Experimental Animals
Exp. Anim. 72(3), 379–388, 2023

Original

# The Kruppel-like factor 4-signal transducer and activator of transcription 5A axis promotes pancreatic fibrosis in mice with caerulein-induced chronic pancreatitis

Xiaoxiang WANG*, Lan YU*, Yao CHEN, Xing XIONG and Hongmei RAN

*Department of Gastroenterology, Chengdu First People's Hospital, No.18 Wanxiang North Road, Wuhou District, Chengdu City, Sichuan Province, 610016, P.R. China*

**Abstract:** Pancreatic fibrosis (PF) is a hallmark of chronic pancreatitis (CP), but its molecular mechanism remains unclear. This study was conducted to explore the role of Kruppel-like factor 4 (KLF4) in PF in CP mice. The CP mouse model was established using caerulein. After KLF4 interference, pathological changes in pancreatic tissues and fibrosis degree were observed by hematoxylin-eosin staining and Masson staining, and levels of Collagen I, Collagen III, and alpha-smooth muscle actin, inflammatory cytokines, KLF4, signal transducer and activator of transcription 5A (STAT5) in pancreatic tissues were measured by enzyme-linked immunosorbent assay, quantitative real-time polymerase chain reaction, Western blot assay, and immunofluorescence. The enrichment of KLF4 on the STAT5 promoter and the binding of KLF4 to the STAT5 promoter were analyzed. The rescue experiments were performed by co-injection of sh-STAT5 and sh-KLF4 to confirm the regulatory mechanism of KLF4. KLF4 was upregulated in CP mice. Inhibition of KLF4 effectively attenuated pancreatic inflammation and PF in mice. KLF4 was enriched on the STAT5 promoter and enhanced the transcriptional and protein levels of STAT5. Overexpression of STAT5 reversed the inhibitory role of silencing KLF4 in PF. In summary, KLF4 promoted the transcription and expression of STAT5, which further facilitated PF in CP mice.

**Key words:** chronic pancreatitis, fibrosis, Kruppel-like factor 4 (KLF4), Masson staining, signal transducer and activator of transcription 5A (STAT5)

## Introduction

Chronic pancreatitis (CP) is a pancreatic fibrosis (PF) syndrome associated with genetic, environmental, and/or other risk factors, characterized by pathological features, including PF, acinar injury, pancreatic calcification, exocrine and endocrine dysfunction [1]. Pancreatic fibrosis (PF) is one of the most typical pathological hallmarks of CP in response to substantial injury or pressure, characterized by excessive deposition of extracellular matrix (ECM) and collagen fibers [2]. In the CP microenvironment, there is an inseparable relationship between PH and pancreatic inflammation as inflammatory mediators [e.g., tumor necrosis factor-alpha (TNF-α) and some ILs] produced by infiltrating inflammatory cells, can transform pancreatic stellate cells from fat-storing cells to myofibroblast-like cells, leading to fibrosis [3, 4]. The incidence and prevalence of CP is rising due to increasing awareness and improved imaging modalities and no curative treatment is available [5, 6]. For this reason, it remains necessary to explore novel molecular biomarkers for PF to extend treatment options for PF.

Kruppel-like factor 4 (KLF4), a zinc-finger transcription factor of the KLF family, plays physiological functions in various tissues and organs, including the pancreas [7, 8]. Of note, KLF4 can either activate or repress inflammatory signals and thus participates in inflamma-

*(Received 24 October 2022 / Accepted 19 March 2023 / Published online in J-STAGE 23 March 2023)*
*Corresponding author: H. Ran. email: ranhongmeicd@163.com*
**Xiaoxiang Wang and Lan Yu are co-first authors.*

tory disorders, such as esophageal epithelial inflammation, nephritis, and atherosclerosis [9–11]. Meanwhile, KLF4 recruitment is potent to accelerate chronic wound healing [12]. Besides, KLF4 can directly promote the expression of profibrotic genes, such as transforming growth factor-β (TGF-β) and connective tissue growth factor (CTGF), contributing to organ fibrosis [13, 14]. Most significantly, KLF4 is noted to be upregulated in mice with acute pancreatitis and promote the progression of pancreatitis [15]. However, its role in PF remains unknown.

On another note, KLF4 has a transcriptional active domain and an inhibitory domain, which can change the positive and negative regulation of its downstream target genes [16]. Signal transducer and activator of transcription 5A (STAT5), one of the best-characterized members of the STAT family, can be activated by plenty of cytokines and growth factors, and persistent activation of STAT5 is prone to chronic inflammation [17]. Intriguingly, inflammatory stress triggers the overproduction of STAT5 and STAT5 ablation attenuates pancreatic inflammation and fibrosis in the animal model of CP [18]. Moreover, the JASPAR database predicted the binding of KLF4 to the STAT5 promoter, suggesting the role of KLF4 in the transcriptional activation of STAT5. Nonetheless, it is unclear whether KLF4 promotes PF by activating STAT5 in CP.

Taking the aforementioned evidence into consideration, it is reasonable to hypothesize that KLF4 participates in the development of PF in the context of CP by regulating STAT5. The caerulein model is the most frequently used model of CP and can cause PF [19]. In the current study, we established the model of CP using injections of caerulein and strived to explore the molecular mechanism PF in the context of CP.

## Materials and Methods

### Establishment and treatment of the pancreatitis mouse model

All animal experiments got the approval of the Animal Care and Use Committee of Chengdu First People's Hospital and followed the *Guide for the Care and Use of Laboratory Animals* [20]. C57BL/6J mice (6–8 weeks, male) were procured from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China, license No: SCXK (Beijing) 2019-0009). According to a prior study [21], Caerulein (MedChemExpress Co., Ltd., Monmouth Junction, NJ, USA) was dissolved in sterile saline and intraperitoneally injected into CP mice (twice every day, with an interval of 8h for consecutive 14 days, at a concentration of 250 $\mu$g/kg). Healthy control (sham) mice were intraperitoneally injected with an equal volume of sterile saline, and the injection times were the same as those in the model group. After 14 days, each mouse was euthanatized (200 mg/kg pentobarbital sodium, i.p.) to remove pancreatic tissue, which was quickly frozen in liquid nitrogen later. There were 12 mice per group, with 6 ones used for histological staining and the remaining 6 ones used for tissue homogenate to measure levels of genes and proteins.

Lentivirus-packaged short hairpin (sh) RNA of KLF4 (sh-KLF4), pcDNA3.1-STAT5 overexpression vector (oe-STAT5), and their controls sh-NC and pcDNA3.1 empty vector (oe-NC) were provided by Hanbio Biotechnology Co., Ltd. (Shanghai, China). Mice were intraperitoneally injected with 200 $\mu$l viruses on the 1st day of the 14-d caerulein injection (virus titer = $1.0 \times 10^{10}$ virus genomes/ml).

