inhibits HFD and KRAS^{G12D} induced pancreatic inflammation

Chronic HFD causes inflammation, which cooperates with oncogenic KRAS to drive PDAC[4]. To understand whether the tumor-suppressive effect of rhFGF21 is through alleviation of pancreatic inflammation, we analyzed the protein expression of COX-2, which was up-regulated in the HFD-fed *Kras$^{G12D/+}$* mice[6]. Indeed, these mice exhibited intense focal staining of COX-2 along the pancreatic ducts; however, rhFGF21 treatment significantly decreased COX-2+ staining to levels comparable to the ND-fed *Kras$^{G12D/+}$* mice (Figure 5A, Supplementary Figure 5A). Furthermore, macrophages, as detected by F4/80+ staining, were abundant in the pancreata of the HFD-fed *Kras$^{G12D/+}$* mice; however, rhFGF21 significantly inhibited HFD-induced macrophage infiltration (Figure 5A, Supplementary Figure 5B). In a similar manner, CD3, as a marker of peripheral T cell infiltration, was significantly elevated under chronic HFD insult, while rhFGF21 alleviated T cell infiltration (Figure 5A, Supplementary Figure 5C). Analysis of extracellular matrix collagen deposition that marks pancreatic fibrosis revealed that although the ND-fed *fElas^{CreERT}* mice displayed a minimal collagen staining that was primarily localized around the blood vessels and the peri-lobular spaces, the HFD-fed *Kras$^{G12D/+}$* mice exhibited intense collagen deposition surrounding the neoplastic ductal structures, indicating a robust fibrotic response. In contrast, FGF21 supplementation significantly reduced collagen levels (Figures 5A, Supplementary Figure 5D).

To better understand the suppressive role of FGF21 in pancreatic inflammation promoted by the cooperative interaction between HFD and KRAS^{G12D}, we analyzed the expression of an array of inflammation-associated cytokines/chemokines and receptors comparatively by qRT-PCR. Of note, the pancreata of the ND-fed *Kras$^{G12D/+}$* mice showed significant increases in the expression of *Cxcl5, Tgfb1, Ccl2, Csf2, Tnf*, and *Tnfrsf11b* in comparison to those of the ND-fed *fElas^{CreERT}* mice, suggesting that pancreatic inflammation is mediated by oncogenic KRAS in this context (Figure 5B–C, Supplementary Figure 5E–H). HFD treatment further enhanced the expression of these genes (Figure 5B–C, Supplementary Figure 5E–H), indicating a significant inflammatory effect of obesogenic HFD on the pancreas. Interestingly, although pancreatic *Ccl17* was not affected by KRAS^{G12D} under the ND conditions, it was markedly upregulated in response to the HFD challenge in the *Kras$^{G12D/+}$* mice (Supplementary Figure 5I). Importantly, rhFGF21 administration significantly suppressed the augmented expression of all these genes (Figure 5B–C, Supplementary Figure 5E–I). These data suggest a prominent anti-inflammatory role of FGF21 in the pancreas. On the other hand, the expression levels of the cytokines that have anti-inflammation or anti-neoplastic functions, including *Ccl19, Ifng, Il21, Cd40l, Ccl21a*, and *Cxcl9*, were significantly downregulated in the HFD-fed *Kras$^{G12D/+}$* mice, while administration of rhFGF21 effectively restored their expression (Supplementary Figure 5J–O).

We have previously shown that RAS activity is an important determinant in pancreatic tumorigenesis, and that inflammation enhances RAS activity[21, 33]. As pharmacological

rhFGF21 significantly suppressed HFD-promoted pancreatic inflammation, we tested if this would lead to a reduction of RAS activity in the pancreata of the HFD-fed *Kras*$^{G12D/+}$ mice. Indeed, we observed that the HFD-enhanced RAS hyperactivity was significantly inhibited by rhFGF21 (Figure 5D, Supplementary Figure 5P). These data indicate that the beneficial effects of FGF21 in protecting the pancreas from neoplastic progression are attributable to the inhibition of the accentuated inflammation and RAS hyperactivation induced by HFD.

Correspondingly, rhFGF21 treatment significantly reduced cyclin D1 and Ki-67 levels in the pancreatic ductal cells of the HFD-fed *Kras*$^{G12D/+}$ mice compared to those without rhFGF21 treatment (Figure 5E, Supplementary Figure 5Q–R). These data indicate that the supplementation of rhFGF21 to compensate for the loss of pancreatic FGF21 inhibits pancreatic tumor cell proliferation induced by the synergistic cooperation between KRAS^{G12D} and HFD.

Pharmacological FGF21 inhibits HFD-induced liver and adipose tissue inflammation

As chronic HFD imposes adverse metabolic effects on adipose tissue and the liver, which are among the direct or indirect targets of endocrine FGF21[9, 10, 16], we therefore tested the anti-inflammatory effects of rhFGF21 on both tissues. The ND-fed *Kras*$^{G12D/+}$ mice showed no significant differences in the inflammation markers compared to the ND-fed *fElas*^{CreERT} mice in either the liver or the adipose tissue (Figure 6A–I, Supplementary Figure 6A–B), suggesting that the liver and adipose tissue do not impose significant inflammatory stress on the pancreas under the ND conditions, and therefore, the observed pancreatic inflammation and associated pathologies in Figure 3 are attributable to KRAS^{G12D} in this context. Thus, FGF21 plays a direct role in restricting KRAS^{G12D}-mediated pancreatic inflammation and associated pathogenesis.

Furthermore, chronic HFD significantly elevated the expression of *Ccl2, Ccl4, CD68*, and *CD11b* in both the liver and adipose tissues, as well as adipose *Tgfbl, Tnf*, and *Ccl11*, while rhFGF21 effectively suppressed these inflammation markers (Figure 6A–I, Supplementary Figure 6A–B). As a metabolic marker of adipose tissue response, adipose *Ucp1* underwent a striking increase of >40 fold with rhFGF21 treatment (Supplementary Figure 6C). Furthermore, the anti-inflammatory and anti-fibrotic adipokine, *Adipoq*, was also significantly elevated upon rhFGF21 treatment (Supplementary Figure 6D). These data suggest that rhFGF21 inhibits the HFD-induced systemic inflammation that otherwise may impinge on the pancreas through secretory cytokines or adipokines.

