

Macrophages-derived p38 α promotes the experimental severe acute pancreatitis by regulating inflammation and autophagy

Hui-Ning Fan^{a,1}, Wei Chen^{a,1}, Li-Na Fan^b, Jing-Tong Wu^c, Jin-Shui Zhu^{a,*}, Jing Zhang^{a,*}

^a Department of Gastroenterology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai 200233, China

^b Department of Gastroenterology, Zhongshan Hospital, Xiamen University, Xiamen 361004, China

^c Department of Gastroenterology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou 310000, China

ARTICLE INFO

Keywords:

Acute pancreatitis
P38 α
Macrophages
Inflammation
ULK1
Autophagy

ABSTRACT

Background: Severe acute pancreatitis (SAP) is a common threat to human health. In the present study, we aimed to investigate the underlying mechanisms by which p38 α in macrophages contributes to SAP. We used conditional knockout of p38 α in macrophages and p38 MAPK inhibitors to understand the effects of p38 α in macrophages on caerulein-induced inflammatory responses in SAP mice models.

Methods and materials: Wild-type (WT) mice were randomly divided into three groups: a control group, SAP group, and SAP + p38MAPK inhibitor (SB203580) group, and mice with a conditional knockout (KO) of p38 α in macrophages were included in a KO + SAP group. We evaluated pancreatic pathology and ultra-structure by hematoxylin and eosin staining and transmission electron microscopy. The pulmonary wet-to-dry weight ratio was calculated. The serum levels of TNF- α and IL-1 β were determined by ELISA. The mRNA and protein expression of inflammatory cytokines TNF- α , IL-1 β , IL-17, IL-18, MIF, and MCP-1 in pancreatic tissues were tested by qRT-PCR and immunohistochemistry analysis. The protein expression of p38, caspase-1, ULK1, LC3B and p62 in pancreatic tissues was examined by Western blotting.

Results: The results indicated that the severity of SAP as well as the expression of the cytokines TNF- α , IL-1 β , IL-17, IL-18 and MCP-1 were higher in the SAP group than those in the control group, but were lower in the SAP + SB203580 and KO + SAP groups as compared with the SAP group. The protein expression of p38, caspase-1, LC3B and p62 was increased in the SAP group than that in the control group, but this result was reversed in the SAP + SB203580 and KO + SAP groups as compared with the SAP group. In addition, the ULK1 level was significantly lower in the SAP group than that in the control group, but was increased in the SAP + SB203580 and KO + SAP groups as compared with the SAP group.

Conclusions: Our findings demonstrated that, macrophage derived p38 α promoted the experimental severe acute pancreatitis by regulating inflammation and autophagy.

1. Introduction

Acute pancreatitis (AP) is a poorly understood inflammatory disease with an increased incidence worldwide each year [1]. Severe acute pancreatitis (SAP), a severe form of AP, is always accompanied by impaired function or multiple system and multiple organ failure [2]. Currently, the clinical prognosis of SAP is poor and the treatment options for SAP are limited. Understanding the mechanisms underlying the pathogenesis of SAP is essential for the development of effective therapies [3,4].

Monocytes/macrophages play a central role in the pathogenesis of

AP, and the activation of macrophages is associated with the severity of AP. Macrophages induce the activation of acinar cells and the systemic generation of inflammatory mediators during AP [5]. The release of proinflammatory cytokines, such as TNF- α , IL-1 β , and IL-6, is associated with activated macrophages [6]. There is increasing evidence that proinflammatory cytokines and oxidative stress are involved in the occurrence of systemic complications of SAP [7].

The activation of inflammation-related NF- κ B and mitogen-activated protein kinases (MAPKs) pathways gives rise to the production of proinflammatory cytokines [8]. p38 MAPK can be triggered by inflammatory cytokines [9] and participate in inflammatory responses

* Corresponding authors at: Department of Gastroenterology, Shanghai Jiao Tong University Affiliated Shanghai Sixth People's Hospital, No. 600 Yishan Road, Shanghai 200233, China.

E-mail addresses: zhujs1803@163.com (J.-S. Zhu), jing5522724@163.com (J. Zhang).

