European Review for Medical and Pharmacological Sciences 2019; 23: 10123-10131

Effect of SOCS3 on lung injury in rats with severe acute pancreatitis through regulating JAK2/STAT3 signaling pathway

M.-Z. QIN[1], M.-B. QIN[2], Z.-H. LIANG[1], G.-D. TANG[1]

[1]Department of Gastroenterology, The First Affiliated Hospital of Guangxi Medical University, Nanning, China
[2]Department of Gastroenterology, The Second Affiliated Hospital of Guangxi Medical University, Nanning, China

Abstract. – OBJECTIVE: To explore the effect of suppressor of cytokine signaling 3 (SOCS3) on the lung injury in rats with severe acute pancreatitis (SAP) by regulating the Janus kinase 2/ signal transducer and activator of transcription 3 (JAK2/STAT3) pathway.

MATERIALS AND METHODS: Sprague-Dawley rats were divided into control group (n=20) and SAP model group (established *via* injection of 5% sodium taurocholate, n=40). Then, SOCS3 was overexpressed using the adenovirus in 20 rats in SAP model group. The serum amylase (AMY) was detected, whether the transfection was successful was verified *via* quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR), the hepatic function indexes were detected, the pathological changes were observed using hematoxylin-eosin (HE) staining, and the wet/dry weight ratio (W/D) was calculated. Moreover, the content of serum inflammatory factors was detected *via* enzyme-linked immunosorbent assay (ELISA) and the expression levels of JAK2/STAT3 signaling pathway genes and proteins were detected through RT-PCR and Western blotting.

RESULTS: The content of AMY in SAP model group was significantly increased, indicating the successful modeling. SOCS3 was significantly increased in transfection group, suggesting that the transfection efficiency was significant. The content of alanine aminotransferase (ALT), and aspartate aminotransferase (AST) in SOCS3 transfection group was significantly lower than in model group. According to the histopathological observation, there were lung injury, pulmonary edema, hemorrhage, severe inflammatory response, and alveolar congestion in SAP model group. There were almost no pathological changes in SOCS3 transfection group. In SOCS3 transfection group, the content of serum interleukin-6 (IL-6), IL-18 and tumor necrosis factor-α (TNF-α), the mRNA and protein expressions of IL-6, JAK2, and STAT3 were all remarkably declined.

CONCLUSIONS: SOCS3 inhibits the activation of the JAK2/STAT3 pathway and the increase of inflammatory factors, promoting the repair of lung injury in SAP rats.

Key Words:
SOCS3, JAK2/STAT3 Signaling pathway, Severe acute pancreatitis, Rats, Lung injury.

Introduction

Severe acute pancreatitis (SAP) is an abnormally severe abdominal organ disorder with a high mortality rate, in which pancreatic elastase and other pro-inflammatory mediators are released into the portal vein and systemic circulation[1,2]. Although the precise aggressive mechanisms of different etiological factors remain unclear, researchers have been working on the exact mechanism of action. Lung injury reduces the gas exchange and causes the oxidative damage, and the protein-rich exudates enter the alveolar space and interstitial tissue[3,4]. The excessive penetration of neutrophils into the lungs has been identified as a key event in acute lung injury (ALI)[5]. The main events in the inflammatory response include the migration of leukocytes from the blood and increased adhesion of endothelial cells[6,7]. At present, the treatment means of SAP mainly focus on the antagonism to such early inflammatory factors as IL-6, but the efficacy is not satisfactory. Therefore, it is urgent to find a new generation of drugs with ideal therapeutic effects. However, the etiology and pathogenesis of ALI are complex, neither specific or effective preven-

Corresponding Author: Guodu Tang, MD; e-mail: tguodu02@126.com

tion strategy nor appropriate treatment for ALI has been found, and the therapeutic mechanism of ALI needs further study. Therefore, deeply understanding its regulatory network is essential for the treatment of ALI in SAP.

The Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway has been widely studied[8]. The JAK/STAT pathway is activated by growth factors and hormones, displaying the advantages and protective effects of JAK2 and STAT3 in inflammatory response[9,10]. Fan et al[11] have demonstrated that soluble factors are dependent on STAT3 in the liver during systemic inflammation. Interleukin-6 (IL-6) is a pro-inflammatory cytokine that can selectively activate STAT3, which plays an important role in the initiation and amplification of the inflammatory process[12]. Furthermore, the exact mechanism of JAK2/STAT3 pathway inhibitors in SAP is related to the severity of the pancreatic disease. Suppressor of cytokine signaling 3 (SOCS3) mediates the inflammatory response induced by interferon (IFN)[13]. The ability of SOCS3 to regulate the pro-inflammatory cytokines has aroused much interest[14,15]. However, the mechanism of SOCS3 in regulating the production of pro-inflammatory cytokines remains unclear, the regulatory function and effect of SOCS3 in lung injury in SAP have not been clarified, and its specific mechanism needs further exploring. Based on this, the effect of SOCS3 on lung injury in SAP rats through the JAK2/STAT3 pathway and its specific mechanism were explored in the present study.