### Histological staining

Pancreatic tissues were fixed in 10% neutral buffered formalin, dehydrated with ethanol, and embedded in paraffin for regular histological examination. Paraffined tissues were cut into 5 $\mu$m sections, followed by hematoxylin-eosin (H&E) and Masson staining, and immunofluorescence. H&E-stained sections from each group were observed at 4–5 random visual fields and subjected to blind scoring according to histological parameters, including edema, inflammatory cell infiltration, acinus necrosis, acinus atrophy, and fibrosis [22], with the scoring criteria shown in Table 1. Masson staining was used to evaluate collagen deposition, and the fibrosis area was quantified by Masson's staining area in ImageJ software. The percentage of fibrosis area was calculated as the ratio of fibrosis area to the entire pancreas area. The immunohistochemical fluorescence staining was conducted to determine the expression of KLF4 (1:1,000, ab214666, Abcam) and STAT5 (1:1,000, ab32043, Abcam) in pancreatic tissue. The area of positive expression was calculated using ImageJ software.

### ELISA

The contents of pancreatitis-related inflammatory cytokines were determined using ELISA kits. According to the manufacturer's protocol (R&D System, Inc., Minneapolis, MN, USA), levels of TNF-α (MTA00B), IL-1β (MLB00C), and IL-6 (M6000B) in pancreatic tissues were measured and the concentration was presented as pg/mg protein.

### Quantitative real-time polymerase chain reaction (qRT-PCR)

The total RNA was extracted from pancreatic tissues

**Table 1.** Histopathologic scoring system of chronic pancreatitis

| Histology | Score | Definition |
|---|---|---|
| Inflammatory cell infiltration | 0 | no infiltrate |
| | 1 | mild infiltrate |
| | 2 | moderate infiltrate |
| | 3 | severe infiltrate |
| Perilobular fibrosis | 0 | Absent |
| | 1 | Mild |
| | 2 | Moderate |
| | 3 | Severe |
| Interlobular fibrosis | 0 | Absent |
| | 1 | Fibrosis between 2 and 3 lobules |
| | 2 | Fibrosis between < 50% of lobules |
| | 3 | Fibrosis between > 50% of lobules |
| Intralobular fibrosis | 0 | Absent |
| | 1 | Fibrosis limited to 1–2 lobule (s) |
| | 2 | Fibrosis in < 50% of lobules |
| | 3 | Fibrosis in > 50% of lobules |
| Acinar atrophy | 0 | Absent |
| | 1 | Focal atrophy in 1–2 lobule (s) |
| | 2 | Focal atrophy in < 50% of lobules |
| | 3 | Focal atrophy in > 50% of lobules |

using the TRIzol reagent and reverse-transcribed into the complementary DNA using a reverse transcription assay kit (Takara, Kyoto, Japan). The mRNA level was determined by qPCR using the SYBR Green Master Mix (Toyobo Co., Ltd., Osaka. Japan). With *GAPDH* as the internal control of mRNA, the relative expression amount was calculated according to the $2^{-\Delta\Delta Ct}$ method [23]. PCR primers are shown in Table 2.

## Western blot assay

The total protein was extracted from the homogenate of pancreatic tissue and protein concentration was quantified using the bicinchoninic acid kit (Invitrogen, Waltham, MA, USA). Subsequently, the protein sample was separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidence fluoride membranes (Millipore, Billerica, MA, USA), followed by 1 h blockade with 5% skim milk. Next, the membranes were incubated with primary antibodies at 4°C overnight, washed thrice with TBS with Tween-20, and incubated with secondary antibody IgG (1:2,000, ab205718, Abcam, Cambridge, MA, USA) at 20°C for 90 min. Eventually, Western bands were quantitatively analyzed using ImageJ version 1.52 software. Primary antibodies included: KLF4 (1:1,000, ab214666, Abcam), STAT5 (1:1,000, ab32043, Abcam), Collagen I (1:1,000, ab260043, Abcam), Collagen III (1:5,000, ab7778, Abcam), alpha-smooth muscle actin (α-SMA, 1:1,000, ab5694, Abcam), and GAPDH (1:10,000, ab181602, Abcam).

**Table 2.** Information of PCR primers

| Gene | Sequence (5'-3') |
|---|---|
| KLF4 | F: GAAGCGACTTCCCCCACTTCCCG |
| | R: GGATGAAGCTGACGCCGAGGTG |
| STAT5 | F: GCGGGCTGGATTCAGGCCCAGCA |
| | R: GGTGCTCCGCCTTCTTCTGCAGCT |
| GAPDH | F: ATGCTGCCCTTACCCCGGGGT |
| | R: TTACTCCTTGGAGGCCATGTAG |
| STAT5 promoter | F: CCCCACCTGCCCAGTGAGTATCT |
| | R: AATGTCTCTCTGTCAACCGTGCT |

## Chromatin immunoprecipitation (ChIP) assay

The ChIP assay was conducted according to the instructions of the ChIP assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Pancreatic tissues were lysed using radioimmunoprecipitation assay buffer (Sigma, St. Louis, MO, USA), fixed with 1% methanal to crosslink DNA and protein, and processed with ultrasonic treatment to fragment DNA. Then, chromatins were incubated with antibodies against KLF4 (ab214666, Abcam) and IgG (ab172730, Abcam) at 4°C overnight and purified using a fragmented DNA purification assay kit (Intron Biotechnology, Seoul, Korea), followed by RT-qPCR analysis. The primer of the STAT5 promoter is shown in Table 2.

## Dual-luciferase assay

The binding site of KLF4 to the promoter sequence of STAT5 was predicted on the JASPAR database (http://jaspar.genereg.net/) [24]. The promoter sequence of STAT5 containing the binding site with KLF4 was in-

serted into luciferase reporter vectors (Promega, Madison, WI, USA) to construct the STAT5 promoter wild-type plasmid (STAT5-WT). Likewise, the promoter sequence of STAT5 containing the mutant binding site was inserted into the vectors to construct STAT5-mutant type (MUT). The above plasmids were co-transfected with oe-KLF4 or oe-NC into 293T cells (ATCC, Manassas, VA, USA) using Lipofectamine 3000. After 48 h, the luciferase activity was analyzed using a dual-luciferase reporter gene assay kit (Promega).

### Statistical analysis

Data statistical analysis and graphing were conducted by SPSS21.0 statistical software (IBM Corp., Armonk, NY, USA) and GraphPad Prism 8.0 software (GraphPad Software Inc., San Diego, CA, USA). Data complied with normal distribution and homogeneity of variance. Data between two groups were analyzed by the *t* test, and data among multiple groups were analyzed by one-way or two-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Data of histological scoring in two panels were analyzed by Mann-Whitney test and in multiple panels were analyzed by Kruskal-Wallis test. A value of $P<0.05$ was indicative of statistical significance, and a value of $P<0.01$ was indicative of highly statistical significance.