¹ Hui-Ning Fan and Wei Chen contributed equally to this article.

<https://doi.org/10.1016/j.intimp.2019.105940>

Received 11 June 2019; Received in revised form 23 September 2019; Accepted 26 September 2019

Available online 23 October 2019

1567-5769/© 2019 Elsevier B.V. All rights reserved.

[10]. p38 α regulates cell cycle, cell differentiation, cell death, tumorigenesis and immune responses [10–12]. Previous studies showed that LPS in macrophages induces the activation of p38 α and in turn activates the downstream cascade of proinflammatory cytokines TNF α , IL-1 and IL-6 [10,13–16]. In the present study, we used specific deletion of p38 α in macrophages or its inhibitor SB203580 to conform the essential role of p38 α in SAP.

Autophagy acts an anti-inflammatory role by regulating innate immune and inflammasome [17]. Basal autophagy maintains pancreatic acinar cell homeostasis [18]. However, impaired autophagy mediates acinar cell vacuole formation and trypsinogen activation in AP models and aggravates the progress of pancreatitis [19]. Excessive cell vacuolization and the accumulation of the lysosomal markers microtubule-associated protein 1 light chain 3 (LC3B) and p62/sequestosome1 (SQSTM1) are indicators of impaired autophagy [20]. A recent study showed that p38 α relieves autophagic control in response to inflammatory signals by inhibiting UNC51-like kinase-1 (ULK1) kinase activity during microglial inflammation [21]. However, whether p38 α in macrophages affects autophagy and inflammatory activity in SAP is still unclear.

2. Materials and methods

2.1. Chemicals and reagents

Caerulein and LPS were obtained from Yeasen (Shanghai, China). SB203580 was obtained from MCE (Shanghai, China). Anti-p38 and anti-caspase-1 antibodies were obtained from Beyotime (Shanghai, China); anti-ULK1, anti-LC3B and anti-p62 antibodies were obtained from ABClonal (Shanghai, China); and anti-TNF- α , anti-IL-1 β , anti-Monocyte chemoattractant protein-1 (MCP-1) and anti-macrophage migration inhibitory factor (MIF) antibodies were obtained from Absin (Shanghai, China). TNF- α and IL-6 enzyme-linked immunosorbent assay (ELISA) kits were obtained from eBioscience (San Diego, CA, USA).

2.2. Animals

Fifteen healthy male C57BL/6 mice weighing 20–22 g were provided by the animal center of Shanghai Sixth People's Hospital. Mice with a macrophage-specific deletion of p38 α were provided by Xiamen University. The p38 α floxed allele was generated by homologous recombination in embryonic stem cells (lexicon), and the first exon (containing ATG) was flanked by two loxP sites as previously described [10,22]. Animals were housed in cages under a controlled temperature of $22 \pm 1^\circ\text{C}$ and 12 h light-dark cycles and were provided by standard laboratory chow and water ad libitum for acclimatization for one week.

2.3. Experimental SAP mice models

Wild-type (WT) mice were randomly divided into three groups: control group ($n = 11$), SAP group ($n = 15$), SB203580 + SAP group (SB, $n = 11$), and mice with a conditional knockout (KO) of p38 α in macrophages were included in a KO + SAP group (KO, $n = 12$). Then, the mice received 7 hourly intraperitoneal (IP) injections of 50 $\mu\text{g}/\text{kg}$ caerulein (Sigma) in sterile saline followed by an IP injection of LPS (10 mg/kg) immediately, while the control mice were given saline. The SB203580 + SAP group was given by IP injection of SB203580 (10 mg/kg) for 30 min before the injection of caerulein. Animals were sacrificed 24 h after the injection of LPS, and the blood and tissue samples were collected. The blood samples were stored at -20°C until use. Pancreas and lung tissue samples were frozen and stored at -80°C . The other portions were fixed in 4% paraformaldehyde for histopathological examination. The experiment was approved by the Ethical and Research Committee of Shanghai Sixth People's Hospital.

Table 1

Histologic Scoring for Acute Hemorrhagic Necrotizing Pancreatitis.