This work aims to investigate the effect of SOCS3 on lung injury in SAP rats through the JAK2/STAT3 pathway and its specific mechanism of action. The effect of SOCS3 on lung injury in SAP rats was clarified *via* adenovirus transfection in *in vivo* experiments and various molecular biological techniques. Finally, the specific molecular mechanism of this effect was explored to reveal the regulatory effect of SOCS3 on lung injury in SAP rats.

Materials and Methods

Animal Grouping and Modeling

Sprague-Dawley rats were divided into control group (n=20) and SAP model group (established with 5% sodium taurocholate, 1 mL/kg, n=40). Then, SOCS3 was overexpressed using the adenovirus in 20 rats in SAP model group. SOCS3 complementary deoxyribonucleic acid (cDNA) was used for the gene-specific primer amplification. After purification, the reaction product was ligated to the vector fragment under the action of T4 DNA and the competent cells were transformed using the ligation product. The adenovirus vector containing SOCS3 was transfected into the rats. All the experimental schemes and methods were approved by the Laboratory Animal Ethics Committee. The serum and an appropriate number of lung tissues were collected and stored for later use.

Detection of Successful Establishment of Animal Model

The serum amylase (AMY) can indicate the occurrence of pancreatitis and provide a reference for the treatment of pancreatitis. The successful establishment of SAP model in this experiment is crucial for the subsequent studies and serum AMY can be used as a detection index for the successful establishment of SAP model. Therefore, after modeling, the blood was taken from the caudal vein and centrifuged to separate the serum. Then, the level of serum AMY was measured, and the changes in AMY were analyzed to determine whether the model was successfully established.

Detection of Transfection Efficiency of SOCS3 Adenovirus

To deeply study the role of SOCS3 in SAP, SOCS3 was transfected into the rats using the adenovirus. Next, the transfection efficiency of SOCS3 in SAP was detected *via* reverse transcription-polymerase chain reaction (RT-PCR) to prepare for the following study of the molecular mechanism of SOCS3 in SAP.

Detection of Serum Hepatic Function Indexes and Determination of Lung W/D

To predict the occurrence of SAP in advance in clinical practice, the hepatic function indexes such as alanine aminotransferase (ALT) were detected. The blood was centrifuged to separate the serum and the serum was placed into the centrifuge tube and detected using a biochemical analyzer. Besides, the water and stains on the surface of lung tissues were sucked dry using the filter paper and the wet weight (W) was measured and recorded using an electronic balance. Then, the lung tissues were carefully baked in a dry box

at 65°C until the weight was not changed, and the dry weight (D) was measured and recorded. Finally, the W/D ratio was calculated.

Observation of Changes in Lung Tissues Via Hematoxylin-Eosin (HE) Staining

The integrity of lung tissues should be ensured before the experiment for the convenience of section preparation. The excised lung tissues previously obtained were soaked in formalin for 5 days, washed with running water for 36 h, dehydrated with gradient alcohol, routinely sliced into sections (about 5 μm in thickness) and deparaffinized, followed by hydration with 95%, 90%, 80%, 75%, and 50% ethanol, respectively. Next, the sections were transparentized, immersed and embedded in paraffin and also prepared into pathological sections. Once the sections were baked dry, they were stained with HE and sealed (Boster, Wuhan, China), followed by tissue observation under a light microscope.

Detection of Content of Inflammatory Factors Via ELISA

The serum samples stored at –80°C were taken out, thawed, and centrifuged at low speed. Then, the supernatant was collected and simply treated and the changes in each index were detected using the kit according to the instructions. Finally, the absorbance was measured using a microplate reader, based on which the content of inflammatory factors in each group was detected.

Detection of Expressions of Related Genes Via RT-PCR

(1) Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA), and the RNA concentration was detected qualified. (2) Then, the RNA was reversely transcribed into cDNA using the RT kit (Invitrogen, Carlsbad, CA, USA), followed by primer amplification using the 20 μL system (2 μL of cDNA, 10 μL of mix, 2 μL of primer, 6 μL of ddH_2O, for a total of 40 cycles). The sequences of target genes and the internal reference glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were designed according to those published in the GenBank (Table I). The expression levels of target genes were detected *via* PCR and the relative expression levels of the related genes were calculated using the $2^{-\Delta\Delta Ct}$ method.