## Results

### KLF4 is upregulated in pancreatic tissues of CP mice

The CP mouse model was established through the injection of caerulein. Pancreatic tissues of CP mice presented evident inflammatory cell infiltration, acinus degeneration (granular or vacuolar-like), and acinus atrophy ($P<0.01$, Fig. 1A). Levels of TNF-α, IL-1β, and IL-6 were increased ($P<0.01$, Fig. 1B). Furthermore, KLF4 was found to be highly expressed in pancreatic tissues of CP mice ($P<0.01$, Figs. 1C–E).

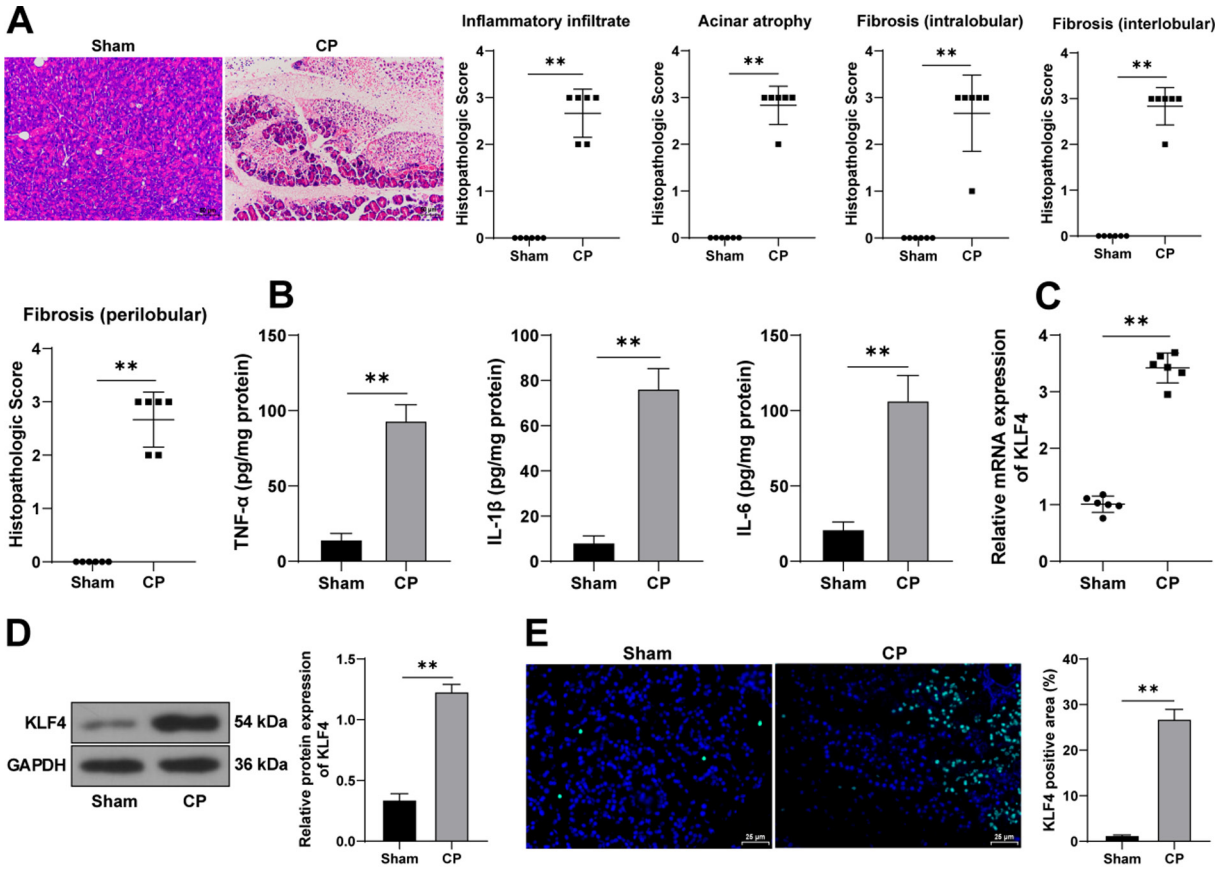


**Fig. 1.** Kruppel-like factor 4 (KLF4) is upregulated in pancreatic tissues of chronic pancreatitis (CP) mice. The CP mouse model was established by injection of caerulein. A: Pathological changes in pancreatic tissues (representative images) were observed by hematoxylin-eosin (H&E) staining; B: Levels of tumor necrosis factor-alpha (TNF-α), IL-1β, and IL-6 in pancreatic tissues were measured by ELISA; C–D: KLF4 expression levels in pancreatic tissues were determined by quantitative real-time polymerase chain reaction (qRT-PCR) (C) and Western blot assay (D); E: KLF4 positive expression was determined by immunofluorescence. n=6, data were presented as mean ± SD. Data in panel A were analyzed by Mann-Whitney test, and data in panels B, C, D, and E were analyzed using the *t* test. $^{**}P<0.01$.

## KLF4 inhibition alleviates inflammation in CP mice

To explore the role of KLF4 in CP, KLF4 expression was reduced in pancreatic tissues using lentivirus-packaged sh-KLF4 ($P$<0.01, Figs. 2A–C). After KLF4 inhibition, the pathological changes in pancreatic tissues were lessened ($P$<0.05, Fig. 2D), and levels of TNF-α, IL-1β, and IL-6 in pancreatic tissues were decreased ($P$<0.01, Fig. 2E), which suggested that KLF4 inhibition alleviated inflammation in CP mice.

## KLF4 inhibition alleviates PF in CP mice

Meanwhile, after KLF4 inhibition, the fibrosis area in pancreatic tissues was reduced ($P$<0.01, Fig. 3A), and levels of Collagen I, Collagen III, and α-SMA were significantly decreased $P$<0.01, Fig. 3B). Above all, KLF4 inhibition alleviated PF in CP mice.