Conditon	Score	Description
Edema	0	Absent
	1	Focally increased between lobules
	2	Diffusely increased between lobules
	3	Tense acini, widely separated lobules
	4	Gross lobular separation
Inflammation	0	Absent
	1	Around ductal margins
	2	In parenchyma ($< 50\%$ of lobules)
	3	In parenchyma (51–75% of lobules)
Hemorrhage	4	Massive collection, abscesses
	0	Absent
	1	Blood in parenchyma ($< 25\%$)
	2	Blood in parenchyma (25–50%)
	3	Blood in parenchyma (50–75%)
Necrosis	4	Blood in 100% of lobules
	0	Absent
	1	Periductal parenchymal destruction
	2	Focal parenchymal necrosis ($< 20\%$)
	3	Diffuse loss of lobules (20–50%)
	4	Severe loss of lobules ($> 50\%$)

2.4. Biochemical analysis

Blood was collected from mice via intracardiac puncture, and the serum was isolated for detection of subsequent amylase and lactate dehydrogenase (LDH) levels in a diagnostic laboratory. Pancreas and lung tissue samples were collected for the detection of myeloperoxidase (MPO) using commercial kits (Biovision, San Francisco, USA) following the manufacturer's guidelines.

2.5. Inflammatory cytokine assay

The concentrations of serum inflammatory cytokine TNF- α and IL-1 β were assayed by ELISA kits (eBioscience, San Diego, USA). The measurements were performed according to the manufacturer's instructions.

2.6. Calculation of pulmonary wet-to-dry weight (W/D) ratios

The blood and water on the surface of the lung were absorbed with filter paper. The wet weight (W) was immediately measured, and then the tissue was placed in an oven for 72 h at 70°C to obtain a constant weight [the dry weight (D)]. The W/D ratio of the lung tissue was measured to assess pulmonary edema.

2.7. qRT-PCR analysis

Total RNA was extracted from pancreatic tissues using TRIzol reagent (Invitrogen Life Technologies, CA, USA), and cDNAs were generated using a first-strand cDNA synthesis kit (Takara, Tokyo, Japan). qRT-PCR was performed with the ABI PRISM 7500 Fast Sequence Detection System (Applied Biosystems, Shanghai, China) using a SYBR Green PCR kit (Applied Biosystems, CA, USA). The amplification reaction conditions were as follows: 95°C for 30 sec, 60°C for 30 sec and 72°C for 60 sec. This procedure was repeated for 30 cycles. Relative mRNA expression was calculated by the comparative Ct ($2^{-\Delta\Delta\text{Ct}}$) method. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the reference gene. The primer sequences were listed as follows: TNF- α forward, 5'-CCAAAGGGATGAGAAGTTCC-3' and reverse, 5'-CTCCACTT GGTGGTTTGCTA-3'; IL-1 β forward, 5'-TTCAGGCAGGC AGTATCA-3' and reverse, 5'-GTC ACAACGAGCAGGTTA-3'; IL-18 forward, 5'-ACAACCTTGGCCGAC TTCAC-3' and reverse, 5'-GGGTTTCAG GCACTTTGAT-3'; IL-17 forward, 5'-GCTCCAGA AGGCCCTCAGA-3' and reverse, 5'-AGCTTTCCTCCGCCATTGA-3'; MIF forward, 5'-CCCAGAA