Pharmacological FGF21 inhibits HFD and KRAS^{G12D} induced liver metastasis and prolongs survival

Next, we determined whether pharmacological administration of rhFGF21 would prolong the overall survival and inhibit liver metastasis in the HFD-fed *Kras*$^{G12D/+}$ mice. We first observed that mice fed a HFD had a substantial increase in body weight; however, this was not evident under rhFGF21 treatment despite a continued HFD consumption (Figure 7A). Furthermore, the survival rate of the HFD-fed *Kras*$^{G12D/+}$ mice significantly decreased compared to the age-matched ND-fed *Kras*$^{G12D/+}$ mice, with median survival rates of 229 days and 352 days, respectively. Treatment with rhFGF21 prolonged the median survival of

the HFD-fed *Kras*$^{G12D/+}$ to 323 days (Figure 7B). *Kras*$^{G12D/+}$ mice fed a ND and concurrently treated with rhFGF21 showed a better survival rate than the ND-fed *Kras*$^{G12D/+}$ mice (Figure 7B), which do not spontaneously develop obesity. Regardless of the nature of the diets and the survival rates, the aged *Kras*$^{G12D/+}$ mice eventually developed PDAC (Figure 7C). The cancerous cells formed ductal structures (closed arrow), showing severe architectural and cytological atypia with frequent mitotic figures and abundant interstitial fibrosis (Figure 7C).

Over 50% of the HFD-fed *Kras*$^{G12D/+}$ mice progressed to liver metastasis. Multiple metastatic nodules with distinct borders were mostly found located on the liver surface in the HFD treatment group (Figure 7D, upper panel). Histological analyses revealed prominent metastatic foci on liver sections of the HFD treatment mice (Figure 7D, middle panel), and CK19 positive staining further confirmed the liver metastases in the HFD-fed *Kras*$^{G12D/+}$ mice. In contrast, the HFD-fed *Kras*$^{G12D/+}$ mice concurrently treated with rhFGF21 failed to develop liver metastases (Figure 7D, lower panel, Supplementary Figure 7A).

In summary, our studies show that KRAS^{G12D}, partly through downregulating *Pparg*, reduces *Fgf21*, which in turn creates a vulnerability to obesogenic HFD challenge, leading to extensive inflammation, RAS hyperactivation, PanIN lesions, and invasive PDAC. Pharmacological supplementation of FGF21 mitigates both pancreatic and systemic inflammation, resulting in the suppression of RAS hyperactivity and PDAC development (Figure 7E).

Discussion

Obesity dramatically increases the risk of pancreatic cancer, which remains one of the most aggressive malignancies[34], and the chronic consumption of a HFD is known as a primary dietary factor in promoting obesity. As the prevalence of obesity has been increasing at an alarming rate, obesity-associated pancreatic cancer poses an even greater threat. Thus, gaining a better understanding of HFD-promoted pancreatic cancer is urgent, as it will provide new insights into possible effective intervention strategies. Experimentally, HFD promotes oncogenic KRAS-mediated PDAC, indicating that mutations in KRAS render mice susceptible to obesogenic HFD insults leading to PDAC. However, the mechanism underlying such vulnerability is unclear. In this study, we made several novel observations concerning the roles of FGF21, metabolic regulator that prevents obesity, in the pancreas and oncogenic KRAS-mediated pancreatic tumorigenesis. First, we find that acinar cells express high levels of *Fgf21, Fgfrl*, and *Klb* under normal feeding conditions, suggesting that acinar cells are both a source and a target of FGF21. Second, we report for the first time that *Fgf21* expression in acinar cells is highly sensitive to mutant KRAS^{G12D}, showing remarkable decrease upon the expression of *Kras*$^{G12D/+}$ at an endogenous level when no overt alterations of pancreatic histology are noted. Third, we show that the significant downregulation of *Fgf21* by mutant KRAS^{G12D} is partly mediated by the reduced PPARG. Fourth, we note that replenishment of the lost FGF21 directly inhibits pancreatic inflammation, transdifferentiation of acinar cells into ductal cells, and PanIN lesions under the ND conditions. Fifth, pharmacological FGF21 significantly mitigates inflammation in both the pancreas and systemic liver and adipose tissue under the HFD conditions. Sixth,

FGF21 supplementation significantly inhibits HFD-promoted pancreatic RAS hyperactivity. Finally, pharmacological FGF21 significantly ameliorates pancreatic pathogenic responses, leading to the significant delay of PDAC development and extension of survival under obesogenic HFD challenge.

We have provided evidence to support that $KRAS^{G12D}$ downregulates pancreatic *FGF21* expression. First, we show that PADC patient tissues and mutant *KRAS-bearing* pancreatic cancer cells exhibit a significant reduction of *FGF21* compared to their normal counterparts. Second, in several $Kras^{G12D/+}$ mouse strains with different Cre drivers, we show that $KRAS^{G12D}$ significantly inhibits *Fgf21* expression. Third, we note that *Pparg, Ppara*, and *Rora*, which were shown to induce *FGF21* expression[9, 10, 30–32] are downregulated by $KRAS^{G12D}$ expression. Finally, treatment of pancreatic cancer cells with PPARG agonist increased *FGF21* expression in a dose-dependent manner, suggesting a role of PPARG in regulating pancreatic *FGF21*. It would be interesting to investigate further the functional roles of PPARG, PPARA, and RORA in regulating pancreatic *FGF21* and pancreatic tumorigenesis.

Our results indicate a prominent role of FGF21 in directly protecting the pancreas from neoplastic progression induced by $KRAS^{G12D}$. First, we show that acinar cells express high levels of FGF21 and its receptor complex, indicating an autocrine/paracrine mode of FGF21 action in the pancreas, which aligns with previous findings showing that FGF21 directly targets acinar cells[18, 19]. Second, the pancreata of the ND-fed $Kras^{G12D/+}$ mice, which do not develop obesity and systemic inflammation, were effectively protected from $KRAS^{G12D}$-driven inflammation and neoplastic progression by rhFGF21, indicating a direct protective effect of FGF21 on the pancreas with minimal systemic influence. Third, we show that rhFGF21 directly inhibits ADM in the *in vitro* 3D explant cultures of acinar clusters, which is not subject to systemic metabolic influence.

Pancreatic function and systemic metabolism are inextricably linked. In the $Kras^{G12D/+}$ mice, chronic consumption of HFD leads to obesity and invasive PDAC, while rhFGF21 ameliorates these detrimental systemic and local pancreatic effects of HFD. We show that HFD induces inflammation in both the pancreas and systemic liver and adipose tissue, while these adverse effects are effectively inhibited by rhFGF21, emphasizing the broad anti-inflammatory role of FGF21. Decreased inflammation was accompanied by the suppression of inflammation-associated fibrosis, PanIN lesions, and PDAC development. Importantly, we observed that the HFD-enhanced pancreatic RAS activity was also significantly reduced by rhFGF21. Furthermore, previous studies have shown that inhibition of local pancreatic inflammation through ablation of COX-2 in acinar cells markedly suppressed HFD and $KRAS^{G12D}$ mediated pancreatic neoplastic progression[6], supporting that the inhibition of local pancreatic inflammation is critical to the suppression of pancreatic tumorigenesis. Collectively, our data suggest that in addition to the noticeable systemic anti-inflammatory effects, the anti-inflammatory effects of rhFGF21 on the pancreas are essential to the suppression of HFD-promoted inflammatory damage and RAS hyperactivation, leading to the protection of the pancreas from neoplastic progression.