## KLF4 enrichment on the STAT5 promoter promotes STAT5 transcription and expression

As a transcription factor, KLF4 can regulate the transcription of downstream target genes. Inflammatory stress robustly increases STAT5 expression and activity, and STAT5 inhibition helps to alleviate pancreatic inflammation and fibrosis in CP mice [18]. Therefore, we speculated that KLF4 may regulate STAT5 expression to participate in the progression of pancreatitis. The JASPAR database (http://jaspar.genereg.net/) predicted that KLF4 can bind to the STAT5 promoter (Fig. 4A). Through the ChIP assay, KLF4 was observed to be enriched on the STAT5 promoter ($P$<0.01, Fig. 4B). In the dual-luciferase assay, KLF4 overexpression vector can effectively increase the relative fluorescence of STAT5-WT but cannot affect the relative fluorescence of STAT5-

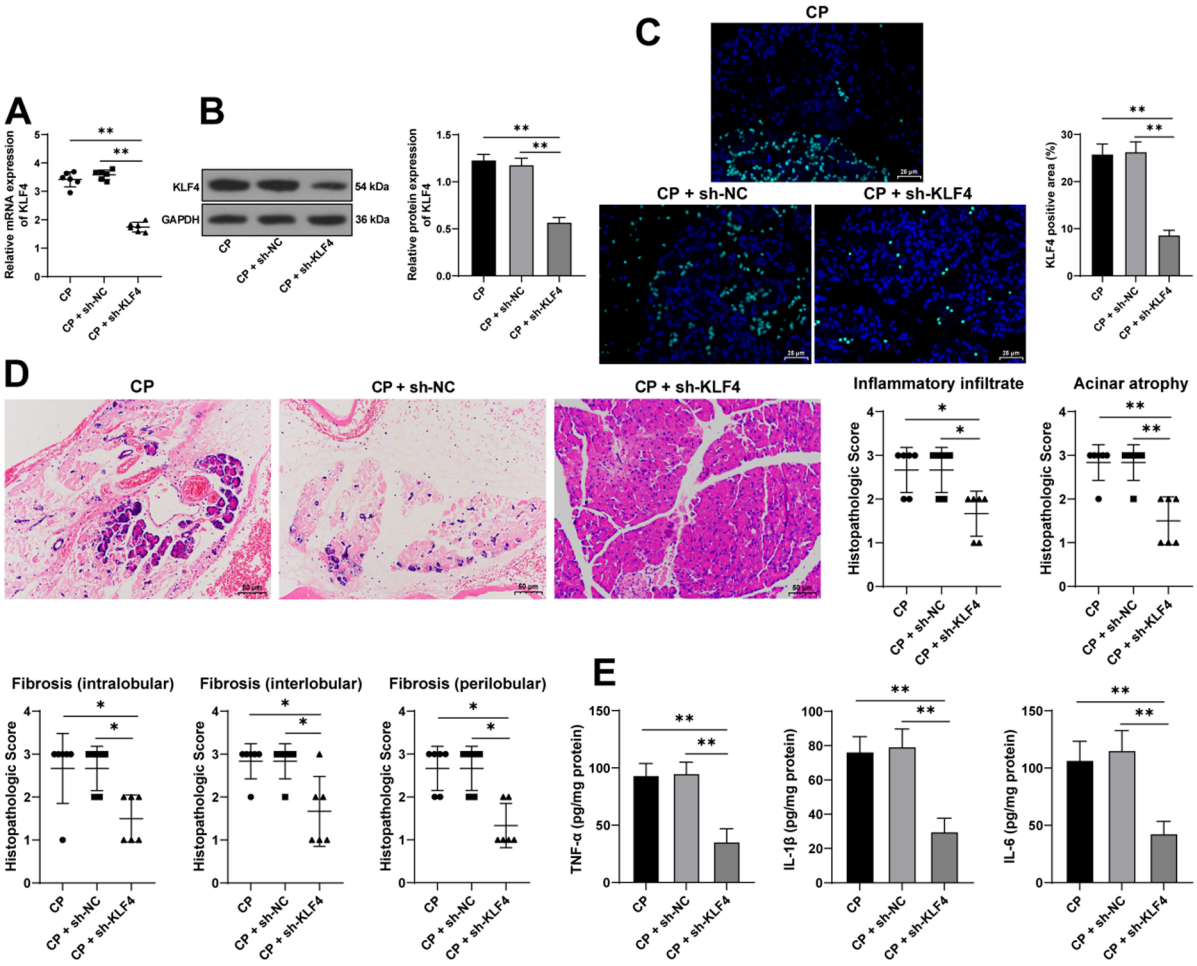


**Fig. 2.** Kruppel-like factor 4 (KLF4) inhibition alleviates inflammation in chronic pancreatitis (CP) mice. CP mice were intraperitoneally injected with lentivirus-packaged sh-KLF4, with sh-NC as the control. A–B: KLF4 expression levels in pancreatic tissues were determined by quantitative real-time polymerase chain reaction (qRT-PCR) (A) and Western blot assay (B); C: KLF4 positive expression was determined by immunofluorescence; D: Pathological changes in pancreatic tissues (representative images) were observed by hematoxylin-eosin (H&E) staining; E: Levels of tumor necrosis factor-alpha (TNF-α), IL-1β, and IL-6 in pancreatic tissues were measured by ELISA. n=6, data were presented as mean ± SD. Data in panels A, B, C, and E were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Data in panel D were analyzed by Kruskal-Wallis test. *$P$<0.05, **$P$<0.01.

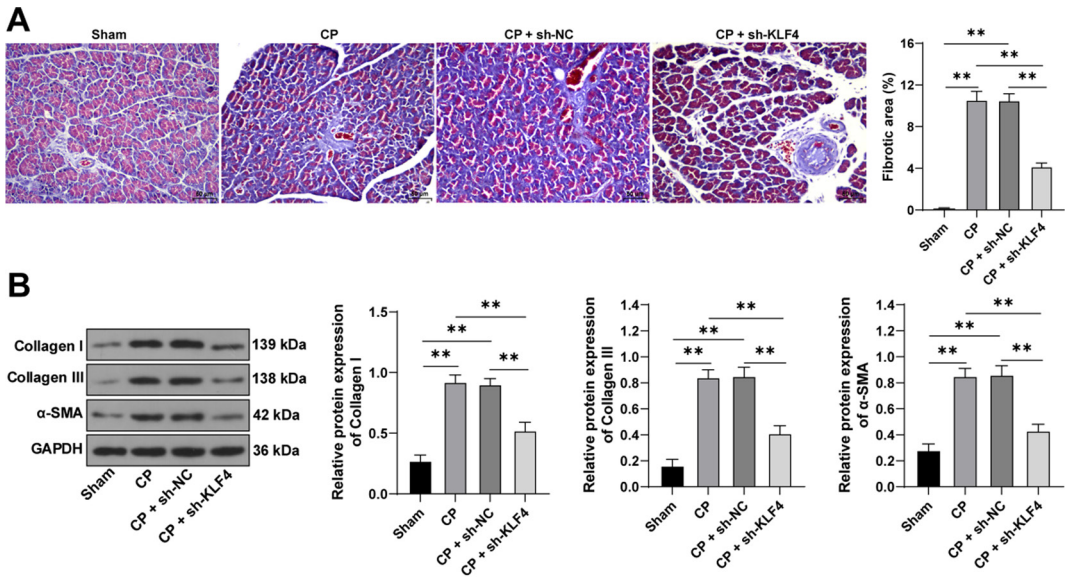


**Fig. 3.** Kruppel-like factor 4 (KLF4) inhibition alleviates pancreatic fibrosis (PF) in chronic pancreatitis (CP) mice. The CP mouse model was established by intraperitoneal injection of caerulein and injected with lentivirus-packaged sh-KLF4 to inhibit sh-KLF4 expression. A: Fibrosis area in pancreatic tissues was observed by Masson staining; B: Levels of Collagen I, Collagen III, and alpha-smooth muscle actin (α-SMA) were determined by Western blot assay. n=6, data were presented as mean ± SD and analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. $^{**}P<0.01$.

