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The JAK2/STAT3 signaling pathway is required for inflammation and cell death induced by cerulein in AR42J cells

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Abstract. – OBJECTIVE: The study was designed to investigate the JAK2/STAT3 signaling pathway in pancreatitis and its association with inflammation and cell death to provide a potential treatment method for pancreatitis.

MATERIALS AND METHODS: The rat pancreatic acinar AR42J cells were used for the study, and they were transfected with JAK2 and STAT3 siRNAs to mimic knockdown condition. Cerulein was used to treat AR42J cells. Western blot and ELISA were employed to detect the expression of related proteins. Flow cytometry was done to analysis the necrosis of AR42J cells.

RESULTS: In this study, we found that cell death and the secretion of IL-6 and TGF-β1 were significantly increased, and the JAK2/STAT3 signaling pathway was activated in cerulein-induced AP. To determine the role of JAK2 and STAT3, JAK2 siRNA and STAT3 siRNA were used to block JAK2 and STAT3, respectively. The levels of IL-6 and TGF-β1 levels in the medium were lower in JAK2 siRNA and STAT3 siRNA-treated cells compared with controls. Flow cytometry analysis showed that the level of cell death, expression of cleaved caspase-3, and the release of LDH were decreased following JAK2 siRNA and STAT3 siRNA treatment.

CONCLUSIONS: These findings point to a novel role for the JAK2/STAT3 signaling pathway in the progression of cerulein-induced AP.

Key Words:
Acute pancreatitis, JAK2, STAT3, Inflammation, Cell death.

Introduction

Acute pancreatitis (AP) is one of the most common disorders of the exocrine pancreas which is characterized by the dysregulation of digestive enzyme production and secretion[1,2]. Although the underlying mechanisms have not been fully elucidated and there is no specific and effective therapy[3,4], AP is believed to originate in the injured acinar cells due to uncontrolled inflammation, which contributes to the death of acinar cells[4]. Indeed, inflammation and parenchymal cell death are prominent pathological phenotypes in patients with AP[5].

Cerulein is an ortholog of the intestinal hormone cholecystokinin and is one of the most-characterized and widely used agents in experimental animal models of pancreatitis[5-7]. Accumulating evidence demonstrates that cerulein induces the death of acinar cells, the formation of edema, and the infiltration of inflammatory cells into the pancreas in mice and rats[8,9]. The mechanism of cerulein action *in vivo* involves the promotion of oxidative stress and the release of pro-inflammatory cytokines[10-12]. However, the effects and molecular mechanisms of cerulein have not been extensively explored through *in vitro* models.

It is well-known that the Janus kinase 2 (JAK2) signal transducer and the activator of transcription 3 (STAT3) pathway plays a key role in modulating immune responses in several cell types[13,14]. This pathway generally transduces signals from activated receptors or intracellular kinases to the nucleus, thereby activating and regulating gene transcription. Its target genes include cytokines, adhesion molecules, and other inflammatory mediators[13,15,16]. On the other hand, JAK2 and STAT3 proteins are stimulated and activated by cytokines[17,18]. Considering that AP is caused by the production and release of various pro-inflammatory cytokines, the JAK2/STAT3 pathway is an important regulator of inflammation and cell death in the pancreas when exposed to an inflammatory stimulus like cerulein[19,20]. In this

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study, we investigated the effect of cerulein on AR42J cells and the specific role of the JAK2/STAT3 pathway in the AP model. We found that cerulein directly activated the JAK2/STAT3 pathway leading to the production of IL-6 and TGF-β1. Downregulating the expression of JAK2 and STAT3 by siRNAs abolished the cerulein-induced production of IL-6 and TGF-β1, and inhibited cell death in AR42J cells. These results suggest that the JAK2/STAT3 signaling pathway mediates the cerulean-induced cell death and inflammation in AR42J cells.

Methods and Materials

Cell Culture and Transfection

The rat pancreatic acinar AR42J cells were cultured in F-12K medium supplemented with 20% fetal bovine serum (FBS, Gibco, Rockville, MD, USA) and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) at 37°C in a humidified atmosphere containing 5% CO_2. To develop an *in vitro* model of acute pancreatitis, AR42J cells were treated with cerulein (10-8 M) and the expression of JAK2, phospho-JAK2, STAT3, and phospho-STAT3 were determined at a different time-points after cerulein treatment. Transfection of AR42J cells with siRNAs was done using Lipofectamine 2000 (11668019, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The plasmid constructs encoding JAK2 and STAT3 siRNAs were used for the transfection. The sequence of JAK2 siRNA (sc-270385) and STAT3 siRNA (sc-270027) and non-targeting siRNA pool (sc-36869) used as a control were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). 48 h after transfection, the effect of siRNAs silencing was tested by Western blot.