Western Blotting

The ratio of lysis buffer was calculated according to the instructions of the protein extraction kit (Beyotime, Shanghai, China). Next, 300 mg of sterile tissues were accurately weighed, placed into a 10 mL Eppendorf (EP) tube, ground under low temperature, rapidly smashed using a homogenizer under low temperature, and added with the protein lysis buffer previously prepared, followed by centrifugation. The supernatant was collected and placed into the EP tube, followed by detection of protein concentration according to the instructions of the bicinchoninic acid (BCA) kit. Western blotting was performed: the protein was loaded for electrophoresis, transferred onto a membrane, incubated with the primary antibody and anti-rabbit secondary antibody. Finally, the protein band was scanned and quantified and the gray value of the band was analyzed.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 21.0 software (IBM, Armonk, NY, USA) was used for the processing of raw experimental data and multiple comparisons were performed for the data. The experimental results obtained were expressed as mean ± standard deviation

Table I. Primer sequences in RT-PCR.

Target gene	Primer sequence (5′-3′)
GAPDH	F: 5'-TGACTTCAACAGCGACACCCA-3' R: 5'-CACCCTGTTGCTGTAGCCAAA-3'
JAK2	F: 5'-GAGGGATCCATGAAATATACAAGCTAT-3' R: 5'-GACCCGTAAATCTGAAGCTAATGC-3'
SOCS3	F: 5'-GACGAATTCTTACGTTGATGCTCTCC-3' R: 5'-AATTAAGGCATCACAGTCCGAGTC-3'
STAT3	F: 5'-TTTGAAGACAGGGACCCTACACAG-3' R: 5'-TCATAGCGGCACATCTCCACA-3'
IL-6	F: 5'-AATCTGCTCTGGTCTTCTTGGAG-3' R: 5'-GTTGGATGGTCTTGGTCCTTAG-3'

($\chi \pm SD$) and $p<0.05$ suggested the significant difference. The bar graph was plotted using the GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA, USA).

Results

Successful Establishment of Animal Model Detected

The content of serum AMY in the caudal vein was directly measured to verify whether the SAP model was successfully established. As shown in Figure 1, the content of serum AMY in SAP model group was significantly higher than in healthy rats ($p<0.05$).

Transfection Efficiency of SOCS3 Adenovirus

To deeply explore the role of SOCS3 in lung tissues in SAP, SOSC3 was overexpressed in rats using the adenovirus transfection technique. Then, the transfection efficiency of SOSC3 was detected *via* PCR. As shown in Figure 2, the expression level of SOSC3 was significantly increased in SOSC3 transfection group ($p<0.05$), close to that in control group.

Detection Results of Hepatic Function and W/D

The important serum biochemical indexes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and ALP play important roles in the lung disease, so their content was detected using the biochemical analyzer. As shown in Table II, the content of AST, ALT, and ALP declined significantly in SOCS3 transfection group, while it was increased significantly in model group ($p<0.05$). Besides, the changes in W/D were consistent with the above trends.

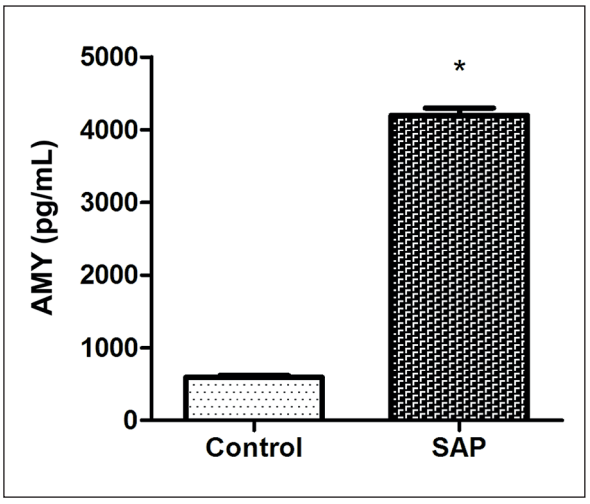

Figure 1. The content of serum AMY in SAP model group is significantly higher than that in healthy rats, suggesting the successful modeling. *$p<0.05$: there is a statistically significant difference.