A

| Matrix ID | Name | Score | Relative score | Sequence ID | Start | End | Strand | Predicted sequence |
|---|---|---|---|---|---|---|---|---|
| MA0039.1 | MA0039.1.Klf4 | 9.520958 | 0.936334338897639 | NC_000077.7:100748177-100750177 | 715 | 724 | + | TAGAAAAAGG |

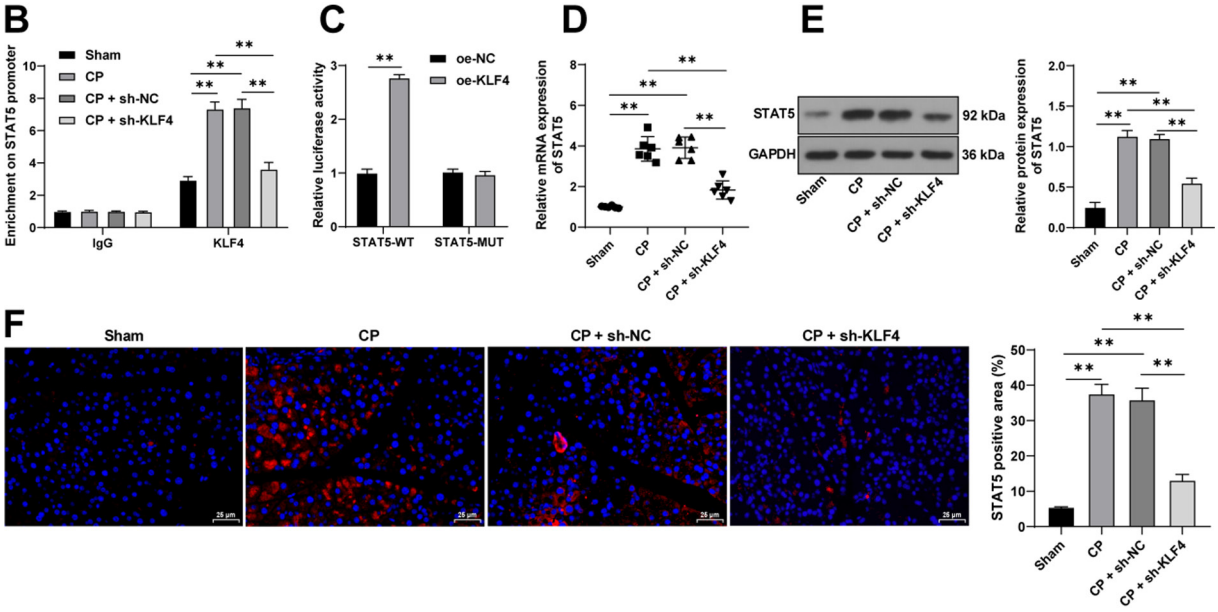


**Fig. 4.** Kruppel-like factor 4 (KLF4) enrichment in the signal transducer and activator of transcription 5A (STAT5) promoter promotes STAT5 transcription and expression. A: The JASPAR database (http://jaspar.genereg.net/) predicted the binding of KLF4 to the STAT5 promoter; B: The enrichment of KLF4 on the STAT5 promoter was determined by ChIP assay, with IgG as the control; C: The binding relationship between KLF4 and the promoter sequence of STAT5 was tested by the dual-luciferase assay; D-E: STAT5 expression levels in pancreatic tissues were determined by quantitative real-time polymerase chain reaction (qRT-PCR) (D) and Western blot assay (E); F: STAT5 positive expression was determined by immunofluorescence. n=6, data were presented as mean ± SD. Dada in panels B and C were analyzed using two-way analysis of variance (ANOVA) and data in panels D, E, and F were analyzed using one-way ANOVA, followed by Tukey's multiple comparison test. $^{**}P<0.01$.

MUT ($P$<0.01, Fig. 4C). In addition, after KLF4 inhibition, the transcriptional and protein levels of STAT5 were both markedly decreased in pancreatic tissues of CP mice ($P$<0.01, Figs. 4D–F). Above all, KLF4 bound to the STAT5 promoter and promoted the transcription and expression of STAT5.

### STAT5 overexpression partly reverses the inhibitory effect of KLF4 inhibition on PF in CP mice

At last, we confirmed the above mechanism via the rescue experiments with oe-STAT5 and sh-KLF4. After injection of lentivirus-packaged oe-STAT5, the expression levels of STAT5 were upregulated in pancreatic tissues ($P$<0.01, Figs. 5A–C). Compared with the CP + sh-KLF4 + oe-NC group, PF was aggravated ($P$<0.05, Figs. 5D and E), along with increased levels of Collagen I, Collagen III, and α-SMA ($P$<0.01, Fig. 5B), and TNF-α, IL-1β, and IL-6 ($P$<0.01, Fig. 5F) in pancreatic tissues in the CP + sh-KLF4 + oe-STAT5 group. These findings suggested that STAT5 overexpression partly reversed the inhibitory effect of KLF4 inhibition on PF in CP mice.

## Discussion

Chronic pancreatitis (CP) is a progressive inflammatory disorder in the pancreas with increasing incidence worldwide and results in irreversible organ damage through the process of pancreatic fibrosis (PF) [1]. PF is associated with the damage caused by increased ECM and collagen fibers, but its molecular mechanism remains unclear [25]. A better understanding of PF underlying mechanism is beneficial to provide alternative treatment options and improve the prognosis of CP patients. In this study, we used the caerulein-induced CP model to explore the molecular mechanism of PF and uncovered that KLF4 and STAT5 were upregulated in CP mice and KLF4 activated STAT5 transcription and protein levels, thus promoting PF.