Western Blot

For Western blot analysis, the cells were homogenized in lysis buffer (1% Nonidet P-40, 20 mM Tris, pH 8.0, 137 mM NaCl, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride (PMSF), sodium butyrate 1 mM, and protease inhibitors) at 4°C. After removal of cellular debris by centrifugation, the supernatant was collected, and protein concentration in the lysate was measured by the Bradford assay (Bio-Rad, Hercules, CA, USA). 50 μg of each sample was boiled in the presence of sample buffer for 5 minutes before separation on 10% sodium dodecyl sulphate (SDS) polyacrylamide gel. Thereafter, the proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The immunoblots were blocked with 5% nonfat dry milk dissolved in phosphate-buffered saline (PBS) for 60 min. The membranes were then incubated overnight at 4°C with primary antibody: rabbit anti-JAK2 (1:500, Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit anti-phospho-JAK2 (1:1000, Cell Signaling, Danvers, MA, USA), rabbit anti-STAT3 (1:1000, Upstate Biotechnology, Lake Placid, NY, USA), rabbit anti-phospho-STAT3 (1:1000, Upstate Biotechnology, Lake Placid, NY, USA), rabbit anti-caspase-3 (1:1000, Upstate Biotechnology, Lake Placid, NY, USA), rabbit anti-β-actin (1:2500, Abcam, Cambridge, MA, USA). Primary antibody incubation was followed by three washes (5 min, rocking, at room temperature) in PBST (PBS containing 0.2% Tween 20) before incubation with goat anti-rabbit secondary antibody conjugated to horseradish peroxidase. The blots were washed three times and the protein expression was detected with Bio-Rad Imaging Systems (Hercules, CA, USA). β-actin was used as the loading control, and the optical densities of protein bands were quantitatively analyzed with Quantity One software (Bio-Rad, Hercules, CA, USA).

Biochemical Assays

The levels of IL-6, TGF-β1, and LDH in the medium were determined by enzyme-linked immunosorbent assay (ELISA) kits (IL-6: R&D System, Minneapolis, MN, USA; TGF-β1: R&D System, Minneapolis, MN, USA). All the procedures were performed according to the manufacturer's instructions. At the end of the assays, fluorescence intensities of the 96 well microplates were read by an assay reader (Tecan, Männedorf, Switzerland). After averaging the results of duplicate wells, the value of IL-6 and TGF-β1 of each sample was calculated as pg/mL medium.

Quantification of Cell Death

Necrosis of AR42J cells was determined by flow cytometry analysis after incubation with cerulein for 24 h using Cell Death Detection kit (BD Biosciences, San Jose, CA, USA) according to the manufacturer's protocol.

Statistical Analysis

All values were expressed as mean ± SEM. The data were analyzed using one-way ANOVA

and New-Keul's test, followed by post hoc test. A p-value < 0.05 was considered statistically significant.

Results

Cerulein Increased Cell Death and Production of Inflammatory Cytokines in AR42J Cells

To determine the effect of cerulein on AR42J cells, we examined the expression of cleaved caspase-3 by Western blot. As shown in Figure 1A, 1B, after culturing for 6 h and 12 h, cerulein increased the level of cell death significantly. Similarly, measurement of inflammatory cytokines by ELISA revealed that the production of IL-6 and TGF-β1 were increased following cerulein treatment (Figure 1C, 1D). These datasets suggest that both cell death and inflammatory cytokines are increased in cerulean-treated AR42J cells.

Cerulein Induced JAK2/STAT3 Activation in AR42J Cells

To determine the effect of cerulein (the analog of cholecystokinin) on the JAK2/STAT3 pathway, we examined the protein expression of JAK2, phospho-JAK2, STAT3, and phospho-STAT3 at different time-point after cerulean treatment. The immunoblots of AR42J cell lysates demonstrated that the expression of pJAK2 and pSTAT3 were slightly increased at 2 h and significantly increased at 6 h and 12 h in the cerulein-treated group (Figure 2A-2D), indicating a marked activation of JAK2 and STAT3 following cerulein administration. We further investigated whether cerulein stimulated the secretion of IL-6 and TGF-β1 in AR42J cells. The level of IL-6 and TGF-β1 in the medium was assessed before or after cerulein treatment by ELISA. We found that the secretion of IL-6 and TGF-β1 was significantly increased following cerulein treatment compared to the controls (Figure 2E-2F). These findings suggest that cerulein induced JAK2/STAT3 activation in AR42J cells.