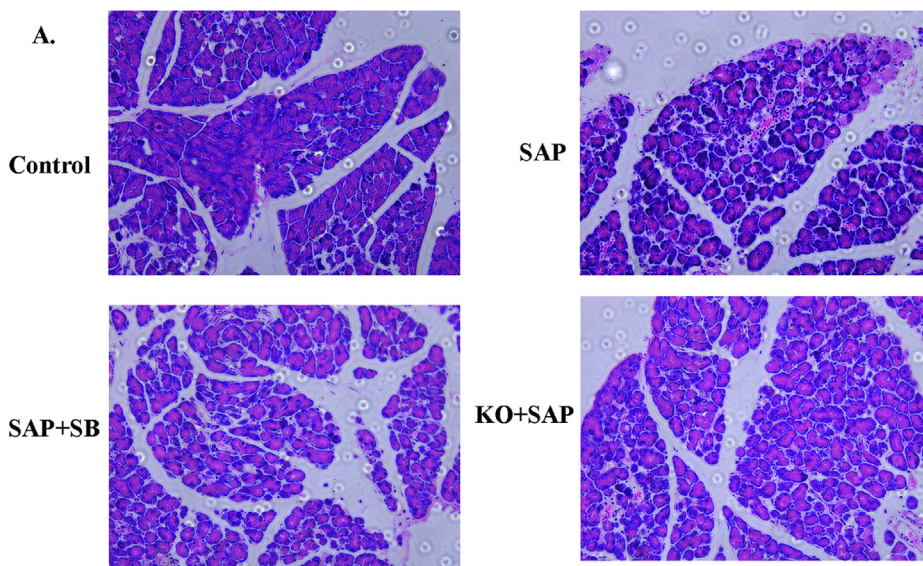
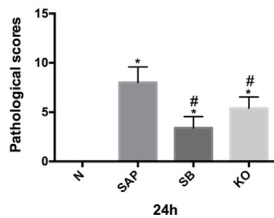


Fig. 1. p38 α KO in macrophages and SB203580 improve pancreatic histopathological injury. (A) Histological changes and pathological scores of pancreatic tissue samples from mice in the NC, SAP, SAP + SB, and SAP + KO groups at the 24-h time point (200 $\times$). (B) Pathological scores at the 24-h time point. (C). The ratio of the wet weight to the dry weight of the same lung (W/D). * P < 0.05 vs. the control group. # P < 0.05 vs. the SAP group.

B.



C.

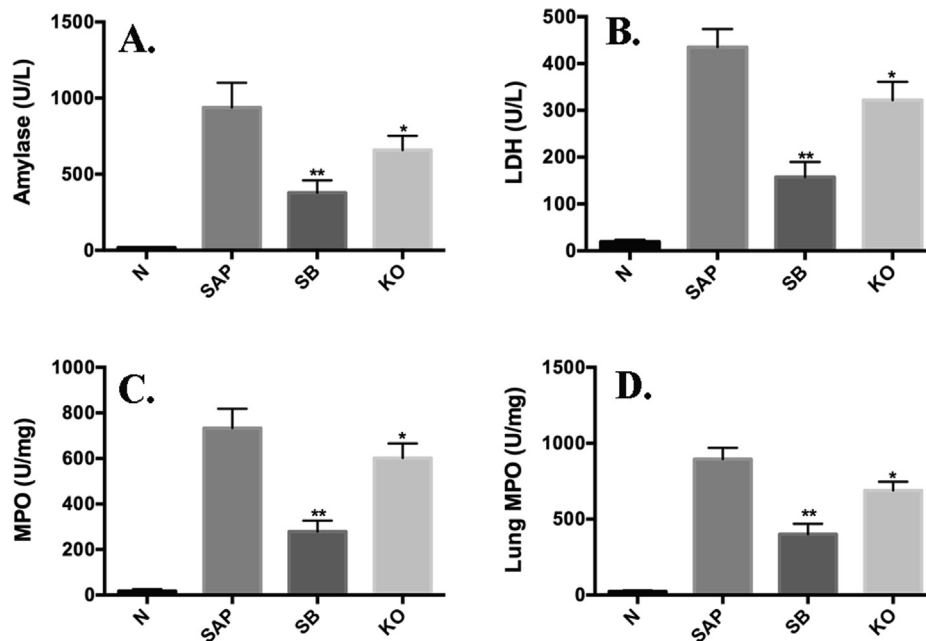
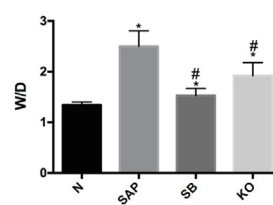


Fig. 2. p38 α KO in macrophages and SB203580 ameliorate experimental SAP. (A) Plasma amylase activity, (B) pancreatic MPO activity, (C) lung MPO activity, and (D) plasma lactate dehydrogenase (LDH) activity at 24 h following administration of caerulein. * P < 0.05 vs. the control group. ** P < 0.01 vs. the control group.

