

Fractalkine Upregulates Inflammation through CX3CR1 and the Jak–Stat Pathway in Severe Acute Pancreatitis Rat Model

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Abstract—Based on the function of chemokine fractalkine (FKN), acting as both adhesion and chemoattractant, FKN plays a role in acute inflammatory response. In this study, we investigated the mechanism of FKN mediated upregulation inflammation in severe acute pancreatitis (SAP) rat models. Western blot, reverse transcriptase-polymerase chain reaction, and immunofluorescence demonstrated that FKN and its receptor CX3CR1 were overexpressed in cerulein-stimulated AR42J cells. AG490 and FKN-siRNA inhibited activation of Janus kinase/signal transducers and activators of transcription (Jak/Stat) in cerulein-stimulated AR42J cells. Following exposure AG490 and FKN-siRNA inhibited tumor necrosis factor-alpha expression by enzyme-linked immunosorbent assay and immunohistochemistry *in vivo* the SAP rat models. These results showed FKN and CX3CR1 were involved inflammatory response in cerulein-stimulated AR42J cells. FKN upregulates inflammation through CX3CR1 and the Jak/Stat pathway in SAP rat models.

KEY WORDS: chemokine; fractalkine; CX3CR1; severe acute pancreatitis; Jak/Stat.

INTRODUCTION

Of the acute pancreatitis (AP) patients, 10–20% develop into a severe disease with systemic inflammation, multi-organ failure, and mortality [1]. Severe acute pancreatitis (SAP) is associated with the release of digestive enzymes to the pancreatic interstitium and to the systemic circulation. At the same time, these increased cytokine production and release can ultimately lead to deleterious local and systemic effects. The release of these inflammatory cytokines triggers an inflammatory cascade which leads to the systemic inflammatory response syndrome. Therefore, inflammatory cytokines play an important role in SAP.

Fractalkine (FKN) possesses a chemokine domain and an extended mucin-like stalk that allows it to function as both a chemoattractant and an adhesion molecule [2]. The accumulating evidences indicate that FKN are involved in the pathogenesis of various inflammatory disorders such as rheumatoid arthritis [3], atherosclerosis [4], acute hepatitis [5], kidney disease [6], and so on. CX3CR1, a receptor for FKN, was shown to mediate both the adhesive and migration functions of FKN. Therefore, FKN and its receptor CX3CR1 play an important role in inflammation. A preliminary study by us showed that the serum FKN, the protein and mRNA of FKN were overexpressed in the SAP rat models. At the same time, FKN was associated with the severity of SAP. Furthermore, we detected the serum FKN level in mild acute pancreatitis (MAP) patients and SAP patients by enzyme-linked immunosorbent assay (ELISA). Although the sample size was small, the results showed that the level of serum FKN increased in patients with MAP, especially in patients developing SAP. Given previous study, we considered FKN as a good marker of the inflammation. Therefore, FKN may play a role in acute inflammatory response and are believed to be an

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important factor in SAP. However, FKN involved in inflammatory mechanism have not been clearly elucidated.

Janus kinase/signal transducers and activators of transcription (Jak/Stat) signaling pathways have been reported to be involved in the immune response of numerous cytokines [7–9]. Cytokines regulate numerous aspects of immune responses. They mediate their responses through activation of the Jak/Stat signaling pathway. AG490 is a Jak2 inhibitor and involved in the synthetically derived tyrphostin family of tyrosine kinase inhibitors [10]. In the present study, we investigated whether the activation of Jak/Stat signaling mediates FKN expression in pancreatic acinar cerulein stimulated in AR42J cells *in vitro* and the SAP rat models *in vivo*.