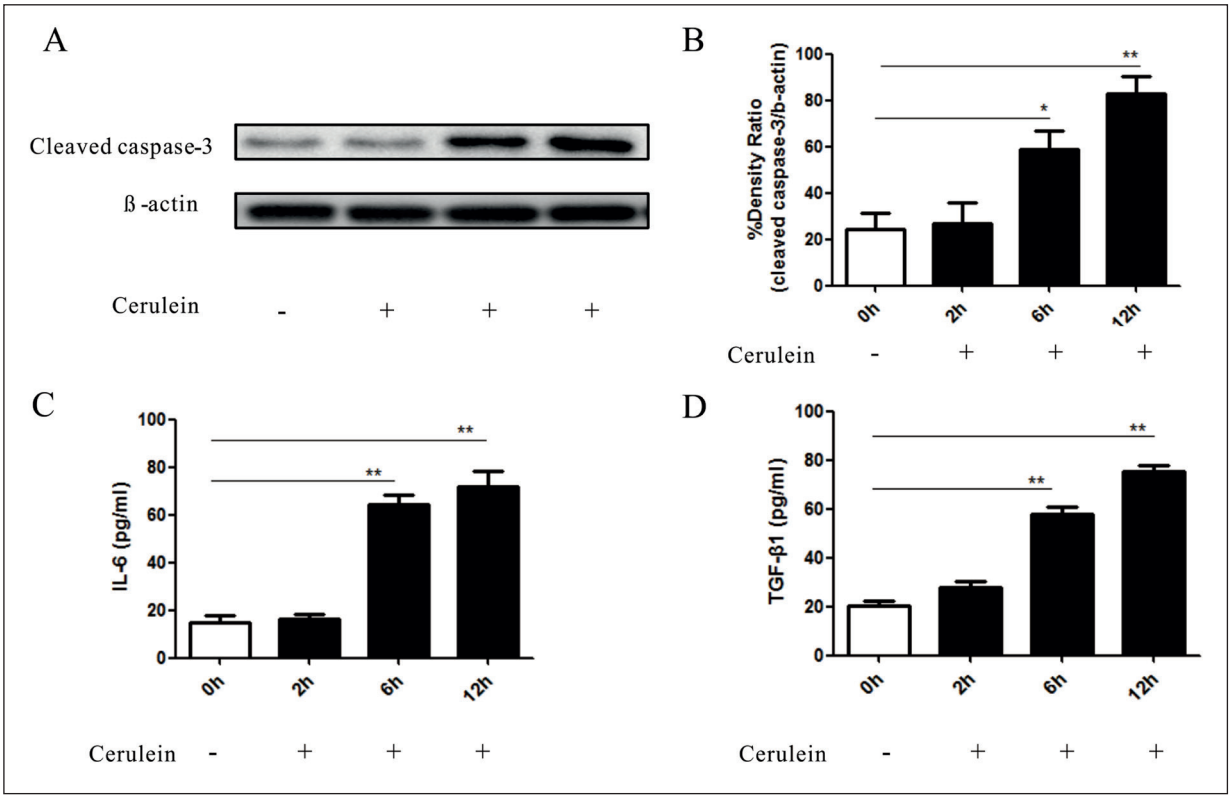

Figure 1. Cerulein promoted cell death and increased the production of inflammatory cytokines. AR42J cells were treated with cerulein for 0, 2, 6, 12 h. Expression of cleaved caspase-3 was determined by Western blot. The level of IL-6 and TGF-β1 in the medium was measured by ELISA. ***A,*** Changes of cleaved caspase-3 in AR42J cells. ***B,*** Density ratio of cleaved caspase-3, $*p < 0.05$, $**p < 0.01$. ***C,*** The level of IL-6 in the medium after treatment with cerulein, $**p < 0.01$. ***D,*** The level of TGF-β1 in the medium after treatment with cerulein, $**p < 0.01$.