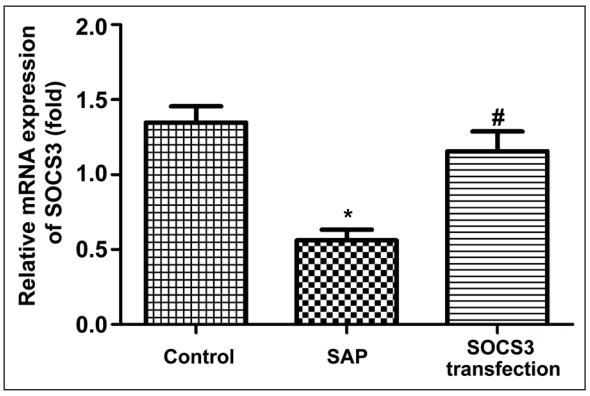

Figure 2. Transfection efficiency of SOCS3. The expression level of SOCS3 is significantly increased in SOCS3 transfection group ($p<0.05$). Control: normal control group, SAP: severe acute pancreatitis model group. There is a statistically significant difference *vs.* control group ($^{*}p<0.05$) and *vs.* model group ($^{\#}p<0.05$).

Changes in Lung Tissues Observed Via HE Staining

As shown in Figure 3, there were lung injury, pulmonary edema, hemorrhage, severe inflammatory response, alveolar congestion, and cellular damage in SAP model group (3A). In SOCS3 transfection group, the cells had normal morphology and histological structure without significant changes compared to control group, and no pathological changes were observed (3B).

Serum TNF-α, IL-6, and IL-18 Content

The content of TNF-α, IL-6, and IL-18 was increased in model group ($p<0.05$), while it declined in SOSC3 transfection group, close to that in control group ($p<0.05$) (Table III).

Gene Detection Results

According to the detection results of gene expression, the mRNA expressions of IL-6, JAK2, and STAT3 remarkably declined in SOSC1 transfection group ($p<0.05$) (Figure 4), indicating that the overexpression of SOCS3 may inhibit the increase of their levels, thereby suppressing the further development of lung injury.

Table II. Changes in content of ALT, ALT, and ALP and W/D.

Group	W/D	AST (U/L)	ALP (U/L)	ALT (U/L)
Model group	10.68 ± 1.24[b]	439.24 ± 6.38[b]	225.85 ± 5.22[b]	142.83 ± 5.65[b]
SOCS3 transfection group	5.24 ± 1.03[a]	226.85 ± 2.88[a]	105.56 ± 4.24[a]	67.41 ± 5.67[a]
Control group	3.39 ± 0.19	219.36 ± 3.58	88.17 ± 5.84	50.47 ± 4.45

Note: The content of AST, ALT, ALP, and W/D decline significantly in SOCS3 transfection group ($p<0.05$). [b]$p<0.05$ model group *vs*. control group, [a]$p<0.05$ SOCS3 transfection group *vs*. model group (the same below).

Expressions of Important Proteins, and Pathway Proteins

To further determine the effect of SOCS3 on the JAK2/STAT3 signaling pathway during the development of lung injury, the protein expressions were detected. It was found that the expression of JAK2/STAT3 in SOCS3 transfection group remarkably declined ($p<0.05$) (Figure 5), demonstrating that the overexpression of SOCS3 can facilitate the recovery of lung injury in SAP.

Discussion

SAP, a disease characterized by acute onset and high mortality rate, often occurs in the human body. There is a wide range of pancreatic injury in the early stage of liver failure[16,17]. It has been proved that inflammatory mediators TNF-α and IL-6 play extremely important roles in regulating SAP and lung injury[18]. Lung injury is a serious complication of SAP, increasing the