MATERIALS AND METHODS

Cell Culture and Transfection

The pancreatic acinar cell lines, AR42J (ATCC, Rockville, MD, USA), was cultured in DEME (Gibco BRL, Gaithersburg, MD, USA) plus 10% fetal bovine serum (Gibco BRL, Gaithersburg, MD, USA) and 1% penicillin/streptomycin (Sigma; St. Louis, MO, USA) in standard conditions (37°C and 5% CO₂). The AR42J cells were plated at a density of 3 × 10⁵/mL in a six-well culture plate and allowed to attach for 12 h. The cells were stimulated with cerulein (10⁻⁸ M). The adenoviral constructs carrying siRNA against FKN was designed and constructed by MingHong Co. LTD, Shanghai, China. The cerulein-stimulated AR42J cells (3 × 10⁵/well) were transfected with siRNA (10 nmol/ml) in six-well plates following the MingHong Co manufacturer's protocol. AG490 (Sigma) was dissolved in dimethylsulphoxide and treated at final concentration of 50 μM.

Animal Model of SAP

Sprague–Dawley rats (220–250 g) were provided by the Experimental Animal Center of Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China. The rats were deprived of food but were allowed access to water for 12 h before the operation. The rats were anesthetized with an intraperitoneal administration of sodium pentobarbital (30%, 0.15 ml/100 g). The biliopancreatic duct was cannulated through the duodenum and the hepatic duct was closed by a small clamp. The SAP rats were induced by

retrograde perfusion of 5% sodium taurocholate (Sigma) in a volume of 1.5 ml/kg using a perfusion pump [11]. The control rats only received an injection of saline solution. Blood was collected by abdominal aorta puncture. After centrifugation for 10 min at 2,500 g/min, the supernatant of the blood was respectively placed into sterilized Eppendorf tubes and stored in a refrigerator at -20°C. Meanwhile, pancreas tissue was frozen in a refrigerator at -80°C until biochemical assays were performed. The experiments were conducted according to the Guidelines of the Shanghai Animal Use and Care Committees and the National Animal Welfare Law.

Western Blotting

The cells were scraped off the plates and lysated in RIPA buffer. Cell lysates (40 μg) were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. The membranes were incubated with antibodies Jak2 (1:1,000; Cell Signaling, Beverly, MA, USA), Stat1 (1:1,000; Cell Signaling), and Stat3 (1:1,000; Cell Signaling,) diluted in TBS-T containing 5% dry milk, phosphor Jak2 (1:1,000; Cell Signaling), phosphor Stat1(1:1,000; Cell Signaling) and phosphor Stat3 (1:1,000; Cell Signaling) diluted in 5% BSA against target proteins overnight at 4°C and the membranes were incubated with the corresponding horseradish peroxidase-conjugated secondary antibody (diluted 1:2,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Western blotting was analyzed by scanning densitometry using the bio-image analysis system (Bio-Rad, Baltimore, MD, USA) for quantification, as previously described [12].

Immunofluorescence

Cells were grown onto 15-mm glass coverslips and fixed with 4% paraformaldehyde. This approach allowed us to impede the access of primary antibodies to the intracellular space, thus detecting proteins expressed on the cell surface. The primary antibodies CX3CR1 (Santa Cruz Biotechnology,) was used at 1:100 dilution. Fluorescein isothiocyanate-conjugated secondary antibodies were from Kangchen Biologic (Kangchen, Shanghai, China). Fluorescent images were acquired by using a charge-coupled device digital camera connected to microscope (Eclipse, TE2000-U, Nikon, Tokyo, Japan).

***In Vivo* SAP Studies**

The injected taurocholate rats with SAP were randomly divided into three groups ($n=5$ for each group): mock (no siRNA and no AG490) group, AG490 group and FKN siRNA group (treated by caudal vein inject). AG490 (0.02 mg/g body weight) and FKN siRNA (20 $\mu\text{g}/100\text{ g}$) were injected once 12 h prior to the first injection of sodium taurocholate to the rats [12]. The mock group used phosphate-buffered saline PBS instead of AG490 and siRNA. Rats were killed under anesthesia at 48 h after the injection of FKN siRNA. Blood and pancreas tissues were collected from the former methods.