CCGCAACT ACA-3' and reverse, 5'-GAGCGAGGCTCAAAGAAC-3'; MCP-1 forward, 5'-CCCTATTCTG ATGGCACT-3' and reverse, 5'-CTATGA GAAACCCAC CACATCT-3'; and GAPDH forward, 5'-TGTGTCCGTCGT GGATCTGA-3' and reverse, 5'-CCTGCTTCAACACCTTCTTGA-3'.

2.8. Western blotting analysis

Tissues from each mouse were homogenized in an ice-cold lysis

buffer containing a cocktail of protease inhibitors. The supernatant fluid of the lysate was collected by centrifugation (4 °C, 12,000 rpm, 10 min). Proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). After electrophoresis, the proteins were electrotransferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, Boston, MA, USA). The membrane was then rinsed with a blocking solution of 5% nonfat milk for 60 min and incubated overnight at 4 °C with primary antibodies. Dilutions for

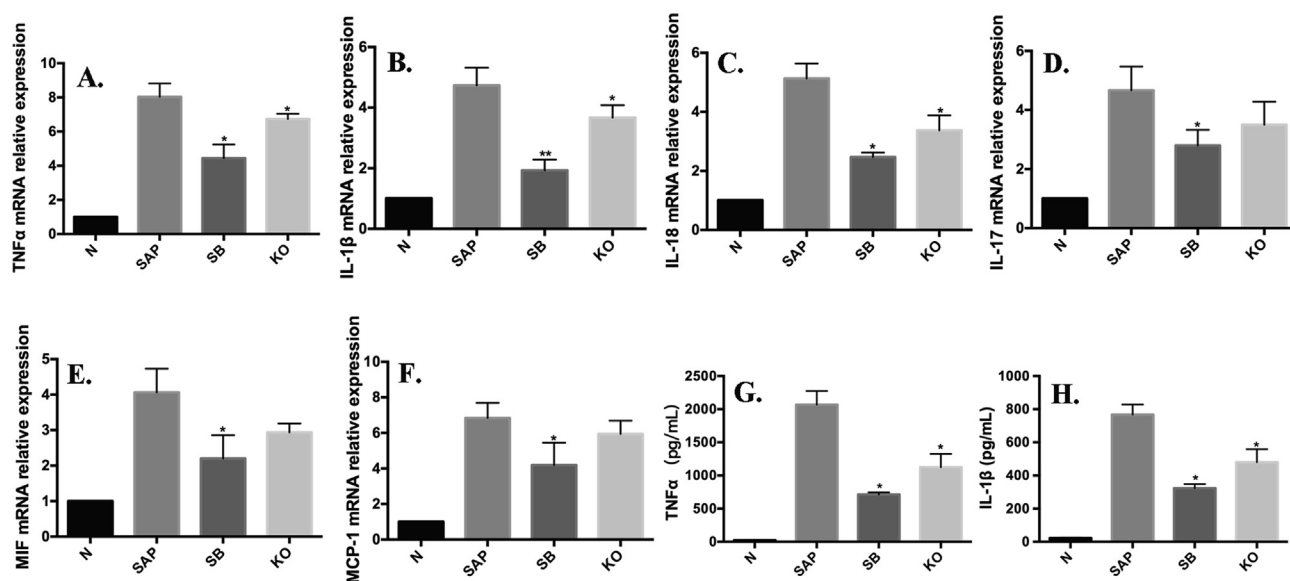


Fig. 3. p38 α KO in macrophages or SB203580 suppresses the production of proinflammatory cytokines in experimental SAP. (A-F) The mRNA expression of proinflammatory cytokines (TNF- α , IL-1 β , IL-18, IL-17, MCP-1 and MIF) in pancreatic tissue was tested by qRT-PCR assay. (G-H) The serum levels of proinflammatory cytokines were tested by ELISA. * P < 0.05 vs. the SAP group. ** P < 0.01 vs. the SAP group.