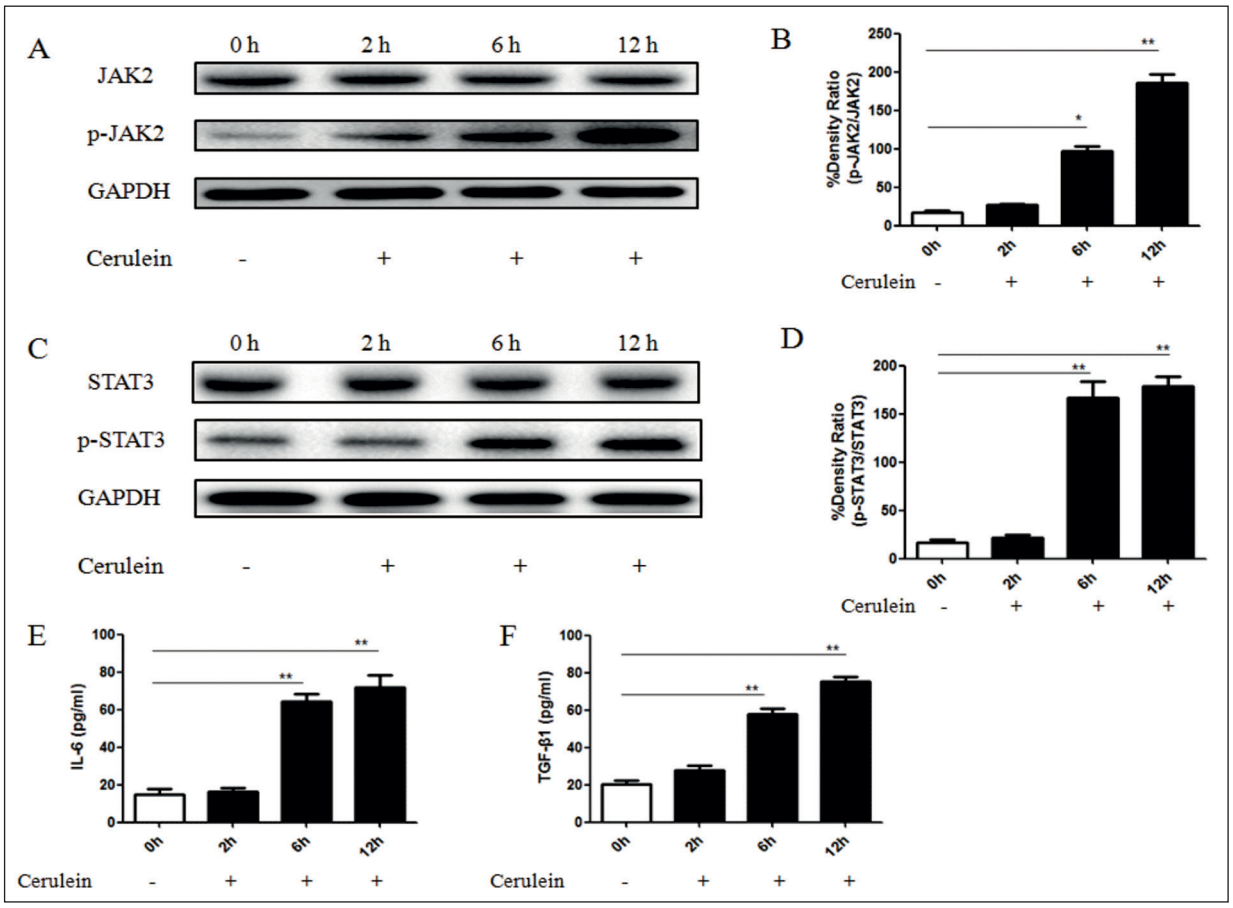

Figure 2. Cerulein activated the JAK2/STAT3 pathway. AR42J cells were treated with cerulein for 0, 2, 6, 12 h. The protein expression of JAK2, p-JAK2, STAT3, and p-STAT3 were determined by Western blot. ***A,*** Changes of JAK2 and p-JAK2 in AR42J cells. ***B,*** Density ratio of p-JAK2 and JAK2, $*p < 0.05$, $**p < 0.01$. ***C,*** Changes of p-STAT3 and STAT3 in AR42J cells. ***D,*** Density ratio of p-STAT3 and STAT3. ***E,*** The secretion of IL-6 following cerulein treatment. ***F,*** The level of TGF-β1 after cerulein culture, $*p < 0.05$, $**p < 0.01$.

Effects of JAK2 and STAT3 siRNAs on Cerulein-Induced Secretion of Cytokines in AR42J Cells

Next, we examined whether the JAK2/STAT3 signaling pathway is required for cerulein-induced cytokines secretion. We used siJAK2 and siSTAT3 to knockdown JAK2 and STAT3, respectively. The efficiency of siRNAs transfection was detected by Western blot. In cerulein-treated AR42J cells, the expression of JAK2 in siJAK2 group and the expression of STAT3 in siSTAT3 group were significantly lower compared with their corresponding control groups (non-targeting siRNA) (Figure 3A, 3B). Moreover, siJAK2 significantly reduced the expression of pJAK2 and pSTAT3 while siSTAT3 reduced the expression of pSTAT3 (Figure 3A, 3B).

The levels of IL-6 and TGF-β1 in siRNAs groups are shown in (Figure 3C-3D). In cerulein-treated siRNA control group, IL-6 was 75.3 ± 3.12 pg/mL and TGF-β1 was 80.1 ± 10.27 pg/mL in the cell media. Suppressing pJAK2 and pSTAT3 expression by siJAK2 and siSTAT3, respectively, led to a decreased secretion of IL-6 (siJAK2: 32.8 ± 3.96 pg/mL; siSTAT3: 39.6 ± 4.02 pg/mL) and TGF-β1 (siJAK2: 54.7 ± 9.11 pg/mL; siSTAT3: 60.5 ± 7.26 pg/mL). These results suggest that inhibiting the JAK2/STAT3 signaling pathway may prevent inflammation by reducing IL-6 and TGF-β1 levels in pancreatic acinar cells.

Reduced JAK2 or STAT3 Expression Decreased Cell Death in Cerulein-Treated AR42J Cells

To further confirm the role of the JAK2/STAT3 pathway on cell death in response to acute pancreatitis, we investigated the apoptosis of AR42J cell treated with cerulein. Compared with control siRNA group, apoptosis level was significantly