Previous studies focused on the pathophysiological roles of FGF21 in the liver and pancreas in the setting of wild-type KRAS[18, 19, 35], which showed that FGF21 exhibits a marked elevation in response to stress-inducing conditions, including the ingestion of a HFD[11]. Unlike these previous studies, however, our study shows that pancreatic FGF21 is negatively regulated by oncogenic KRAS. By abrogating FGF21, oncogenic KRAS allows the malevolent metabolic effect of chronic HFD consumption to take its maximal toll on the pancreas. In light of this fact, the loss of pancreatic FGF21 specifically by oncogenic KRAS is significant, as this breaks a stress-defensive barrier in the path towards PDAC development.

We observed multiple small pancreatic cysts in the HFD-fed *Kras*$^{G12D/+}$ mice; however, rhFGF21 treatment effectively inhibited this phenomenon, suggesting that inflammation is likely the cause of pancreatic cysts. In patients, mucinous cystic lesions are precursors to invasive pancreatic cancer. Therefore, it is possible that FGF21 holds the potential to reverse mucinous pancreatic cysts and reduce the risk of pancreatic cancer in such patients.

In summary, our findings reveal a previously unknown critical vulnerability conferred by oncogenic KRAS, highlight pancreatic FGF21 as an important downstream target of oncogenic KRAS, and identify pharmacological FGF21 as a putative intervention strategy to hinder pancreatic inflammation and cancer development in the context of obesity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Abbreviations:

ADM	acinar-to-ductal metaplasia
CCL17	C-C chemokine ligand 17
CCL21a	C-C chemokine ligand 21A
CD3	cluster of differentiation 3
CD11b	macrophage cluster of differentiation 11
CD40L	Cluster of differentiation 40 ligand

CD68	cluster of differentiation 68
CK19	cytokeratin-19
COX2	cyclooxygenase-2
CXCL5	C-X-C motif chemokine ligand 5
CXCL9	C-X-C motif chemokine ligand 9
F4/80	EGF-like module-containing mucin-like hormone receptor-like 1
FGF	fibroblast growth factor
FGFR	fibroblast growth factor receptor
CSF2 (GM-CSF)	granulocyte-macrophage colony-stimulating factor
HFD	high-fat diet
HPDE	human pancreatic ductal epithelial cells
IFNG	interferon gamma
IL21	interleukin 21
Ki-67	proliferation-related Ki-67 antigen
KLB	Klotho beta
KRAS	Kirsten rat sarcoma-2 viral (v-Kiras2) oncogene homolog
CCL2 (MCP1)	Monocyte/macrophage chemoattractant protein 1
CCL4 (MIP1b)	macrophage inflammatory protein 1 beta
MIST1	muscle, intestine and stomach expression 1
MUC5	mucin 5 subtypes A and C
PanIN	pancreatic intraepithelial neoplasia
PDAC	pancreatic ductal adenocarcinoma
PPARA	peroxisome proliferator-activated receptor alpha
PPARG	peroxisome proliferator-activated receptor gamma
RORA	retinoid-related orphan receptor alpha
TGFB1	transforming growth factor beta
TNF	tumor necrosis factor alpha
TNFRSF11b	TNF receptor superfamily member 11b

UCP1	uncoupling protein 1

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Author names in bold designate shared co-first authorship.

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WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT:

A high-fat diet (HFD) promotes obesity, which increases the risk of pancreatic cancer. In mice, a HFD and expression of oncogenic KRAS lead to the development of pancreatic cancer. We investigated how the expression of oncogenic KRAS regulates fibroblast growth factor 21 (FGF21), which prevents obesity, and the effects of FGF21 administration on pancreatic tumorigenesis.

NEW FINDINGS:

Normal acinar cells from mice and humans express high levels of FGF21. In mice, acinar expression of oncogenic KRAS significantly reduces FGF21 expression. When these mice are placed on a HFD, they develop extensive inflammation, pancreatic cysts, pancreatic intraepithelial neoplasias, and pancreatic adenocarcinomas—all were reduced by injection of FGF21. FGF21 reduces HFD-promoted RAS activity.

LIMITATIONS:

These studies were performed in mice—more studies of humans are needed.

IMPACT:

FGF21 is downregulated by oncogenic KRAS. FGF21 might be given to patients for the prevention or treatment of pancreatic cancer.