Animal Model Assessment

Pancreas tissue, including mock group, AG490 group and FKN siRNA group, were fixed in 4% formaldehyde and dehydrated ethanol. Tissue samples were impregnated in paraffin wax and cut into blocks (4 μm). After being stained by hematoxylin–eosin, pancreas tissue sections were examined by light microscopy. For this study case, each tissue sample was examined with five randomly chosen microscopic fields and the degrees of pancreatic injury were assessed with histological score by the previously described method [13]. Serum amylase activity was determined by spectrophotometric assay using detection kit (Nanjing Jiancheng Bioengineering Research Institute; Nanjing, China). Neutrophilic infiltration in pancreatic tissue was assessed by measuring myeloperoxidase (MPO) activity (MPO detection kit, Nanjing Jiancheng Bioengineering Research Institute) [14].

Enzyme-Linked Immunosorbent Assay

Serum levels of FKN and tumor necrosis factor- α (TNF- α) were examined with ELISA using kit (Maibio Co. LTD, Shanghai, China). Tests were made according to the instructions with the kits [14].

Reverse Transcriptase-Polymerase Chain Reaction

The total RNA was extracted with Trizol (TaKaRa Biotechnology Co, Ltd; Dalian, China) reagent for each group. The cDNA was synthesized using the Prime Script™ RT reagent kit (TaKaRa Biotechnology Co, Ltd), and the reverse transcription was then performed on 1 μg RNA sample by adding PrimeScript reagents. After the reverse transcription, the reverse transcriptase-polymerase chain reaction (RT-PCR) reactions were

performed in 25 μL volumes. The primers were indicated as follows: the primer sequence of FKN (141 bp): the forward primer, 5'-CTG CCC TGA CTA GAA ATG GT-3', the reverse primer, 5'-CAG TCG GTT CCA AAG TAA GG-3'; the primer sequence of CX3CR1 (390 bp): the forward primer, 5'-CCTGC CCTTG CTTAT CAT-3', the reverse primer, 5'-CTCCT CTGGG ACTCT GTTG-3'; the primer sequence of TNF- α (250 bp): the forward primer, 5'-TCTCA TTCCT GCTCG TGG-3', the reverse primer, 5'-CCATT GGCCA GGAGG GCGTT GG-3'; the primer sequence of GAPDH (460 bp): the forward primer: 5'-ACC ACA GTC CAT GCC ATC AC-3', the reverse primer: 5'-TCC ACC ACC CTG TTG CTG TA-3'. The band densities were normalized to the GAPDH band densities and the results were represented as the ratio. The densities of the cDNA bands were analyzed by scanning densitometry using Gel Doc 2000 software (Bio-Rad, Baltimore, MD, USA).

Immunohistochemical Staining Analysis

These sections embedded in paraffin wax were de-waxed, rehydrated in gradient alcohol, and endogenous peroxidase activity was blocked with 3% H_2O_2 for 5 min, and then followed by using TNF- α antibody (diluted 1:100; Santa Cruz Biotechnology) overnight at 4°C, followed by Ultra Sensitive™ S-P kit (Maixin Biotech, Inc., Fuzhou, China). At last, the bindings were visualized by the diaminobenzidine, the sections were counterstained with haematoxylin. The positive signals were detected as cytoplasm and nucleus staining presenting yellow color.

Survival Study

Mock group, AG490 group, and FKN siRNA group were further studied in survival. These rat models were not prohibited diet. The survival time was recorded for 48 h.

Statistical Analysis

Statistics was done by SPSS program at 11.0 versions (SPSS, Chicago, IL, USA). All data were represented as the mean \pm standard deviation. The one-way analysis of variance with Dunnett's multiple comparison tests were used for comparisons. Survival analysis was conducted with a Kaplan–Meier analysis. $P<0.05$ was considered as statistically significant.