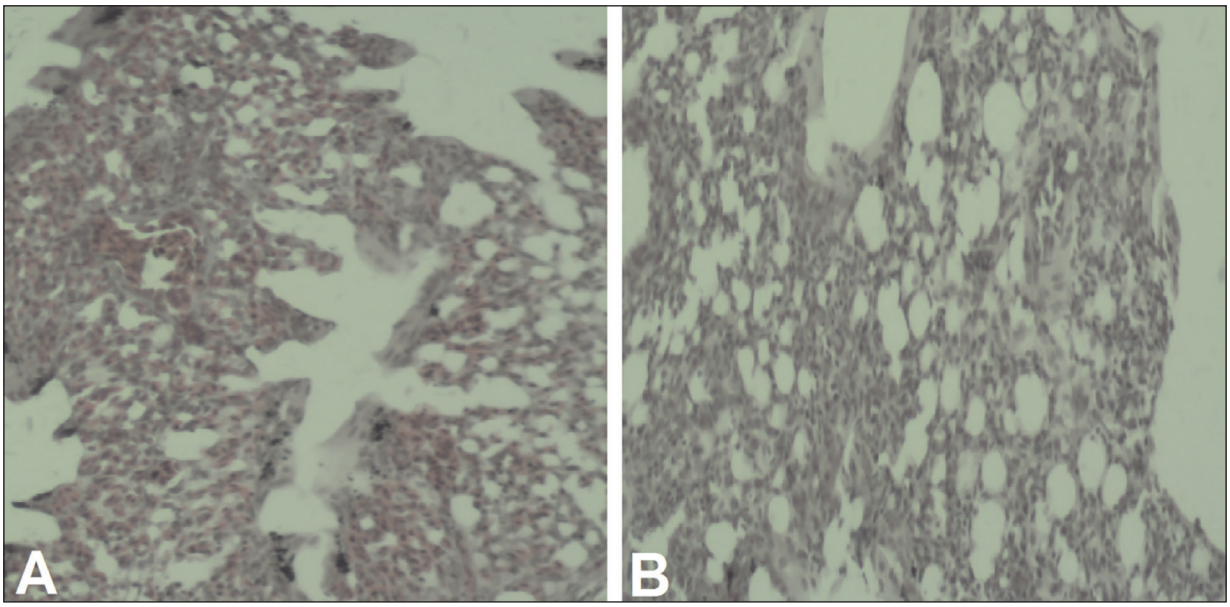

Figure 3. HE staining. There are lung injury, severe inflammatory response, alveolar congestion and cellular damage in SAP model group (**A,** magnification ×200). No pathological changes are observed in SOCS3 transfection group (**B,** magnification ×200).

Table III. Serum TNF-α, IL-6, and IL-18 content (pg/mL).

Group	TNF-α	IL-6	IL-18
Model group	252.45 ± 1.27[b]	298.45 ± 3.45[b]	400.51 ± 5.54[b]
SOCS3 transfection group	100.85 ± 2.15[a]	65.25 ± 6.47[a]	74.75 ± 3.14[a]
Control group	55.34 ± 3.73	29.15 ± 2.72	20.45 ± 5.52

Note: The content of TNF-α, IL-6, and IL-18 decline in SOSC3 transfection group ($p<0.05$) ([a,b]$p<0.05$).

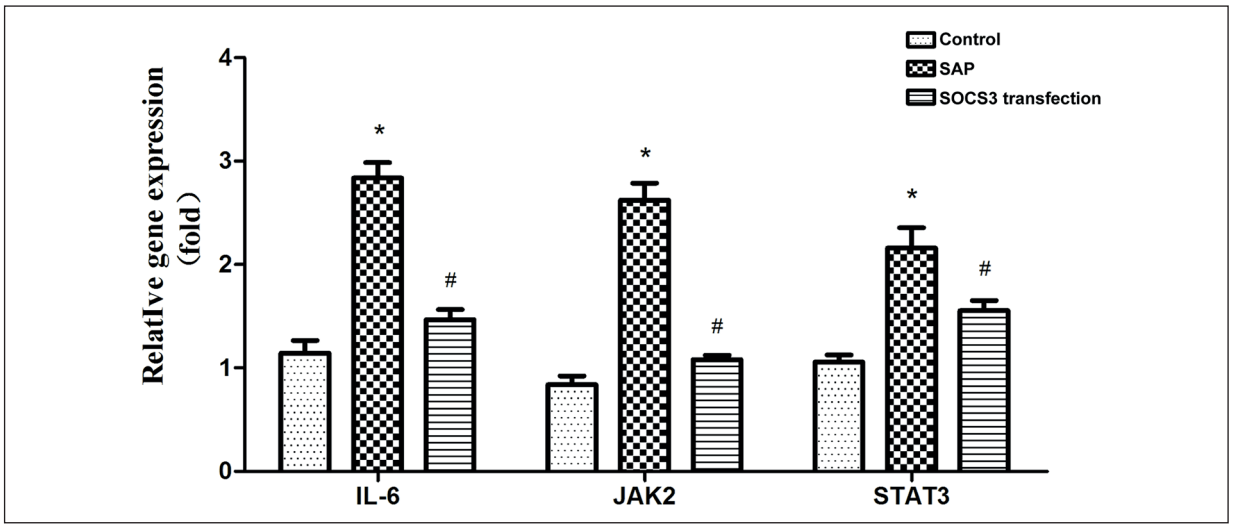

Figure 4. Detection results of gene expression. mRNA expressions of IL-6, JAK2, and STAT3 remarkably decline in SOSC1 transfection group ($p<0.05$).