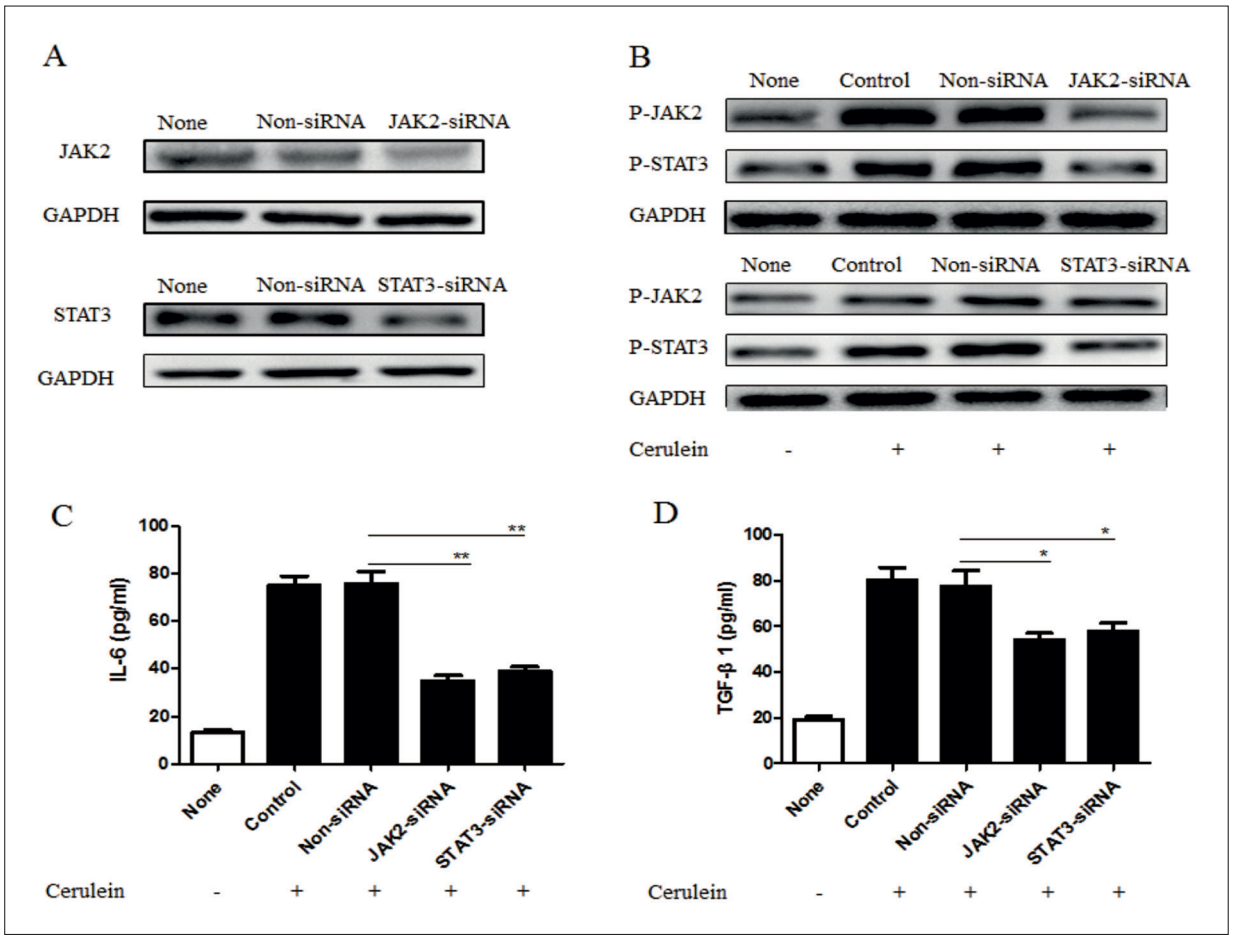

Figure 3. The JAK2/STAT3 signaling pathway is required for cerulein-induced cytokines secretion. In cerulein-treated AR42J cells, the JAK2/STAT3 signaling pathway was activated while silencing JAK2 and STAT3 expression (siRNA) reduced IL-6 and TGF-β1 production. ***A,*** Expression of JAK2 and STAT3 as determined by Western blot. ***B,*** Expression of p-JAK2 and p-STAT3 as determined by Western blot. ***C,*** The level of IL-6 in the medium after treatment with JAK2 and STAT3 siRNA, $^{**}p < 0.01$. ***D,*** The level of TGF-β1 in the medium after treatment with JAK2 and STAT3 siRNA, $^{**}p < 0.01$.

reduced in siJAK2 and siSTAT3 groups (Figure 4A, 4B). Western blot analysis of the expression of cleaved caspase-3 revealed that its protein levels were significantly decreased in siJAK2 and siSTAT3 groups compared to the control siRNA groups (Figure 4C, 4D). Additionally, the release of LDH was also decreased in siJAK2 and siSTAT3 groups (Figure 4E). The results implied that down-regulation of JAK2 and STAT3 may decrease apoptosis induced by cerulein.

Discussion

Acute pancreatitis (AP) is an inflammatory disease characterized by premature activation of zymogens leading to acinar cell injury[21-24] and the release of chemokines and cytokines that trigger an inflammatory response. Pain is the major symptom of AP which usually resolves within 1 week[25-27]. However, severe AP may cause tissue auto-digestion, multiorgan failure and even death[28-30]. In the present work, cerulein significantly induced cytokines (IL-6 and TGF-β1) production, cell death and activation of the JAK2/STAT3 pathway 6 h after cerulein treatment in AR42J cells. Using siJAK2 and siSTAT3 to knockdown JAK2 and STAT3 gene expression, we showed that the level of IL-6 and TGF-β1 significantly decreased, and the expression of cleaved caspase-3 and release of LDH were also decreased in siJAK2 or siSTAT3 groups.

Since AP is an inflammatory condition, it is important to explore the molecular mechanisms