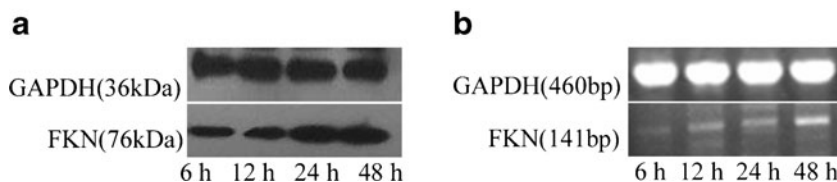


Fig. 1. Cerulein increases the expression of FKN in AR42J cells. Cultured AR42J cells were exposed to cerulein for each time point. **a** The protein of FKN was increased at 6 h by Western blot and in a time-dependent manner. **b** The mRNA of FKN was increased at 6 h by RT-PCR and in a time-dependent manner.

RESULTS

FKN was Expression in Cerulein-Stimulated AR42J Cells

Protein and mRNA expression of FKN were determined by cerulein-stimulated AR42J cells (Fig. 1). Western blot and RT-PCR showed that FKN expression was observed at 6-h culture and increased in a time-dependent manner up to 48 h. We concluded from these data that the expression of FKN is a usual feature of the cerulein-stimulated AR42J cells.

Exposure to FKN Increase TNF- α Expression in Ar42jcells

FKN was overexpression in cerulein-stimulated AR42J cells. We examined whether FKN could stimulate inflammatory response in AR42J cells. AR42J cells were exposed to increasing concentrations of FKN, and the effect on TNF- α expression was determined up to 48 h. We found that the level of protein of TNF- α increased in an FKN dose- and time-dependent fashion (Fig. 2).

FKN Receptor CX3CR1 was Expression in Cerulein-Stimulated AR42Jcells

Because FKN increased TNF- α expression in AR42J cells, we considered that FKN receptor CX3CR1 might be existed in AR42J cells. Protein and mRNA expression (Fig. 3a) of CX3CR1 were determined by cerulein-stimulated AR42J cells. Western blot and RT-PCR showed that CX3CR1 expression was observed at 6-h culture and increased in a time-dependent manner up

to 48 h. Furthermore, CX3CR1 expression at the membrane level in cerulein-stimulated AR42J cells was established by using an antibody for the extra cellular portion and detected by immunofluorescence (Fig. 3b).

FKN and CX3CR1 Activates TNF- α through in Cerulein-Stimulated AR42J Cells

We determined whether FKN could activate TNF- α in cerulein-stimulated AR42J cells through its receptor CX3CR1. We transfected FKN siRNA into cultured cerulein-stimulated AR42J cells. Protein of CX3CR1 and TNF- α were expressed less than compared with cerulein-stimulated AR42J cells transfected with control siRNA by Western blot (Fig. 4a). Furthermore, mRNA of CX3CR1 and TNF- α decreased than transfected with control siRNA by RT-PCR (Fig. 4b). These results indicated that the FKN and CX3CR1 did active TNF- α in cerulein-stimulated AR42J cells.

AG490 and FKN-siRNA Inhibited Activation of Jak2, Stat1, and Stat3 in Cerulein-Stimulated AR42J Cells

To study the mechanism of FKN-mediated upregulation inflammation, we examined the activation of Jak2, Stat1, and Stat3 in cerulein-stimulated AR42J cells. At the same time, we observed the change of phosphorylated Jak2, phosphorylated Stat1, and phosphorylated Stat3 in cerulein-stimulated AR42J cells (Fig. 5). These results demonstrated that cerulein-activated Jak2/Stat1 and Jak2/Stat3 signaling in cerulein-stimulated AR42J cells. As shown in Fig. 5, 12-h pretreatment of the Jak2 inhibitor AG490 and FKN-siRNA suppressed the acti-

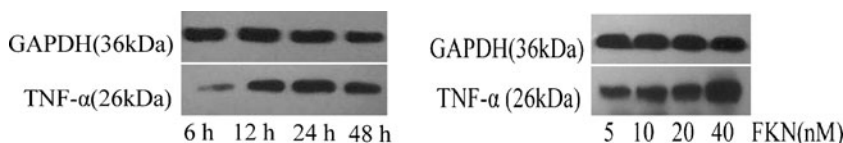


Fig. 2. FKN increases TNF- α protein in AR42Jcells. AR42J cells were exposed to FKN for the indicated doses and times. Cells were harvested and Western blot was performed for quantitation of TNF- α protein. TNF- α protein increased in a dose- and time-dependent fashion.