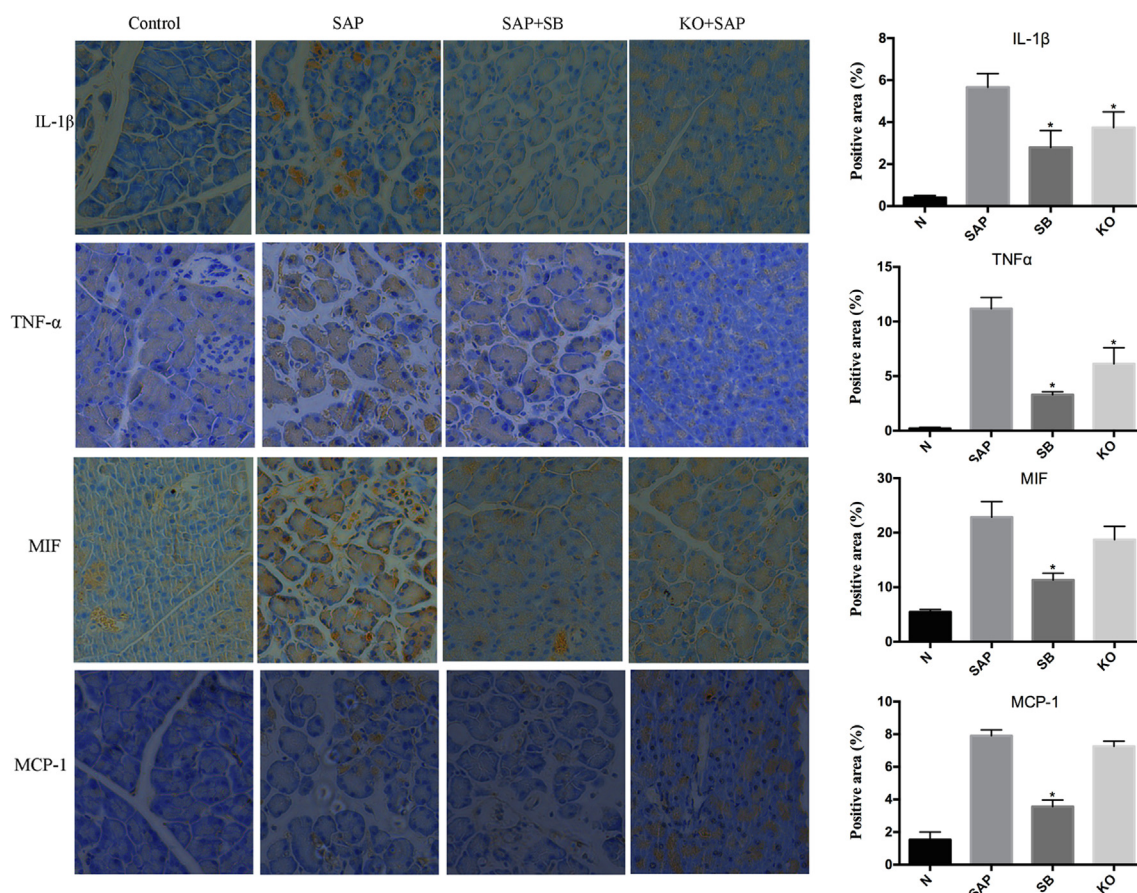


Fig. 4. p38 α KO in macrophages or SB203580 suppresses the production of proinflammatory cytokines in experimental SAP. The protein expression levels of proinflammatory cytokines (TNF- α , IL-1 β , IL-18, IL-17, MCP-1 and MIF) in pancreatic tissue were tested by IHC. * P < 0.05 vs. the SAP group. ** P < 0.01 vs. the SAP group.

primary antibody were as follows: anti-p38 MAPK (1:1000), anti-caspase-1 (1:1000), anti-LC3B (1:500), anti-ULK1 (1:1000) and p62 (1:1000), and was followed by an incubation with secondary antibodies at room temperature for 1 h. The proteins were visualized using an

enhanced chemiluminescence (ECL) system (Thermo Scientific). Protein expression levels were normalized to the level of GAPDH.