KLF4 emerges as a crucial regulator of inflammatory disorders and wound healing [12, 26]. For instance, KLF4 activates nuclear factor-kappa B to enhance esophageal epithelial inflammation [10], and KLF4 facilitates the transformation of myeloid-derived suppressor cells into fibrocytes to accelerate wound healing of pressure ulcers [27]. Intriguingly, inhibition of KLF4 boosts the proliferation of pancreatic acinar cells and suppresses inflammation, thus retarding the progression of acute pancreatitis [15]. In this study, the caerulein treatment induces inflammatory cell infiltration, acinus degeneration, and atrophy, increased levels of TNF-α, IL-1β, and IL-6, and upregulated the expression levels of KLF4, whereas KLF4 knockdown attenuated the above histopathological changes and inflammatory responses in CP mice. In addition, PF is characterized by collagen deposition that is reflected by elevated levels of Collagen I and Collagen III [28]. α-SMA is a marker of the activation of pancreatic stellate cells and α-SMA production increases the secretion of collagens to play a vital role in the process of PF [29]. In caerulein-induced CP mice, levels of Collagen I, Collagen III, and α-SMA were decreased by KLF4 knockdown. In agreement with our results, KLF4 upregulates the expression of profibrotic genes secreted from tubular epithelial cells, such as TGF-β, CTGF, and α-SMA, leading to renal fibrosis [30], whereas KLF4 depletion buffers fibrocyte generation in parallel to decreased levels of Th2 cytokines in mice with asthma, contributing to attenuating lung fibrosis [31]. Altogether, our findings suggested that inhibition of KLF4 attenuated both inflammatory responses and PF in caerulein-induced CP mice.

KLF4 is able to transactivate the transcription of downstream targets by binding to the gene promoters [32, 33]. Abnormal activity of STAT5 has been found in a variety of inflammatory environments, such as atherosclerosis, cardiac inflammation, and airway inflammation [34–36]. The Janus kinase/STAT5 pathway plays a role in mediating chronic inflammation and wound healing in gastrointestinal diseases [37]. Loss of STAT5 has been demonstrated to alleviate inflammatory reactions and PF in CP mice by enhancing neutrophil infiltration [18]. First, our bioinformatics and experimental data confirmed the binding of KLF4 to the STAT5 promoter. Then, the transcriptional and protein levels of STAT5 in pancreatic tissues were both declined after KLF4 inhibition in CP mice, suggesting that KLF4 may bind to the STAT5 promoter to enhance both the transcription and translation of STAT5. Furthermore, our rescue experiments showed that STAT5 overexpression partly reversed the inhibitory effects of silencing KLF4 on inflammation and PF in CP mice. In agreement with our results, STAT5 inhibition reduces ECM deposition and expression of profibrotic genes in mesangial cells, thus ameliorating renal fibrosis [38]. In addition, STAT5 is likely to induce TGF-β1 to function in liver fibrosis [39] and TGF-β1 can activate the phosphorylation of JAK1, STAT1, STAT3, STAT5, Smad1, Smad3, and Smad5 in alveolar epithelial cells [40]. In this regard, we speculated that TGF-β might be a downstream effector of the KLF4-STAT5 axis. However, the interaction between STAT5 and TGF-β in pancreatic tissue is complex and warrants more experiments to validate their regulatory relationship. Moreover, KLF4 has also been shown to directly transactivate TGF-β1 in cardiac myofibroblast differen-