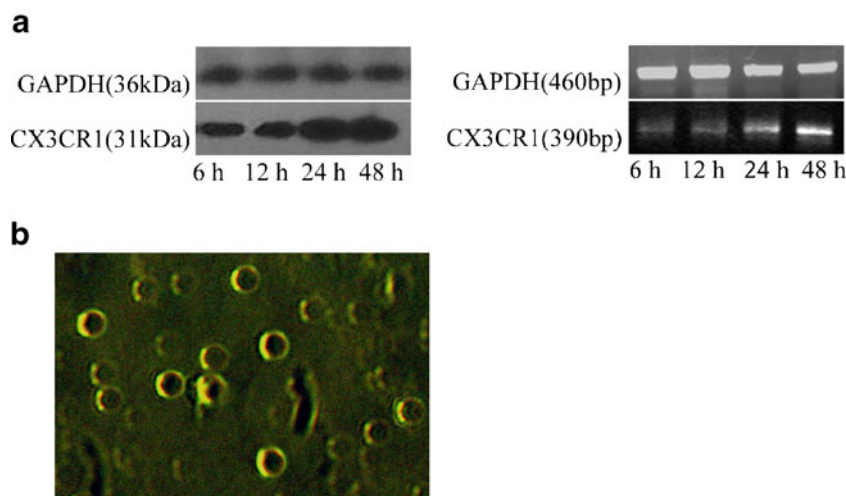


Fig. 3. **a** Time-response of cerulein-stimulated AR42J cells for protein expression of CX3CR1, as determined by Western blot and RT-PCR. Cultured AR42J cells were exposed to cerulein for each time point. The protein and mRNA of CX3CR1 was increased at 6 h by Western blot and RT-PCR in a time-dependent manner. **b** CX3CR1 localization by fluorescein isothiocyanate in cerulein-stimulated AR42J cells.

vation of Jak2, Stat1, and Stat3 in cerulein-stimulated AR42J cells. These results show that AG490 and FKN-siRNA may be beneficial in pancreatitis by inhibiting the activation of Jak2/Stat1 and Jak2/Stat3 inflammatory signaling in cerulein-stimulated AR42J cells.

The Severity of Acute Pancreatitis

The levels of serum amylase and MPO activity were shown in Table 1. Amylases and the MPO activity in rats with SAP were significantly increased compared with the group with treated FKN-siRNA and AG490 ($P < 0.05$). In the SAP group, interstitial edema, inflammatory cell infiltration, focal necrosis, and interstitial hemorrhage were observed. Histological score of pancreatic injury were also significantly increased compared with the group with treated FKN-siRNA and AG490 ($P < 0.05$).

AG490 and FKN-siRNA Inhibit TNF- α Expression *In Vivo* SAP Rat Model

We established the SAP rat model and treated them with FKN siRNA and AG490. All rats were killed under anesthesia at 48 h after treated with FKN siRNA, AG490, and sham operation. We detected the level of serum TNF- α and FKN by ELISA. The values of serum TNF- α and FKN in each group were represented in Fig. 6a. The serum TNF- α and FKN level elevated in the SAP than the controlled group ($P < 0.05$). The values of serum TNF- α was descended after induced FKN siRNA and AG490 than the SAP group ($P < 0.05$). Furthermore, immunohistochemistry showed TNF- α decreased after injection FKN-siRNA and AG490 in SAP rat model (Fig. 6b). Combined together, treatment of FKN siRNA and AG490 can inhibit inflammation in SAP rat model, indicating that FKN siRNA may serve as a novel therapeutic agent for treating SAP with FKN overexpression.