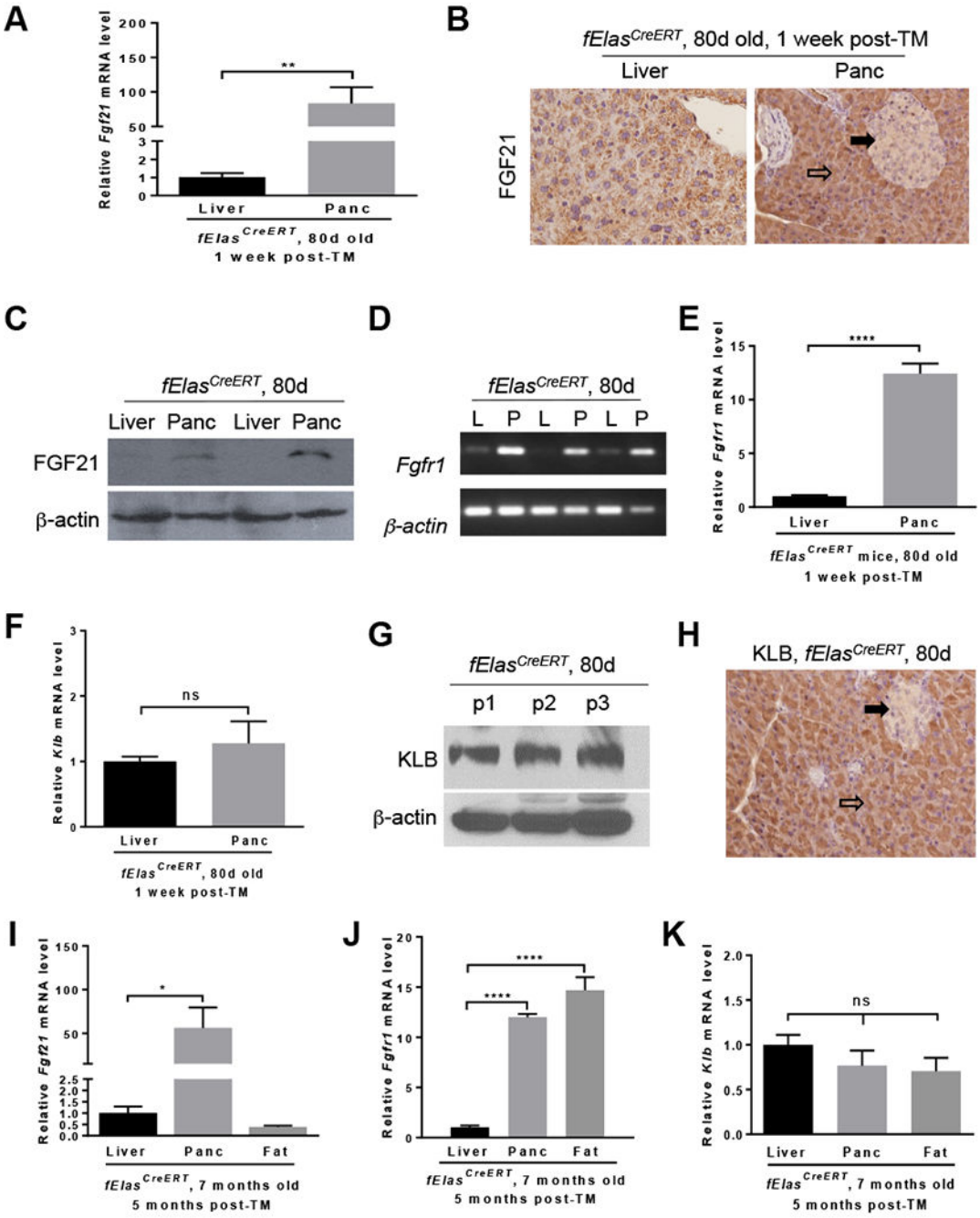

Figure 1. Pancreatic acinar cells are both a source and a target of FGF21.

All mice in A-H were fed a ND, induced by TM at 70 days of age, and sacrificed one week post-TM. (A) qRT-PCR analysis of *Fgf21* expression in the liver (n=11) and pancreas (Panc, n=11) of *fElas*^{CreERT} mice. (B) IHC analysis of FGF21 on the liver and pancreas sections. 200x. (C) Western blot analysis for FGF21 levels. (D) Semi-quantitative RT-PCR analysis for *Fgfrl* in the liver (L) and pancreas (P). (E-F) qRT-PCR analysis for *Fgfrl* and *Klb* in the liver (n=9) and pancreas (n=9). (G) Western blot analysis for KLB in the pancreas (p). (H) IHC analysis of KLB in pancreatic sections. 200x. All mice in I-K were fed a ND, induced

by TM at 70 days of age, and sacrificed at 7 months of age. Comparative qRT-PCR analysis for *Fgf21* (I), *Fgfr1* (J) and *Klb* (K) in the liver (n=7), pancreas (n=7), and adipose tissue (n=7) of $fElas^{CreERT}$ mice. Open arrow, acinar cells. Closed arrow, islet cells. Data are means ± SEM. *, $p<0.05$; **, $p<0.01$; ****, $p<0.0001$; ns, not significant.

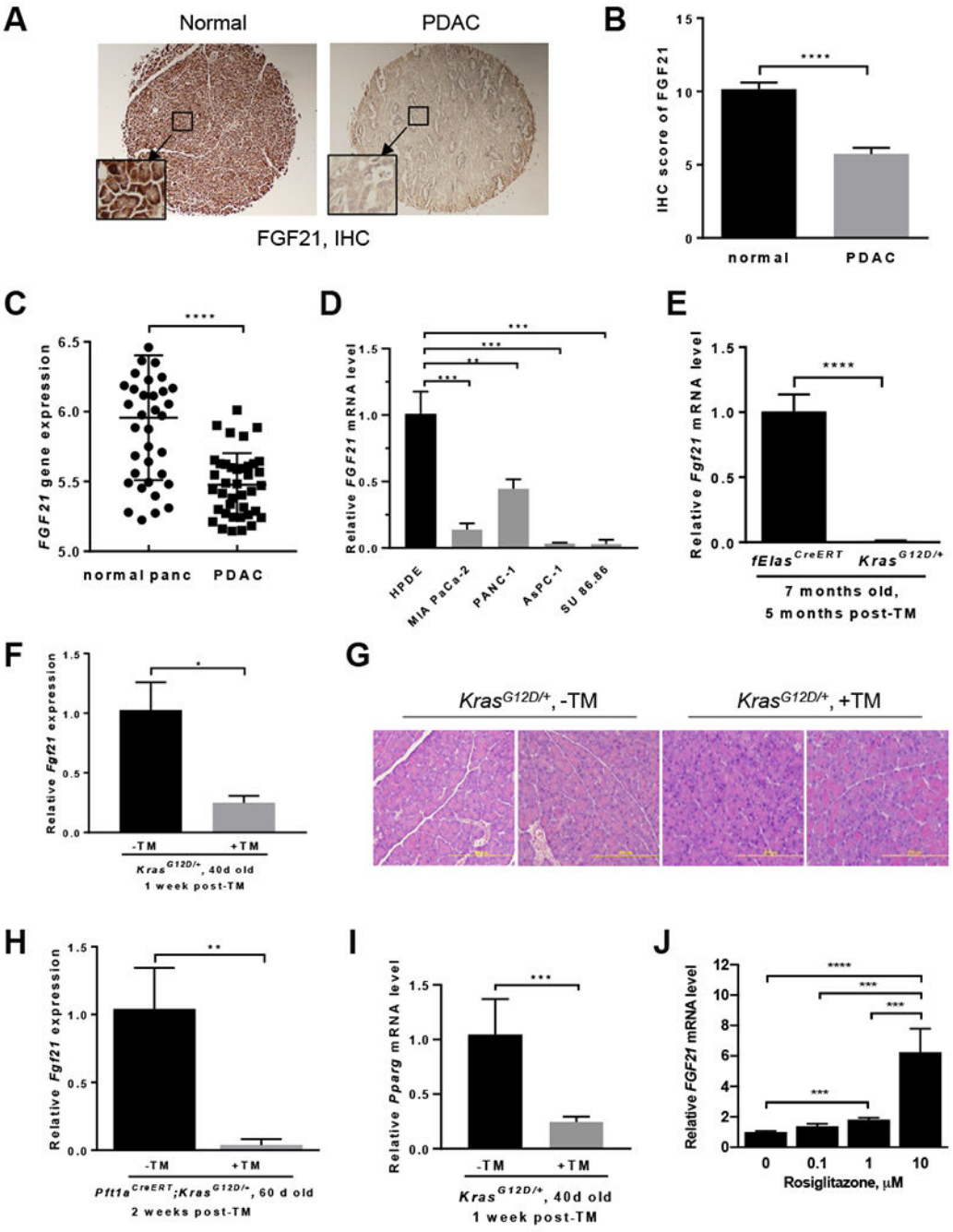

Figure 2. KRAS^{G12D} downregulates *Fgf21* gene expression in acinar cells.