risk of systemic inflammation and death[19]. The congestion of monocytes and macrophages in the local inflammatory region in lung tissues is a key event in SAP and ALI, and intercellular adhesion molecule-1 plays an important role in mediating the inflammatory response[20]. ALI is a pulmonary inflammatory injury mainly characterized by polymorphonuclear cell infiltration[21]. Lung injury in SAP is a complex and the dynamic process regulated by a variety of cellular components and cytokines, which involves the influence of multiple genes and regulatory factors on lung injury. Therefore, deeply understanding the specific molecular regulatory network of SOCS3 is essential for the treatment of lung injury. In the present investigation, the level of serum AMY in SAP model group was significantly increased, indicating that the SAP model was successfully established and could be used for subsequent studies. To further explore the role of SOCS3 in lung tissues, SOSC3 was overexpressed using the adenovirus transfection technique. Then, the transfection efficiency of SOSC3 was detected. The results showed that the expression level of SOSC3 was significantly increased in SOSC3 transfection group, and the content of AST, ALT, and ALP declined significantly, while it was increased significantly in model group ($p<0.05$). Besides, the changes in W/D were consistent with the above trends. The above findings demonstrate that the hepatic function indexes have evident changes in the case of lung injury, indicating the occurrence and development of the disease. Immune and inflammatory factors play important roles in initiating and promoting the development of SAP[22]. It has been found[23] that the level of

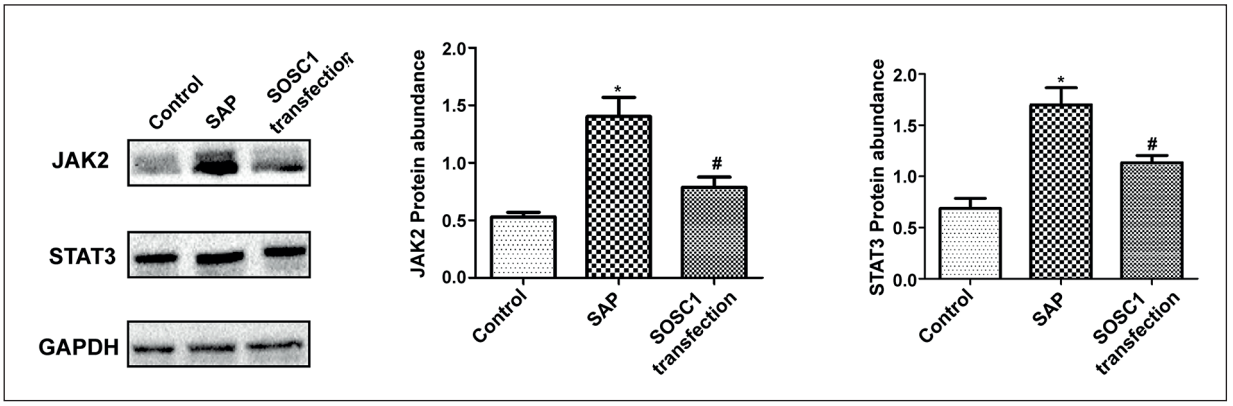

Figure 5. Protein expressions. The expression of JAK2/STAT3 in SOCS3 transfection group remarkably decline (*,#$p<0.05$).

TNF-α is significantly increased in SAP, promoting the development of lung injury. Studies have proved that inflammation plays an indispensable role in the occurrence and development of SAP. In this work, the content of TNF-α, IL-6, and IL-18 was increased in model group ($p<0.05$), suggesting that the increased levels of IL-6 and TNF-α further promote the development of SAP and further aggravate the inflammatory response. After the overexpression of SOCS3, the levels of them declined, close to or even lower than those in control group ($p<0.05$), and the condition of the disease was also improved, suggesting that SOCS3 has a good protective effect on SAP. The conclusion in this study is consistent with the above findings, which demonstrates that SOCS3 is able to inhibit excessive inflammatory cytokines, prevent the excessive production from causing irreversible damage to cells, and stimulate various anti-inflammatory substances, resisting the inflammatory injury. In addition, the morphological observation showed that there were lung injury, pulmonary edema, hemorrhage, severe inflammatory response, alveolar congestion, and cellular damage in SAP model group. In SOCS3 transfection group, the cells had normal morphology and histological structure without significant changes compared to control group, and no pathological changes were observed, consistent with previous studies[24,25].