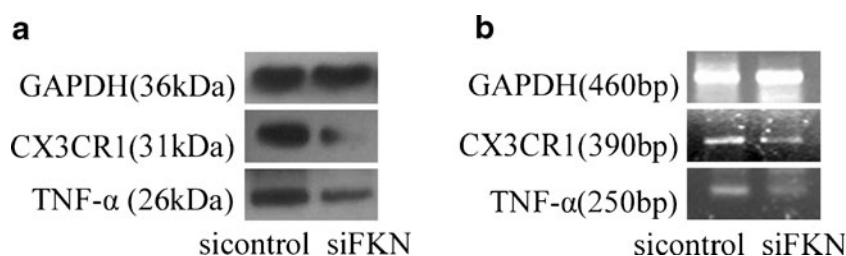


Fig. 4. FKN siRNA was able to remarkably suppress CX3CR1 and TNF- α overexpression in cerulein-stimulated AR42J cells. **a** Showed representative western blot of protein detected with CX3CR1, TNF- α and GAPDH antibodies in each group. **b** Showed representative RT-PCR of mRNA of CX3CR1 and TNF- α (measured as the ratio of CX3CR1 and TNF- α to GAPDH by band density) in each group.

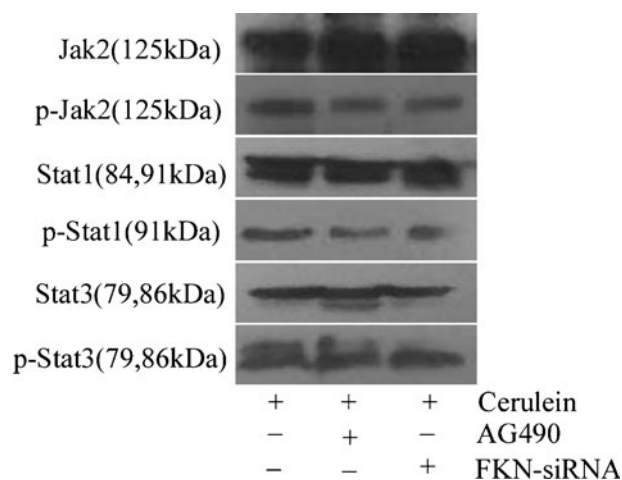


Fig. 5. FKN activates the Jak2/Stat1 and Jak2/Stat3 in cerulein-stimulated AR42J cells. Cerulein-stimulated AR42J cells were treated with (+) or without (-) AG490 and FKN-siRNA for 12 h. Phosphorylated and nonphosphorylated forms of each Jak2, Stat1, and Stat3 were determined in the cells by Western blot analysis.

Rats Survival Analysis

The average survival time of SAP group was 39.40 ± 2.49 h, 95% confidence interval (CI) 34.53–44.27; AG490 group was 46.90 ± 0.61 h, 95% CI 45.71–48.09; FKN-siRNA group was 45.80 ± 1.10 , 95% CI 43.64–47.96 (Fig. 7). The rats with injection AG490 and FKN-siRNA group had longevity compared with the SAP rats during the experimental time.

DISCUSSION

Chemokines have conserved cysteine residues and are classified into C, CC, CXC, and CX3C subfamilies. FKN is the unique member of the CX3C chemokine subfamily. Because of FKN chemokine and adhesion function, it can capture leukocytes, activate multiple inflammatory passages, and caused vessel injury by coordinating with other cytokines [2]. Recently, the