(A) IHC analysis for FGF21 levels in tissue arrays of human normal pancreas (n=45) and PDAC (n=59) (40x). *Inset:* 200x. (B) Quantitation of human FGF21 levels as described in 2A. (C) Human *FGF21* expression in 39 pairs of normal and tumor tissues resected from pancreatic cancer patients as collected in the GEO database (https://www.ncbi.nlm.nih.gov/geo/tools/profileGraph.cgi?ID=GDS4103:221433_at). (D) qRT-PCR analysis of *FGF21* in human pancreatic cancer cell lines carrying *KRAS* mutations. Human normal pancreatic ductal epithelial (HPDE) cells served as a control. All mice in E-I were

fed a ND. (E) qRT-PCR analysis of pancreatic *Fgf21* in 7-month-old $Kras^{G12D/+}$ (n=11) or $fElas^{CreERT}$ mice (n=5) mice five months post-TM. (F) qRT-PCR analysis of pancreatic *Fgf21* in 40-day-old $Kras^{G12D/+}$ mice one week post-TM (n=7) or without TM (n=9). (G) Representative histology of the pancreata as in 2F. 200x. (H) qRT-PCR analysis of pancreatic *Fgf21* of two-month-old $Ptf1a^{CreERT};Kras^{G12D/+}$ mice two weeks post-TM (n=3) or without TM (n=3). (I) qRT-PCR analysis of pancreatic *Pparg* in 40-day-old $Kras^{G12D/+}$ mice one week post-TM (n=7) or without TM (n=9). (J) qRT-PCR analysis of *FGF21* in Panc-1 cells with Rosiglitazone treatment. Data are mean ± SD. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; **** $p<0.0001$.

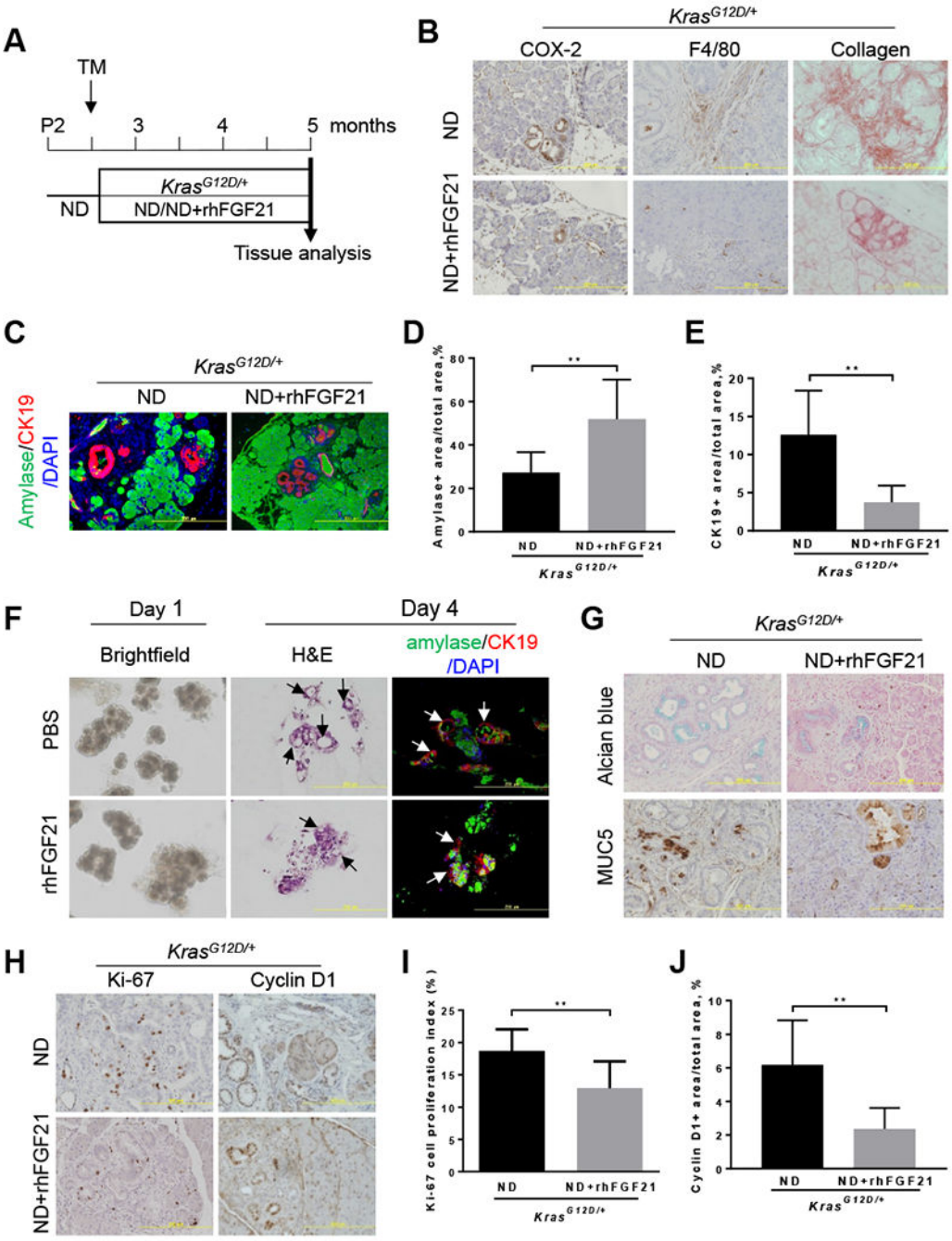

Figure 3. Replenishment of FGF21 suppresses KRAS^{G12D}-induced pancreatic inflammation and PanIN lesions under ND conditions.

(A) Experimental scheme for B-E and G-J: 70-day-old ND-fed *Kras*$^{G12D/+}$;*fElas*^{CreERT} mice were treated with TM at 70 days of age to induce *Kras*$^{G12D/+}$ expression in acinar cells, and then treated with rhFGF21 (0.5 mg/kg body weight/day) (n=10) or PBS (n=8) for ten weeks. (B) IHC staining of COX-2 and F4/80, and Sirius Red staining of collagen on pancreatic sections of *Kras*$^{G12D/+}$ mice with indicated treatments. 200x. (C) Co-immunofluorescence (Co-IF) analysis of amylase and CK19 as indicated. 200x. (D-E) Quantitation of amylase+ areas or CK19+ areas on pancreatic sections (n=4) as described in 3C. (F) The explanted

acinar cell 3D clusters isolated from 80-day-old $Kras^{G12D/+}$ mice one week post-TM were treated daily with rhFGF21 or PBS and then analyzed by brightfield images on day 1, H&E staining on day 4, and Co-IF staining of amylase and CK19 on day 4. (G) Alcian blue staining of acidic mucins and IHC staining of MUC5. (H) IHC analyses of Ki-67 (ND, n=4; ND+rhFGF21, n=4) and Cyclin D1 (ND, n=5; ND+rhFGF21, n=3). (I-J) Quantitation of Ki-67 and Cyclin D1 levels in 3H. Results were expressed as mean ± SD. **, $p<0.01$.