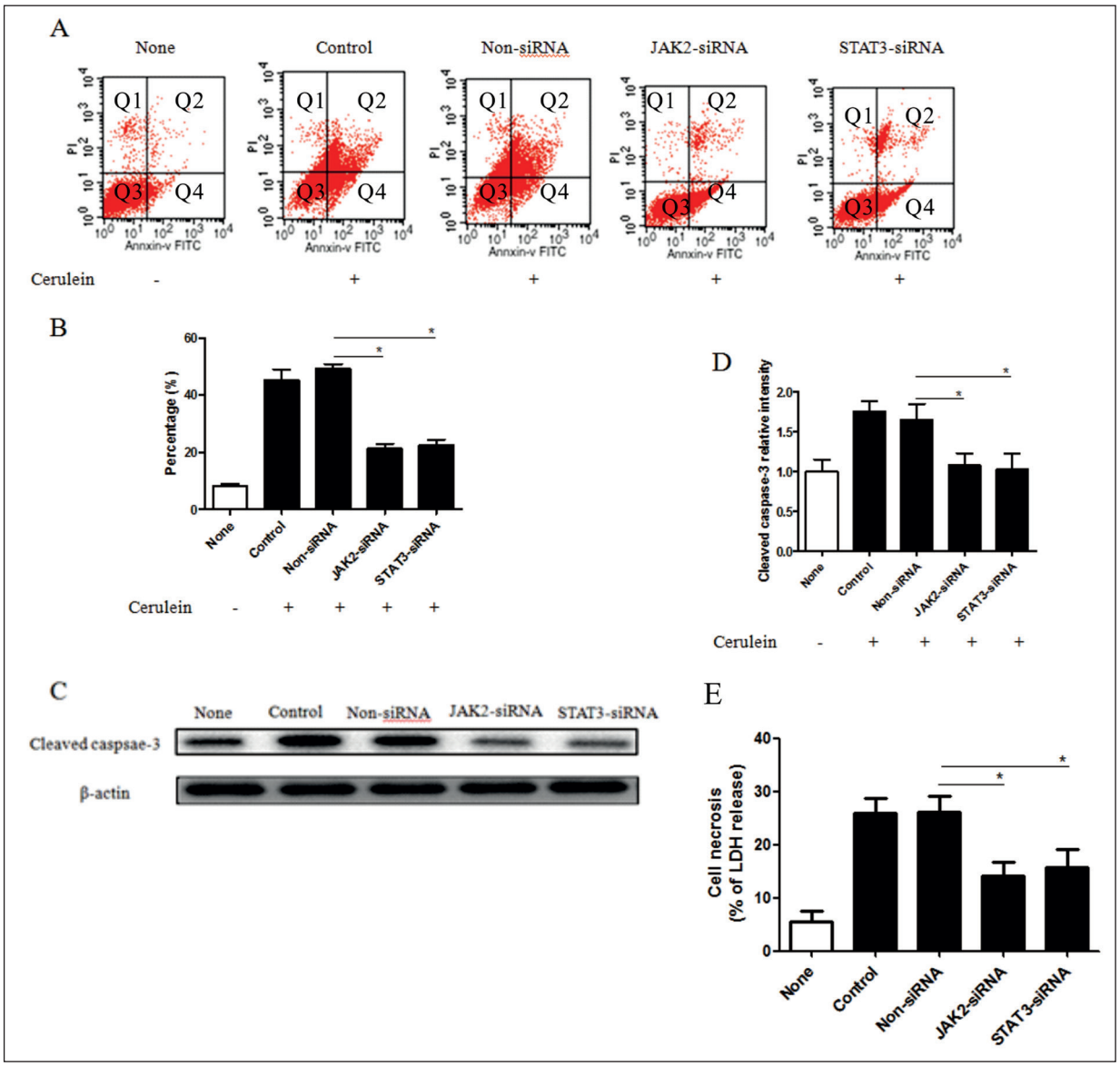

Figure 4. Effects of JAK2-siRNA and STAT3-siRNA on cell apoptosis and necrosis in cerulein-treated AR42J cells. ***A,*** Cell apoptosis as measured by flow cytometry: apoptotic cells are presented in the lower right quadrant of the figure (Q4), dead cells are shown in the upper right quadrant (Q2), living cells are shown in the lower left quadrant (Q3), and cell debris are shown in the upper left quadrant (Q1). ***B,*** Percentage of cell apoptosis and necrosis in cerulein-treated AR42J cells, $*p < 0.05$, $**p < 0.01$. ***C,*** Expression of cleaved caspase-3 as determined by Western blot. ***D,*** Density ratio of cleaved caspase-3, $*p < 0.05$, $**p$ 0.01. ***E,*** LDH release in medium, $*p < 0.05$.

of inflammation in the progress of AP[5,31]. The JAK/STAT (signal transducer and activator of transcription) cascade is an essential inflammatory signaling pathway that modulates immune responses. STAT targeted genes include cytokines, adhesion molecules, and other inflammatory mediators[32-34]. In this study, we found that cerulein directly activated JAK2 and STAT3 proteins and markedly increased the production of cytokines IL-6 and TGF-β1. IL-6 is a well-known proinflammatory cytokine which reflects the severity of pancreatitis. In the pancreas, TGF-β1 regulates the remodeling of the extracellular matrix and is considered to be an important contributing factor to the pathogenesis of pancreatitis[35]. The TGF-β1 expression is enhanced in human chronic pancreatitis. Fibrosis and inflammation were found in the pancreas of transgenic mice

overexpressing TGF-β1. Therefore, the expression of IL-6 and TGF-β1 is important to the development of pancreatitis[36]. Interestingly, blocking JAK2 and STAT3 decreased the expression of IL-6 and TGF-β1 significantly. Hence, we demonstrated that the JAK2/STAT3 pathway is required for inflammatory responses in cerulean-treated AR42J cells.