impact of FKN has been recognized in the progression of inflammatory diseases [3–6]. At the same time, CX3CR1, the unique receptor for FKN, is expressed on subsets of peripheral CD4⁺ and CD8⁺ T cells, nature killer cells, and monocytes [15]. CX3CR1 is preferentially expressed by cells representing a proinflammatory, Th1, and/or cytotoxic phenotype, and the interaction of FKN and CX3CR1 has been proposed as an amplification process for polarized Th1/proinflammatory responses [16, 17]. Therefore, FKN and CX3CR1 have been thought to play an important role in inflammatory diseases. In this study, FKN and CX3CR1 were overexpressed in cerulein-stimulated AR42J cells by Western blot and RT-PCR (Fig. 1, Fig. 3a) and increased in a time-dependent manner. At the same time, TNF- α was increased in a dose- and time-dependent fashion in AR42J cells exposed of FKN. These results showed FKN and CX3CR1 were involved in inflammatory response in pancreatic acinar cells. Our preliminary study showed that serum FKN, the protein, and mRNA of FKN were overexpressed in the SAP rat models. FKN can induce leukocyte adhesion and transmigration into injured tissue [18, 19]. FKN and CX3CR1 interaction preferentially mediates arrest and migration of inflammatory cells [16]. Garten *et al.* reported that the release of FKN from the endothelial membrane-bound molecule may occur by the action of different proteases, such as TNF- α converting enzyme [20]. CX3CR1 and TNF- α were decreased after FKN-siRNA treatment in cerulein-stimulated AR42J cells (Fig. 4). Some studies have suggested that the interaction of FKN and CX3CR1 contributes to the pathogenesis of atherosclerosis [21, 22] and kidney diseases [6]. Therefore, FKN and CX3CR1 play an important part in attracting and activating of leukocytes in inflamed tissue, and these properties make them attractive candidate mediators in the pathogenesis of SAP.

In the present study, the increase in serum levels of TNF- α was accompanied with the edematous and inflammatory pancreatic damages. The serum levels of

Table 1. Serum Amylase, Pancreatic MPO Activity, and Histological Score of Pancreatic Injury in Each Group

	SAP	SAP treated FKN-siRNA	SAP treated AG490
Serum amylase (μ /dL)	62.93 \pm 4.55	41.89 \pm 5.58*	39.41 \pm 6.03*
Pancreatic MPO activity (U/g)	0.28 \pm 0.014	0.19 \pm 0.018*	0.17 \pm 0.015*
Histological score of pancreatic injury	10.8 \pm 0.84	4.6 \pm 0.89*	4.2 \pm 0.84*

Values are means \pm SD

* P <0.05 vs. SAP group

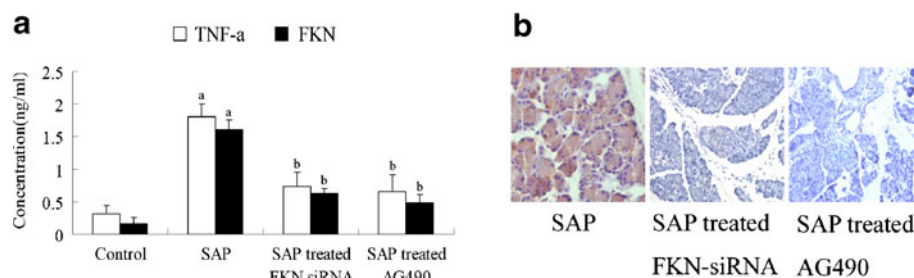


Fig. 6. **a** The serum TNF- α and FKN concentration of each group vs. controlled group, $P < 0.05$; **b** vs. SAP group, $P < 0.05$. **b** Confirmation of reduced TNF- α in response to AG490 and FKN siRNA treatment of the pancreas tissue ($\times 40$) in SAP rat model by immunostaining.

TNF- α and pancreatic damage determined the severity of inflammatory response in SAP rat models. These changes were significantly inhibited by treatment of FKN-siRNA. RNA interference mediated by siRNA is an important defense mechanism that binds to complementary target mRNA, while specifically targeting these sequences for degradation, resulting in the inhibition of protein expression to prevent and/or treat disease. siRNA has already been demonstrated as a potent therapy for targeting a wide variety of diseases [23, 24]. In addition, siRNA have been extensively tested against inflammation diseases in animal models [25]. Adenovirus-mediated methods of transferring siRNA as recent studies have shown this method to be a more efficient delivery mechanism [26, 27]. In this study, we utilized an adenovirus-mediated method to transfer siRNA by using FKN siRNA to target FKN overexpression and suppressed CX3CR1 and TNF- α in cerulein-stimulated AR42J cell (Fig. 4). These results showed that FKN and CX3CR1 were upregulated inflammation *in vitro*. Furthermore, FKN might be served as a novel and effective therapeutic target for SAP.