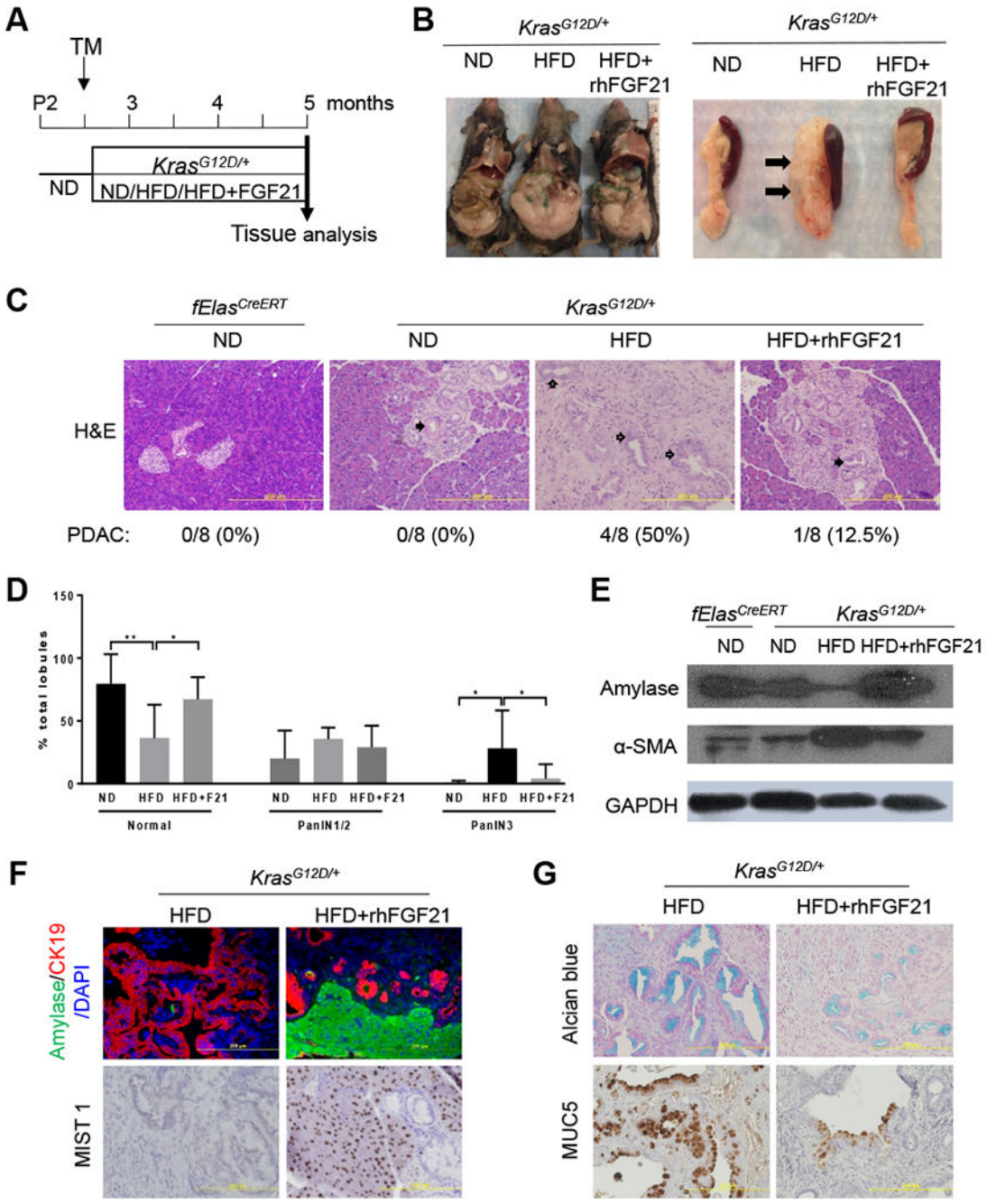

Figure 4. Supplementation of FGF21 suppresses HFD and KRAS^{G12D} induced PanIN lesions and PDAC.

(A) Experimental scheme: 70-day-old ND-fed *Kras$^{G12D/+}$*;*fElas^{CreERT}* mice were treated with TM to induce *Kras$^{G12D/+}$* expression and randomly separated into ND, HFD, or HFD with rhFGF21 treatment groups (n=8 per group) for ten weeks. The age-matched ND-fed *fElas^{CreERT}* mice (n=8) served as controls. (B) Gross images of *Kras$^{G12D/+}$* mice (left) and the pancreata (right) after the indicated treatments. Closed arrows, multiple pancreatic cysts. (C) Histology of representative pancreata. Closed arrows, PanIN lesions. Open arrows, cancerous ductal structure. 100x. (D) Average grades of PanIN1/2 and PanIN3 lesions as in

4C. F21, rhFGF21. (E) Western blot analysis of pancreatic amylase and α-SMA as indicated. (F) Co-IF analysis of pancreatic amylase and CK19 or IHC analysis of MIST1. 200x. (G) Alcian blue+ staining of acidic mucins and IHC staining of MUC5. 200x. Data are mean + SD. *, $p<0.05$; **, $p<0.01$.