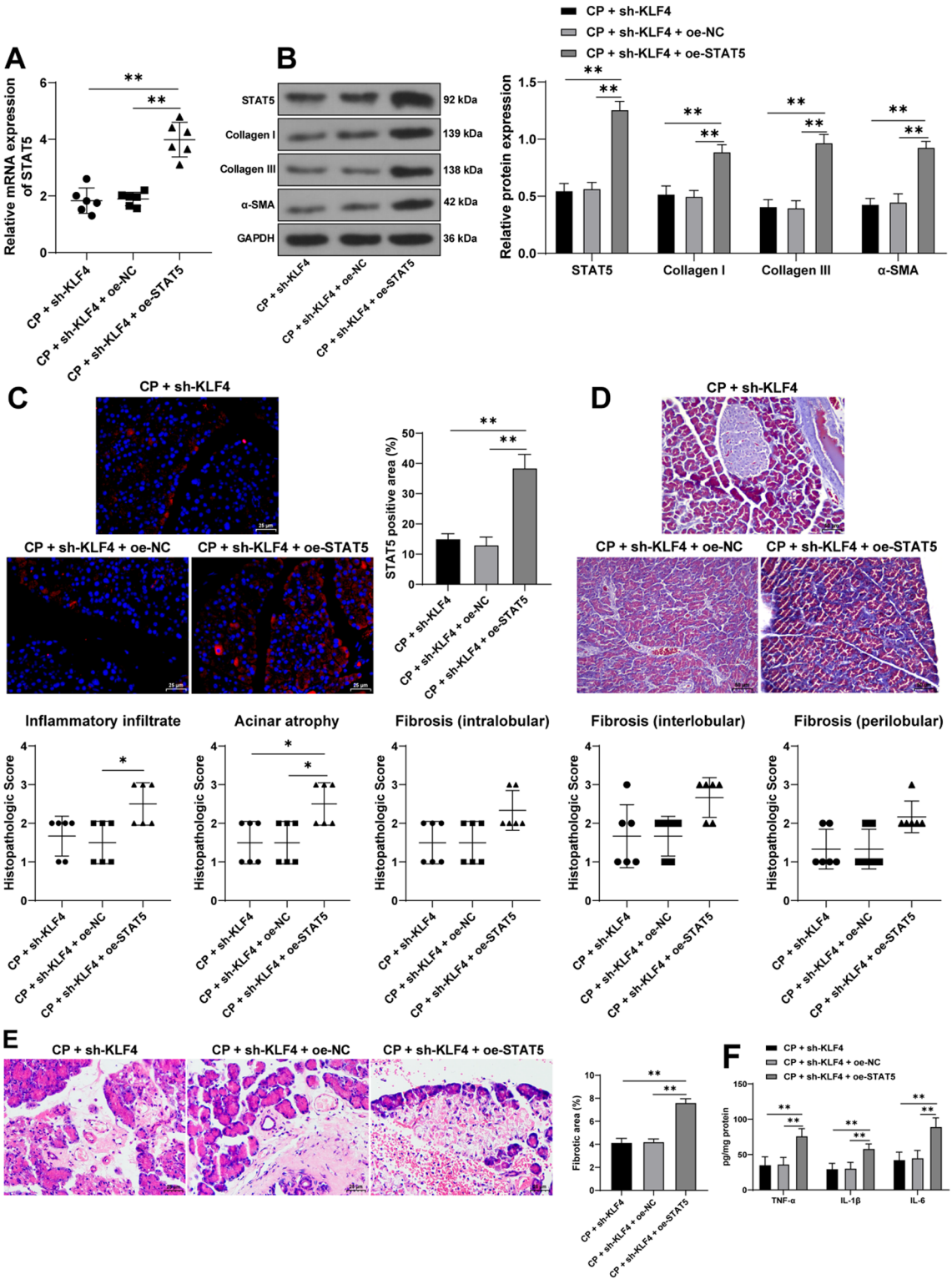


**Fig. 5.** Signal transducer and activator of transcription 5A (STAT5) overexpression partly reverses the inhibitory effect of Kruppel-like factor 4 (KLF4) inhibition on pancreatic fibrosis (PF) in chronic pancreatitis (CP) mice. CP mice were intraperitoneally injected with lentivirus-packaged pcDNA3.1-STAT5 overexpression vector (oe-STAT5), with empty vector (oe-NC) as the control, followed by rescue experiments with sh-KLF4. A: mRNA levels of *STAT5* in pancreatic tissues were determined by quantitative real-time polymerase chain reaction (qRT-PCR); B: Protein levels of STAT5, Collagen I, Collagen III, and alpha-smooth muscle actin (α-SMA) in pancreatic tissues were determined by Western blot assay; C: STAT5 positive expression was determined by immunofluorescence; D: Pathological changes in pancreatic tissues (representative images) were observed by hematoxylin-eosin (H&E) staining; E: Fibrosis area in pancreatic tissues was observed by Masson staining; F: Levels of tumor necrosis factor-alpha (TNF-α), IL-1β, and IL-6 in pancreatic tissues were measured by ELISA. n=6, data were presented as mean ± SD. Data in panels A, C, and E were analyzed by one-way analysis of variance (ANOVA), and data in panels B and F were analyzed by two-way ANOVA, followed by Tukey's multiple comparison test. Data in panel D were analyzed by Kruskal-Wallis test. **P*<0.05, ***P*<0.01.

tiation [41], suggesting that the KLF4 may also participate in the exacerbation of CP in a STAT5-independent manner.

To conclude, our study for the first time validated the profibrotic role of the KLF4-STAT5 axis in caerulein-induced CP mice, which provides potential targets for the diagnosis and treatment of PF. However, our findings were all obtained from animal experiments, lacking validation from clinical data, so it takes a long period of time to apply our theoretical knowledge to the clinic. Besides, we did not further investigate the cell type by which KLF4 function in CP and specific pathological features that KLF4 regulates. Moreover, since we only explored STAT5 as a downstream target gene of KLF4, whether other downstream targets of KLF4 play a role in PF remains to be investigated. Since the CP model was established by caerulein, the differences between caerulein-induced CP mice and CP in the clinic may affect the reliability of our findings. In the future, more studies are essential to further investigate the role of KLF4 in PF through the establishment of more CP mouse models with other drugs and treatments and further investigate the regulatory relationship between KLF4 and STAT5.

## Acknowledgments

We would like to thank all the patients and investigators who participated in the study.

## References

1. Mann R, Boregowda U, Vyas N, Gajendran M, Umapathy CP, Sayana H, et al. Current advances in the management of chronic pancreatitis. Dis Mon. 2021; 67: 101225. [Medline] [CrossRef]
2. Huang CT, Lee TH, Lin CK, Chen CY, Yang YF, Liang YJ. Pancreatic fibrosis (early chronic pancreatitis) as emerging diagnosis in structural causes of dyspepsia: evidence from endoscopic ultrasonography and shear wave elastography. Diagnostics (Basel). 2021; 11: 1252. [Medline]
3. Ng B, Viswanathan S, Widjaja AA, Lim WW, Shekeran SG, Goh JWT, et al. IL11 activates pancreatic stellate cells and causes pancreatic inflammation, fibrosis and atrophy in a mouse model of pancreatitis. Int J Mol Sci. 2022; 23: 3549. [Medline] [CrossRef]
4. Kandikattu HK, Venkateshaiah SU, Mishra A. Chronic pancreatitis and the development of pancreatic cancer. Endocr Metab Immune Disord Drug Targets. 2020; 20: 1182–1210. [Medline] [CrossRef]
5. Sirtl S, Beyer G, Mayerle J. Clinical and translational markers of severity and prognosis in chronic pancreatitis. Curr Opin Gastroenterol. 2022; 38: 501–508. [Medline] [CrossRef]
6. Beyer G, Habtezion A, Werner J, Lerch MM, Mayerle J. Chronic pancreatitis. Lancet. 2020; 396: 499–512. [Medline] [CrossRef]
7. Yang Z, Li D, Liu Z, Miao X, Yang L, Zou Q, et al. BIRC7 and KLF4 expression in benign and malignant lesions of pancreas and their clinicopathological significance. Cancer Biomark. 2016; 17: 437–444. [Medline] [CrossRef]
8. Ghaleb AM, Yang VW. Krüppel-like factor 4 (KLF4): What we currently know. Gene. 2017; 611: 27–37. [Medline] [CrossRef]
9. Wen Y, Lu X, Ren J, Privratsky JR, Yang B, Rudemiller NP, et al. KLF4 in macrophages attenuates TNF alpha-mediated kidney injury and fibrosis. J Am Soc Nephrol. 2019; 30: 1925–1938. [Medline] [CrossRef]
10. Shaverdashvili K, Padlo J, Weinblatt D, Jia Y, Jiang W, Rao D, et al. KLF4 activates NFκB signaling and esophageal epithelial inflammation via the Rho-related GTP-binding protein RHOF. PLoS One. 2019; 14: e0215746. [Medline] [CrossRef]
11. Li W, Wang J, Li Z. ALK5 deficiency inhibits macrophage inflammation and lipid loading by targeting KLF4. Biosci Rep. 2020; 40: BSR20194188. [Medline]
12. Yang X, Mathis BJ, Huang Y, Li W, Shi Y. KLF4 promotes diabetic chronic wound healing by suppressing Th17 cell differentiation in an mdsc-dependent manner. J Diabetes Res. 2021; 2021: 7945117. [Medline] [CrossRef]
13. Shen K, Li R, Zhang X, Qu G, Li R, Wang Y, et al. Acetyl oxygen benzoate engeletin ester promotes KLF4 degradation leading to the attenuation of pulmonary fibrosis via inhibiting TGFβ1-smad/p38MAPK-lnc865/lnc556-miR-29b-2-5p-STAT3 signal pathway. Aging (Albany NY). 2021; 13: 13807–13821. [Medline] [CrossRef]
14. Gu J, Qiu M, Lu Y, Ji Y, Qian Z, Sun W. Piperlongumine attenuates angiotensin-II-induced cardiac hypertrophy and fibrosis by inhibiting Akt-FoxO1 signalling. Phytomedicine. 2021; 82: 153461. [Medline] [CrossRef]
15. Deng X, He Y, Miao X, Yu B. ATF4-mediated histone deacetylase HDAC1 promotes the progression of acute pancreatitis. Cell Death Dis. 2021; 12: 5. [Medline] [CrossRef]
16. Cui J, Shi M, Quan M, Xie K. Regulation of EMT by KLF4 in gastrointestinal cancer. Curr Cancer Drug Targets. 2013; 13: 986–995. [Medline] [CrossRef]
17. Loh CY, Arya A, Naema AF, Wong WF, Sethi G, Looi CY. Signal transducer and activator of transcription (STATs) proteins in cancer and inflammation: functions and therapeutic implication. Front Oncol. 2019; 9: 48. [Medline] [CrossRef]
18. Lin Y, Chen Y, Feng W, Zhang J, Hua R, Yin B, et al. STAT5 promotes chronic pancreatitis by enhancing GM-CSF-dependent neutrophil augmentation. J Leukoc Biol. 2021; 110: 293–300. [Medline] [CrossRef]
19. Lerch MM, Gorelick FS. Models of acute and chronic pancreatitis. Gastroenterology. 2013; 144: 1180–1193. [Medline] [CrossRef]
20. National Academy of Sciences. The National Academies Collection: Reports funded by National Institutes of Health; 2011.
21. Hung J, Awasthi R, Klibanov AL, Kelly KA. Identification of novel ligands for targeted antifibrotic therapy of chronic pancreatitis. Int J Nanomedicine. 2021; 16: 5495–5512. [Medline] [CrossRef]
22. El-Hamoly T, Hajnády Z, Nagy-Pénzes M, Bakondi E, Regdon Z, Demény MA, et al. Poly(ADP-Ribose) polymerase 1 promotes inflammation and fibrosis in a mouse model of chronic pancreatitis. Int J Mol Sci. 2021; 22: 3593. [Medline] [CrossRef]
23. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001; 25: 402–408. [Medline] [CrossRef]
24. Castro-Mondragon JA, Riudavets-Puig R, Rauluseviciute I, Lemma RB, Turchi L, Blanc-Mathieu R, et al. JASPAR 2022: the 9th release of the open-access database of transcription factor binding profiles. Nucleic Acids Res. 2022; 50:(D1): D165–D173. [Medline] [CrossRef]
25. Huang C, Iovanna J, Santofimia-Castaño P. Targeting fibrosis: the bridge that connects pancreatitis and pancreatic cancer. Int J Mol Sci. 2021; 22: 4970. [Medline]