Although Damm et al[26] have shown that the JAK/STAT pathway regulates the expression of pro-inflammatory factors during the development of SAP, little is known about its molecular mechanism in mediating ALI in SAP. The JAK/STAT signaling pathway is widely involved in inflammatory response[27]. JAK2 binds to STAT3 through cytokine receptors to activate the cytokine signaling cascade[28,29], while TNF-α activates JAK2 and STAT3 in pancreatic injury[30], blocking the JAK2/STAT3 signaling pathway to prevent the lethal effects of excessive systemic inflammatory response in SAP. However, the effect of the JAK2/STAT3 signaling pathway in SAP on systemic inflammatory response and its correlation with the severity of pancreatic disease are still unclear. Besides, Li et al[31] have shown that after injection of sodium taurocholate, JAK2 and STAT3 are activated rapidly, and their protein expressions are significantly increased, which may indicate that pro-inflammatory cytokines can regulate the JAK2/STAT3 pathway. Based on this, many genes or proteins that can regulate this pathway are expected to be potential targets for the treatment of lung injury in SAP. In the present report the mRNA expressions of IL-6, JAK2 and STAT3 remarkably declined in SOSC1 transfection group ($p<0.05$), indicating that the overexpression of SOCS3 may inhibit the increase of their levels. Moreover, the pathway proteins also significantly declined in SOCS3 transfection group, demonstrating that the overexpression of SOCS3 can facilitate the recovery of lung injury in SAP. Therefore SOCS3, as a key regulator of the JAK2/STAT3 signaling pathway, seems to be an effective target for the treatment of lung injury in SAP. Further exploration into the JAK2/STAT3 pathway can find more therapeutic targets. To sum up, the above results indicate that SOCS3 affects lung injury in SAP by down-regulating the JAK2/STAT3 signaling pathway. However, there are still some deficiencies in this study. For example, the work was conducted at a certain stage of the disease, but dynamic changes in the development of the disease were not observed so research can be carried out at multiple time points in the future.

Conclusions

After the overexpression of SOCS3, the inflammatory level significantly declined, and the levels of IL-6 mRNA and JAK2/STAT3 pathway genes and proteins are all significantly down-regulated. Therefore, SOCS3 may exert a regulatory effect on SAP and such an effect is realized mainly through the mediation of the JAK2/STAT3 pathway. This study provides a basis for the prevention and treatment of SAP.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding Acknowledgements

Natural Science Foundation of China (8156040100).

References

1) Shen HN, Lu CL, Li CY. Effect of diabetes on severity and hospital mortality in patients with acute pancreatitis: a national population-based study. Diabetes Care 2012; 35: 1061-1066.
2) Banks PA, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, Tsiotos GG, Vege SS; Acute Pancreatitis Classification Working Group. Classification

of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. Gut 2013; 62: 102-111.

3) Storme L, Aubry E, Rakza T, Houeijeh A, Debarge V, Tourneux P, Deruelle P, Pennaforte T; French Congenital Diaphragmatic Hernia Study Group. Pathophysiology of persistent pulmonary hypertension of the newborn: impact of the perinatal environment. Arch Cardiovasc Dis 2013; 106: 169-177.

4) Shields CJ, Winter DC, Redmond HP. Lung injury in acute pancreatitis: mechanisms, prevention, and therapy. Curr Opin Crit Care 2002; 8: 158-163.

5) Yuan Q, Jiang YW, Ma TT, Fang QH, Pan L. Attenuating effect of ginsenoside Rb1 on LPS-induced lung injury in rats. J Inflamm (Lond) 2014; 11: 40.

6) Rao RM, Yang L, Garcia-Cardena G, Luscinskas FW. Endothelial-dependent mechanisms of leukocyte recruitment to the vascular wall. Circ Res 2007; 101: 234-247.

7) Williams MR, Azcutia V, Newton G, Alcaide P, Luscinskas FW. Emerging mechanisms of neutrophil recruitment across endothelium. Trends Immunol 2011; 32: 461-469.

8) Boengler K, Hilfiker-Kleiner D, Drexler H, Heusch G, Schulz R. The myocardial JAK/STAT pathway: from protection to failure. Pharmacol Ther 2008; 120: 172-185.

9) O'Shea JJ, Schwartz DM, Villarino AV, Gadina M, McInnes IB, Laurence A. The JAK-STAT pathway: impact on human disease and therapeutic intervention. Annu Rev Med 2015; 66: 311-328.

10) Powers N, Srivastava A. JAK/STAT signaling is involved in air sac primordium development of Drosophila melanogaster. FEBS Lett 2019; 593: 658-669.

11) Fan J, Chen Q, Wei L, Zhou X, Wang R, Zhang H. Asiatic acid ameliorates CCl4-induced liver fibrosis in rats: involvement of Nrf2/ARE, NF- κB/IκBα, and JAK1/STAT3 signaling pathways. Drug Des Devel Ther 2018; 12: 3595-3605.

12) Chen SC, Chang YL, Wang DL, Cheng JJ. Herbal remedy magnolol suppresses IL-6-induced STAT3 activation and gene expression in endothelial cells. Br J Pharmacol 2006; 148: 226-232.