The development of acute pancreatitis is a complex process that is characterized by inflammation and parenchymal cell death[5,37]. It has been shown[38,39] that the occurrence of cell death in different inflammatory diseases is not similar. As for acute pancreatitis, the severity of inflammation is directly correlated with necrosis and inversely correlated with apoptosis. Therefore, shifting death responses from necrosis to apoptosis has a therapeutic value[40]. In this research, we demonstrated that necrosis and apoptosis of AR42J cells were increased following cerulein treatment in addition to the increased expression of cleaved caspase-3 and release of LDH. Administration of JAK2 siRNA and STAT3 siRNA showed a therapeutic effect on cerulein-induced pancreatitis.

Conclusions

The activation of JAK2/STAT3 signaling pathway increased the production of cytokines and cell death which were eliminated by blocking JAK2 and STAT3 expression. Our findings indicates that the JAK2/STAT3 signaling pathway is a potential therapeutic target in the management of acute pancreatitis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Whitcomb DC. Clinical practice. Acute pancreatitis. N Engl J Med 2006; 354: 2142-2150.
2) Li H, Qian Z, Liu Z, Liu X, Han X, Kang H. Risk factors and outcome of acute renal failure in patients with severe acute pancreatitis. J Crit Care 2010; 25: 225-229.
3) Papachristou GI, Clermont G, Sharma A, Yadav D, Whitcomb DC. Risk and markers of severe acute pancreatitis. Gastroenterol Clin North Am 2007; 36: 277-296.
4) Sarmiento N, Sanchez-Bernal C, Ayra M, Perez N, Hernandez-Hernandez A, Calvo JJ, Sanchez-Yague J. Changes in the expression and dynamics of SHP-1 and SHP-2 during cerulein-induced acute pancreatitis in rats. Biochim Biophys Acta 2008; 1782: 271-279.
5) Kim H. Cerulein pancreatitis: oxidative stress, inflammation, and apoptosis. Gut Liver 2008; 2: 74-80.
6) Lerch MM, Gorelick FS. Models of acute and chronic pancreatitis. Gastroenterology 2013; 144: 1180-1193.
7) Sarmiento N, Sanchez-Bernal C, Perez N, Sardina JL, Mangas A, Calvo JJ, Sanchez-Yague J. Rolipram and SP600125 suppress the early increase in PTP1B expression during cerulein-induced pancreatitis in rats. Pancreas 2010; 39: 639-645.
8) Mareninova OA, Sung KF, Hong P, Lugea A, Pandol SJ, Gukovsky I, Gukovskaya AS. Cell death in pancreatitis: caspases protect from necrotizing pancreatitis. J Biol Chem 2006; 281: 3370-3381.
9) Garg PK, Madan K, Pande GK, Khanna S, Sathyanarayan G, Bohidar NP, Tandon RK. Association of extent and infection of pancreatic necrosis with organ failure and death in acute necrotizing pancreatitis. Clin Gastroenterol Hepatol 2005; 3: 159-166.
10) Bettaieb A, Xi Y, Hosein E, Coggins N, Bachaalany S, Wiede F, Perez S, Griffey SM, Sastre J, Tiganis T, Haj FG. Pancreatic T cell protein-tyrosine phosphatase deficiency ameliorates cerulein-induced acute pancreatitis. Cell Commun Signal 2014; 12: 13.
11) Vaquero E, Gukovsky I, Zaninovic V, Gukovskaya AS, Pandol SJ. Localized pancreatic NF-kappaB activation and inflammatory response in taurocholate-induced pancreatitis. Am J Physiol Gastrointest Liver Physiol 2001; 280: G1197-G1208.
12) Kubisch CH, Sans MD, Arumugam T, Ernst SA, Williams JA, Logsdon CD. Early activation of endoplasmic reticulum stress is associated with arginine-induced acute pancreatitis. Am J Physiol Gastrointest Liver Physiol 2006; 291: G238-G245.
13) Liu F, Zhao X, Perna F, Wang L, Koppikar P, Abdel-Wahab O, Harr MW, Levine RL, Xu H, Tefferi A, Deblasio A, Hatlen M, Menendez S, Nimer SD. JAK2V617F-mediated phosphorylation of PRMT5 downregulates its methyltransferase activity and promotes myeloproliferation. Cancer Cell 2011; 19: 283-294.
14) Yu JH, Kim KH, Kim H. SOCS 3 and PPAR-gamma ligands inhibit the expression of IL-6 and TGF-beta1 by regulating JAK2/STAT3 signaling in pancreas. Int J Biochem Cell Biol 2008; 40: 677-688.
15) Griffiths DS, Li J, Dawson MA, Trotter MW, Cheng YH, Smith AM, Mansfield W, Liu P, Kouzarides T, Nichols J, Bannister AJ, Green AR, Gottgens B. LIF-independent JAK signalling to chromatin in embryonic stem cells uncovered from an adult stem cell disease. Nat Cell Biol 2011; 13: 13-21.

16) Berishaj M, Gao SP, Ahmed S, Leslie K, Al-Ahmadie H, Gerald WL, Bornmann W, Bromberg JF. Stat3 is tyrosine-phosphorylated through the interleukin-6/glycoprotein 130/Janus kinase pathway in breast cancer. Breast Cancer Res 2007; 9: R32.

17) Bromberg J, Wang TC. Inflammation and cancer: IL-6 and STAT3 complete the link. Cancer Cell 2009; 15: 79-80.