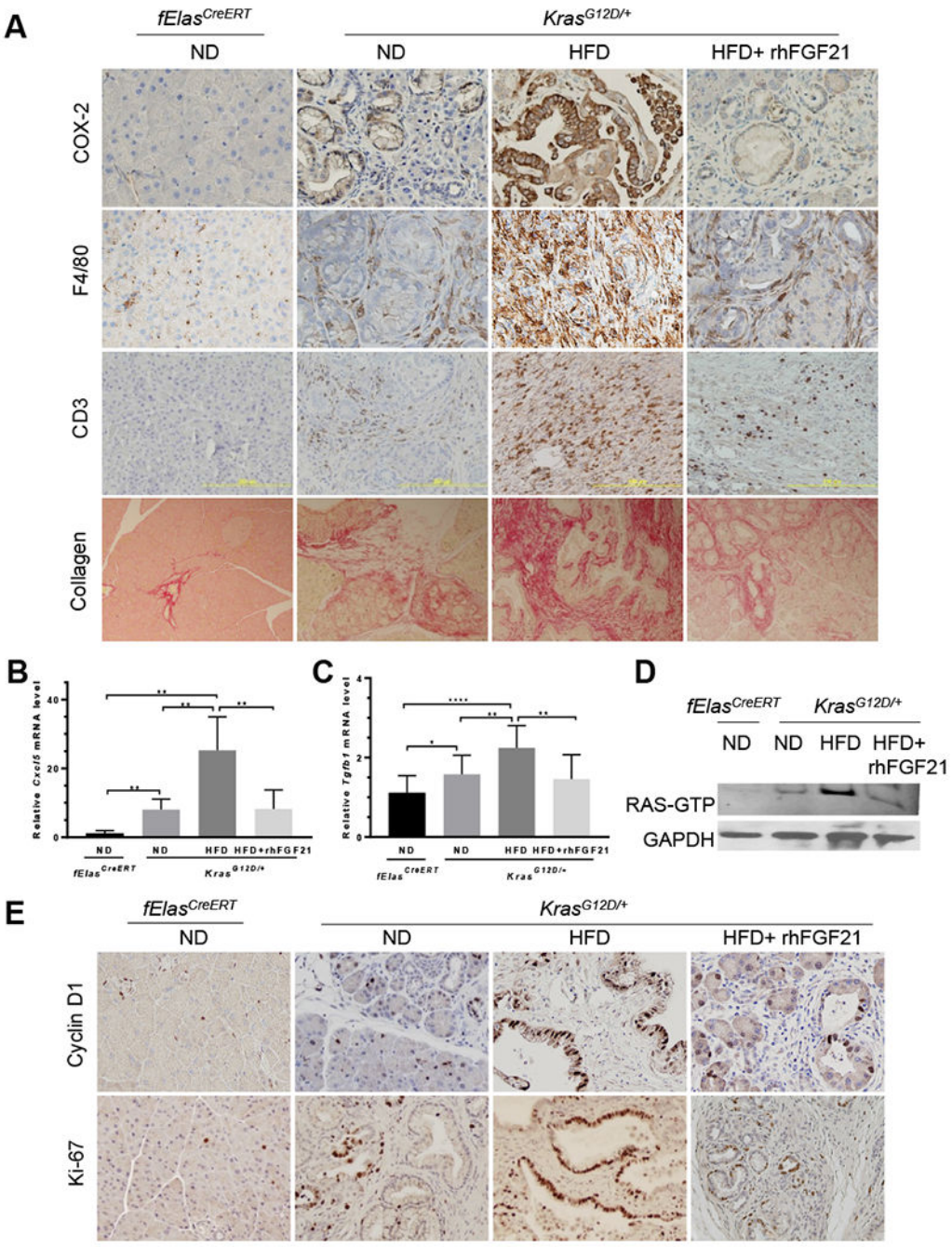

Figure 5. Pharmacological FGF21 inhibits HFD and KRAS^{G12D} induced pancreatic inflammation and ductal cell proliferation.

All the samples were described as in Figure 4A. (A) IHC staining of COX-2, F4/80, and CD3, as well as Sirius Red staining for collagen on pancreatic sections of *Kras*$^{G12D/+}$ mice. 200x. (B-C) qRT-PCR analysis of pancreatic Cxc/5 and *Tgfbl* expression in *Kras*$^{G12D/+}$ mice with ND (n=4), HFD (n=4) or HFD+rhFGF21 (n=5). The age-matched ND-fed *fElas*^{CreERT} mice (n=5) served as a control. (D) Pancreatic RAS activity with the indicated treatments. (E) IHC staining of pancreatic cyclin D1 and Ki-67. 200x. Data are mean ± SD. *, p<0.05; **, p<0.01; ****, p<0.0001.

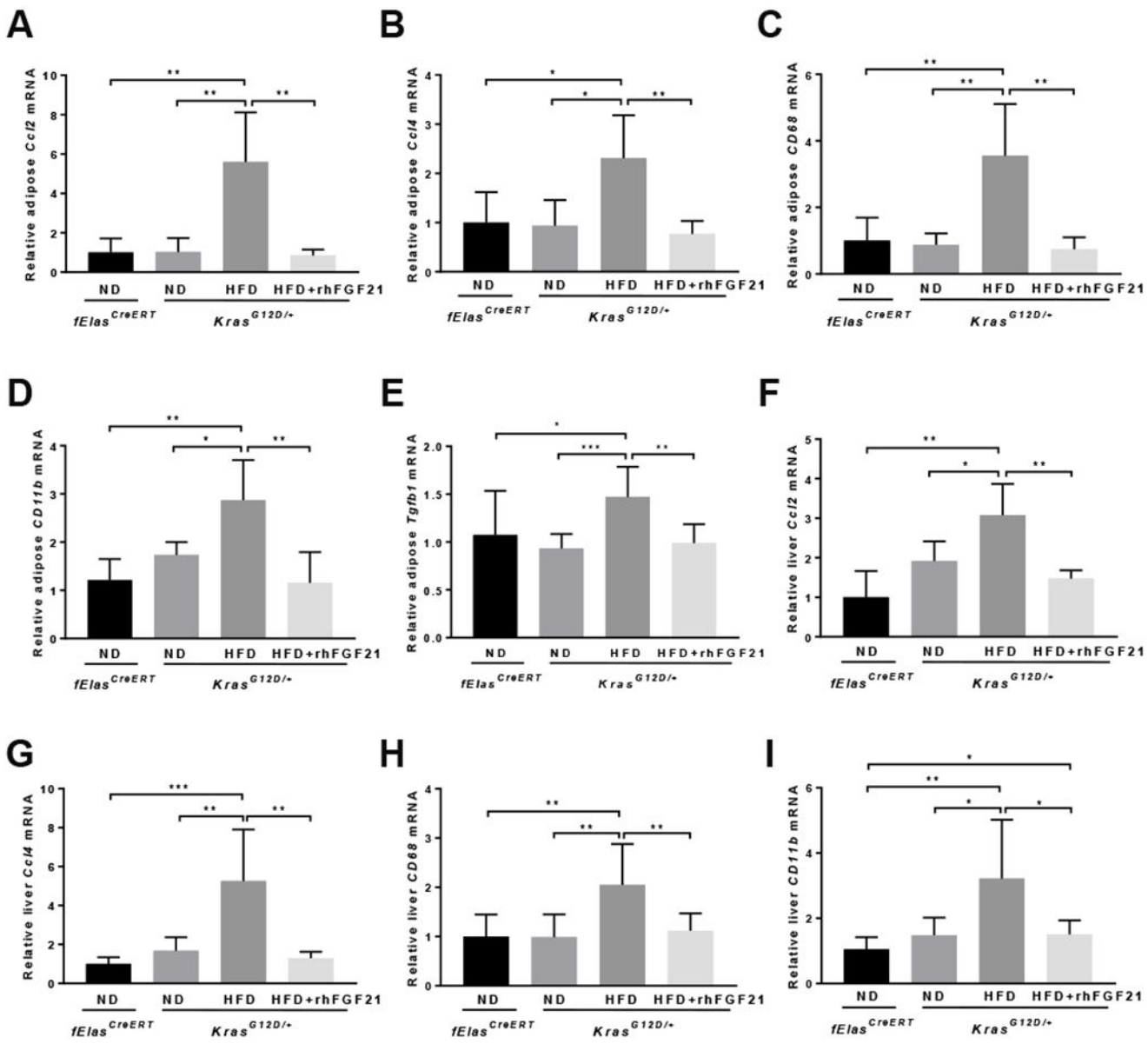

Figure 6. Pharmacological FGF21 inhibits HFD-promoted liver and adipose tissue inflammation