13) Chinen T, Kobayashi T, Ogata H, Takaesu G, Takaki H, Hashimoto M, Yagita H, Nawata H, Yoshimura A. Suppressor of cytokine signaling-1 regulates inflammatory bowel disease in which both IFNgamma and IL-4 are involved. Gastroenterology 2006; 130: 373-388.

14) Mansell A, Smith R, Doyle SL, Gray P, Fenner JE, Crack PJ, Nicholson SE, Hilton DJ, O'Neill LA, Hertzog PJ. Suppressor of cytokine signaling 1 negatively regulates Toll-like receptor signaling by mediating Mal degradation. Nat Immunol 2006; 7: 148-155.

15) Baetz A, Frey M, Heeg K, Dalpke AH. Suppressor of cytokine signaling (SOCS) proteins indirectly regulate toll-like receptor signaling in innate immune cells. J Biol Chem 2004; 279: 54708-54715.

16) 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.

17) Geisler F, Algül H, Riemann M, Schmid R. Questioning current concepts in acute pancreatitis: endotoxin contamination of porcine pancreatic elastase is responsible for experimental pancreatitis-associated distant organ failure. J Immunol 2005; 174: 6431-6439.

18) Jiang Y, An Y, Jiang D, Wu B, Yang Y, Sun D. TNF-α regulating interleukin-33 induces acute pancreatic inflammation in rats. Ann Clin Lab Sci 2016; 46: 54-59.

19) Wenhong D, Jia Y, Weixing W, Xiaoyan C, Chen C, Sheng X, Hao J. Zerumbone attenuates the severity of acute necrotizing pancreatitis and pancreatitis-induced hepatic injury. Mediators Inflamm 2012; 2012: 156507.

20) Zhang XH, Li ML, Wang B, Guo MX, Zhu RM. Caspase-1 inhibition alleviates acute renal injury in rats with severe acute pancreatitis. World J Gastroenterol 2014; 20: 10457-10463.

21) Castillo RL, Carrasco Loza R, Romero-Dapueto C. Pathophysiological approaches of acute respiratory distress syndrome: novel bases for study of lung injury. Open Respir Med J 2015; 9: 83-91.

22) Bertoni AG, Burke GL, Owusu JA, Carnethon MR, Vaidya D, Barr RG, Jenny NS, Ouyang P, Rotter JI. Inflammation and the incidence of type 2 diabetes: the Multi-Ethnic Study of Atherosclerosis (MESA). Diabetes Care 2010; 33: 804-810.

23) Naka T, Nishimoto N, Kishimoto T. The paradigm of IL-6: from basic science to medicine. Arthritis Res 2002; 4 Suppl 3: S233-S242.

24) Han X, Wang Y, Chen H, Zhang J, Xu C, Li J, Li M. Enhancement of ICAM-1 via the JAK2/STAT3 signaling pathway in a rat model of severe acute pancreatitis-associated lung injury. Exp Ther Med 2016; 11: 788-796.

25) Maheshwari R, Balaraman R, Sen AK, Shukla D, Seth A. Effect of concomitant administration of coenzyme Q10 with sitagliptin on experimentally induced diabetic nephropathy in rats. Ren Fail 2017; 39: 130-139.

26) Damm J, Harden LM, Gerstberger R, Roth J, Rummel C. The putative JAK-STAT inhibitor AG490 exacerbates LPS-fever, reduces sickness behavior, and alters the expression of pro- and anti-inflammatory genes in the rat brain. Neuropharmacology 2013; 71: 98-111.

27) 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.

28) Agrawal S, Gollapudi S, Su H, Gupta S. Leptin activates human B cells to secrete TNF-α, IL-6, and IL-10 via JAK2/STAT3 and p38MAPK/ERK1/2 signaling pathway. J Clin Immunol 2011; 31: 472-478.

29) Wu L, Li H, Zheng SZ, Liu X, Cai H, Cai BC. Da-Huang-Fu-Zi-Tang attenuates liver injury in rats with severe acute pancreatitis. J Ethnopharmacol 2013; 150: 960-966.

30) Robinson K, Vona-Davis L, Riggs D, Jackson B, McFadden D. Peptide YY attenuates STAT1 and STAT3 activation induced by TNF-alpha in acinar cell line AR42J. J Am Coll Surg 2006; 202: 788-796.

31) Li M, Zhang X, Wang B, Xu X, Wu X, Guo M, Wang F. Effect of JAK2/STAT3 signaling pathway on liver injury associated with severe acute pancreatitis in rats. Exp Ther Med 2018; 16: 2013-2021.