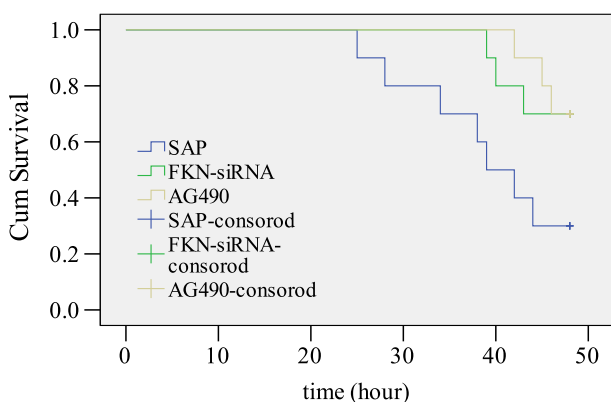


Fig. 7. Alterations in the survival rate during 48 h in each group. The survival rate was estimated by the Kaplan–Meier method.

Cytokines regulate numerous aspects of immune response. They mediate their responses through activation of the Jak/Stat signaling pathway. Jaks represent a family of four nonreceptor tyrosine kinases, Jak1, Jak2, Jak3, and Tyk2. These kinases selectively phosphorylate Stats, leading to their activation [28]. Once activated, Stat plays a critical role in regulating innate and acquired host immune responses. The Jak/Stat pathway is well-known to be activated by the family of cytokine receptors and to mediate a wide variety of biological effects, such as immune responses, differentiation, cell survival, proliferation, or oncogenesis. Activated Jaks in turn phosphorylate the receptor that recruits the Stat protein [8, 29]. AG490 is a Jak2 inhibitor and involved in the synthetically derived tyrphostin family of tyrosine kinase inhibitors [10]. The molecular mechanism is responsible for cell activation by FKN remain largely unknown. On the basis of this observation, we became interested in elucidating, in the cerulein-stimulated AR42J cells, the effect of FKN on the Jak/Stat signal transduction pathway. The cerulein-stimulated AR42J cells were pretreated with AG490 and FKN-siRNA. Figure 5 showed AG490 and FKN-siRNA effectively inhibited the activation of Jak2 phosphorylation in cerulein-stimulated AR42J cells. Likewise, Stat1 and Stat3 phosphorylation was blocked by AG490 and FKN-siRNA. Furthermore, AG490 and FKN-siRNA significantly reduced ceruelin TNF- α and FKN expression in SAP rat model, indicating that FKN expression is dependent on Jak2/Stat3 signaling. We also observed that the rats with injection AG490 and FKN-siRNA group had longevity compared with the SAP rats during the experimental time. AG490 and FKN-siRNA may negatively regulate inflammation in the episodes of acute pancreatitis. Therefore, we inferred FKN, which upregulated inflammation, must be activated through Jak2/Stat signaling, a key pathway in inflammation. Presumably, FKN may be caused by inactivation of Jak2, which

would be required to catalyze the event of Stat phosphorylation. These data provide evidence to indicate that FKN activates Stat1 and Stat3 through the activation of Jak2. Further study should be elucidated how FKN can be activated the Jak/Stat pathway in the future. The results also showed the important relevance of Jak2 inhibition to the treatment of SAP.

In this study, we demonstrated that the cerulein-stimulated AR42J cells express both FKN and its receptor CX3CR1. FKN and CX3CR1 trigger Jak2/Stat inflammatory signaling and may result in important inflammatory events in SAP rat model. We suggest Jak2/Stat activation as the molecular mechanism underlying pathogenesis of severe acute pancreatitis and chemokine FKN may become attractive targets in the clinical therapy of inflammatory diseases.

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