18) Grivennikov S, Karin E, Terzic J, Mucida D, Yu GY, Vallabhapurapu S, Scheller J, Rose-John S, Cheroutre H, Eckmann L, Karin M. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. Cancer Cell 2009; 15: 103-113.

19) Zhu S, Zhang C, Weng Q, Ye B. Curcumin protects against acute renal injury by suppressing JAK2/STAT3 pathway in severe acute pancreatitis in rats. Exp Ther Med 2017; 14: 1669-1674.

20) Shields BJ, Wiede F, Gurzov EN, Wee K, Hauser C, Zhu HJ, Molloy TJ, O'Toole SA, Daly RJ, Sutherland RL, Mitchell CA, McLean CA, Tiganis T. TCPTP regulates SFK and STAT3 signaling and is lost in triple-negative breast cancers. Mol Cell Biol 2013; 33: 557-570.

21) Baron TH, Morgan DE. Acute necrotizing pancreatitis. N Engl J Med 1999; 340: 1412-1417.

22) Jin Y, Xu H, Chen Y, Wu J, Jin F, Wu Q, Yao XM. Therapeutic effect of Bifidobacterium combined with early enteral nutrition in the treatment of severe acute pancreatitis: a pilot study. Eur Rev Med Pharmacol Sci 2018; 22: 4018-4024.

23) Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. Gastroenterology 2013; 144: 1252-1261.

24) Roberts SE, Akbari A, Thorne K, Atkinson M, Evans PA. The incidence of acute pancreatitis: impact of social deprivation, alcohol consumption, seasonal and demographic factors. Aliment Pharmacol Ther 2013; 38: 539-548.

25) Singh VK, Moran RA, Afghani E, De-Madaria E. Treating acute pancreatitis: what's new? Expert Rev Gastroenterol Hepatol 2015; 9: 901-911.

26) Saluja AK, Donovan EA, Yamanaka K, Yamaguchi Y, Hofbauer B, Steer ML. Cerulein-induced in vitro activation of trypsinogen in rat pancreatic acini is mediated by cathepsin B. Gastroenterology 1997; 113: 304-310.

27) Naruse S. Molecular pathophysiology of pancreatitis. Intern Med 2003; 42: 288-289.

28) Lerch MM, Gorelick FS. Models of acute and chronic pancreatitis. Gastroenterology 2013; 144: 1180-1193.

29) Jensen RT, Wank SA, Rowley WH, Sato S, Gardner JD. Interaction of CCK with pancreatic acinar cells. Trends Pharmacol Sci 1989; 10: 418-423.

30) Willemer S, Elsasser HP, Adler G. Hormone-induced pancreatitis. Eur Surg Res 1992; 24 Suppl 1: 29-39.

31) Yu JH, Kim KH, Kim H. Suppression of IL-1beta expression by the Jak 2 inhibitor AG490 in cerulein-stimulated pancreatic acinar cells. Biochem Pharmacol 2006; 72: 1555-1562.

32) Yang X, He G, Hao Y, Chen C, Li M, Wang Y, Zhang G, Yu Z. The role of the JAK2-STAT3 pathway in pro-inflammatory responses of EMF-stimulated N9 microglial cells. J Neuroinflammation 2010; 7: 54.

33) Yu JH, Kim H. Role of janus kinase/signal transducers and activators of transcription in the pathogenesis of pancreatitis and pancreatic cancer. Gut Liver 2012; 6: 417-422.

34) Horiguchi A, Asano T, Kuroda K, Sato A, Asakuma J, Ito K, Hayakawa M, Sumitomo M, Asano T. STAT3 inhibitor WP1066 as a novel therapeutic agent for renal cell carcinoma. Br J Cancer 2010; 102: 1592-1599.

35) Brands MW, Banes-Berceli AK, Inscho EW, Al-Azawi H, Allen AJ, Labazi H. Interleukin 6 knockout prevents angiotensin II hypertension: role of renal vasoconstriction and janus kinase 2/signal transducer and activator of transcription 3 activation. Hypertension 2010; 56: 879-884.

36) Ju KD, Lim JW, Kim KH, Kim H. Potential role of NADPH oxidase-mediated activation of Jak2/Stat3 and mitogen-activated protein kinases and expression of TGF-beta1 in the pathophysiology of acute pancreatitis. Inflamm Res 2011; 60: 791-800.

37) Gukovskaya AS, Gukovsky I, Jung Y, Mouria M, Pandol SJ. Cholecystokinin induces caspase activation and mitochondrial dysfunction in pancreatic acinar cells. Roles in cell injury processes of pancreatitis. J Biol Chem 2002; 277: 22595-22604.

38) Gunther C, Neumann H, Neurath MF, Becker C. Apoptosis, necrosis and necroptosis: cell death regulation in the intestinal epithelium. Gut 2013; 62: 1062-1071.

39) Jilling T, Lu J, Jackson M, Caplan MS. Intestinal epithelial apoptosis initiates gross bowel necrosis in an experimental rat model of neonatal necrotizing enterocolitis. Pediatr Res 2004; 55: 622-629.

40) Mareninova OA, Sung KF, Hong P, Lugea A, Pandol SJ, Gukovsky I, Gukovskaya AS. Cell death in pancreatitis: caspases protect from necrotizing pancreatitis. J Biol Chem 2006; 281: 3370-3381.