All mice were induced by TM at 70 days of age and then treated as indicated for 10 weeks prior to analyses. qRT-PCR analysis of *Ccl2, Ccl4, CD68, CD11b*, and *Tgfbl* in adipose tissues (A-E) and *Ccl2, Ccl4, CD68*, and *CD11b* in the liver (F-I) of $Kras^{G12D/+}$ mice with ND (n=5), HFD (n=6), or HFD+rhFGF21 (n=5). The ND-fed $fElas^{CreERT}$ mice (n=5) were controls. Data are mean ± SD. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

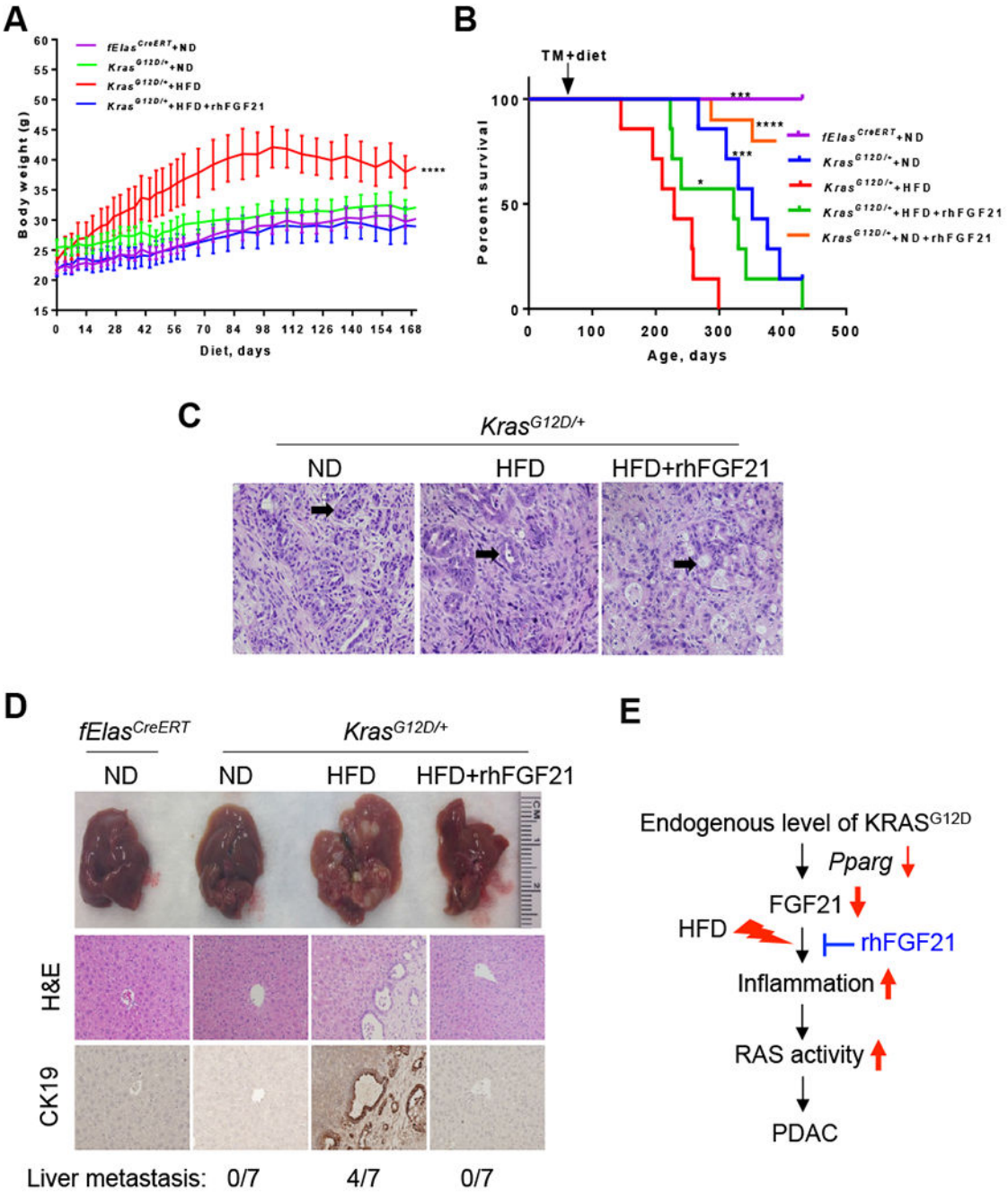

Figure 7. Pharmacological FGF21 prevents liver metastasis and prolongs KRAS^{G12D} mice survival.

70-day-old mice were treated with TM to induce *fElas*^{CreERT} or *Kras*$^{G12D/+}$ expression, and then fed a ND or a HFD with or without rhFGF21 until they aged. The ND-fed *fElas*^{CreERT} mice (n=7) were controls. (A) Body weight of the *Kras*$^{G12D/+}$ mice with ND (n=7), HFD (n=7), or HFD+rhFGF21 treatment (n=7) until the HFD group aged. (B) Survival of the *Kras*$^{G12D/+}$ mice with ND (n=7), ND+rhFGF21 (n=10), HFD (n=7), or HFD+rhFGF21 treatment (n=7). The HFD-fed *Kras*$^{G12D/+}$ mice served as a control for statistical comparison. (C) Histological changes in pancreata of *Kras*$^{G12D/+}$ mice with the indicated

treatments upon aging. 200x. (D) Liver metastasis. Upper panel, gross images of the livers. Middle panel, H&E stained sections. Lower panel, IHC analysis of CK19. (E) Schematic summary of the role of pancreatic FGF21 in the HFD and oncogenic KRAS mediated pancreatic tumorigenesis. 200x. Data are mean ± SD. *, $p<0.05$; ***, $p<0.001$; ****, $p<0.0001$.