26. Bulut GB, Alencar GF, Owsiany KM, Nguyen AT, Karnewar S, Haskins RM, et al. KLF4 (Kruppel-like factor 4)-dependent perivascular plasticity contributes to adipose tissue inflammation. Arterioscler Thromb Vasc Biol. 2021; 41: 284–301. [Medline]
27. Ou L, Shi Y, Dong W, Liu C, Schmidt TJ, Nagarkatti P, et al. Kruppel-like factor KLF4 facilitates cutaneous wound healing by promoting fibrocyte generation from myeloid-derived suppressor cells. J Invest Dermatol. 2015; 135: 1425–1434. [Medline] [CrossRef]
28. Zhang SK, Cui NQ, Zhuo YZ, Hu JG, Liu JH, Li DH, et al. Modified xiaochaihu decoction () promotes collagen degradation and inhibits pancreatic fibrosis in chronic pancreatitis rats. Chin J Integr Med. 2020; 26: 599–603. [Medline] [CrossRef]
29. Liu P, Zhu L, Zou G, Ke H. Matrine suppresses pancreatic fibrosis by regulating TGF-beta/Smad signaling in rats. Yonsei Med J. 2019; 60: 79–87. [Medline] [CrossRef]
30. Xu D, Chen PP, Zheng PQ, Yin F, Cheng Q, Zhou ZL, et al. KLF4 initiates sustained YAP activation to promote renal fibrosis in mice after ischemia-reperfusion kidney injury. Acta Pharmacol Sin. 2021; 42: 436–450. [Medline] [CrossRef]
31. Nimpong JA, Gebregziabher W, Singh UP, Nagarkatti P, Nagarkatti M, Hodge J, et al. Deficiency of KLF4 compromises the lung function in an acute mouse model of allergic asthma. Biochem Biophys Res Commun. 2017; 493: 598–603. [Medline] [CrossRef]
32. Zhang X, Chen J, Sun L, Xu Y. SIRT1 deacetylates KLF4 to activate Claudin-5 transcription in ovarian cancer cells. J Cell Biochem. 2018; 119: 2418–2426. [Medline] [CrossRef]
33. Huang R, Fu Y, Deng Y. KLF4 transactivates TRIM29 expression and modulates keratin network. Biochem Biophys Rep. 2021; 28: 101117. [Medline]
34. Wang X, Ding X, Yan J, Lu Z, Cao H, Ni X, et al. STAT5 inhibitor attenuates atherosclerosis via inhibition of inflammation: the role of STAT5 in atherosclerosis. Am J Transl Res. 2021; 13: 1422–1431. [Medline]
35. Jin G, Wang L, Ma J. Inhibiting STAT5 significantly attenuated Ang II-induced cardiac dysfunction and inflammation. Eur J Pharmacol. 2022; 915: 174689. [Medline] [CrossRef]
36. Huang S, Wang J, Liu F, Dong L. Alternatively activated macrophages promote airway inflammation through JAK3-STAT5-Fra2 in asthma. Inflamm Res. 2022; 71: 873–885. [Medline] [CrossRef]
37. Surbek M, Tse W, Moriggl R, Han X. A centric view of JAK/STAT5 in intestinal homeostasis, infection, and inflammation. Cytokine. 2021; 139: 155392. [Medline] [CrossRef]
38. Li S, Guo X, Zhang T, Wang N, Li J, Xu P, et al. Fibroblast growth factor 21 ameliorates high glucose-induced fibrogenesis in mesangial cells through inhibiting STAT5 signaling pathway. Biomed Pharmacother. 2017; 93: 695–704. [Medline] [CrossRef]
39. Abu El Makarem MA, El-Sagheer GM, Abu El-Ella MA. The role of signal transducer and activator of transcription 5 and transforming growth factor-beta1 in hepatic fibrosis induced by chronic hepatitis c virus infection in egyptian patients. Med Princ Pract. 2018; 27: 115–121. [Medline] [CrossRef]
40. Dong Z, Tai W, Lei W, Wang Y, Li Z, Zhang T. IL-27 inhibits the TGF-β1-induced epithelial-mesenchymal transition in alveolar epithelial cells. BMC Cell Biol. 2016; 17: 7. [Medline] [CrossRef]
41. Zhang Y, Wang Y, Liu Y, Wang N, Qi Y, Du J. Krüppel-like factor 4 transcriptionally regulates TGF-β1 and contributes to cardiac myofibroblast differentiation. PLoS One. 2013; 8: e63424. [Medline] [CrossRef]