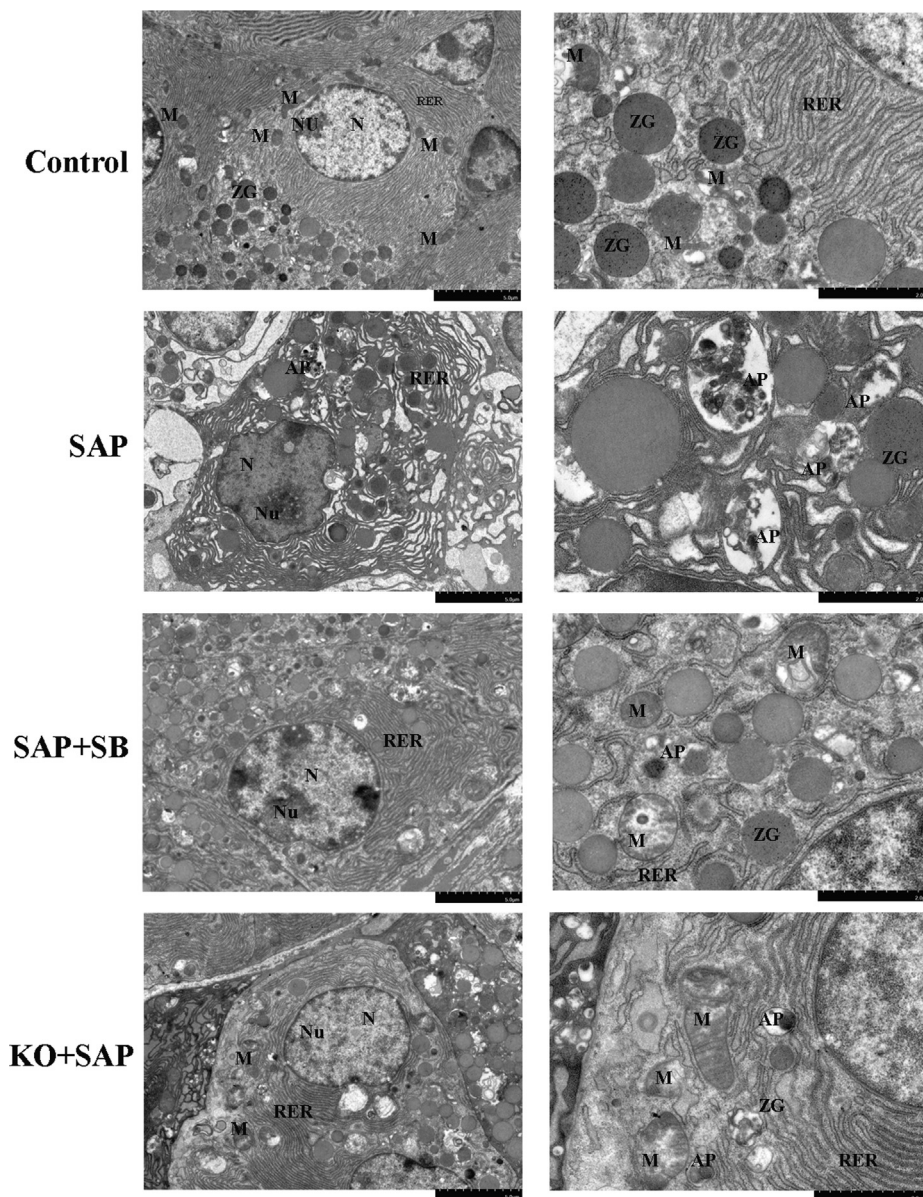


Fig. 5. p38 α KO in macrophages or SB203580 alters the morphological structure of pancreatic cells in SAP. Electron micrograph of pancreatic acinar cell in control group showed normal mitochondria [M], nucleus [N], nucleolus [Nu], zymogen granules [ZG], rough endoplasmic reticulum [RER]. The pancreatic acinar cell in SAP group showed severe edema, the nucleus [N] was pyknotic, the number of mitochondria [M] was small, and most of mitochondrial cristae were disappeared, the RER was severely expanded, the size of ZG was varied and impaired autophagy was increased, including abnormal aggregation of autophagosomes. The pancreatic acinar cell in SB and KO groups showed relatively mild acinar cell damage, normal nuclear structure, obvious swelling of mitochondria, a small amount of RER expansion, uniform size of ZG, and a small amount of damaged autophagy. Scale bar = 5 μ m and Scale bar = 2 μ m.

2.9. Histopathological examination

Pancreatic tissue from each mouse was incised and fixed in 4% paraformaldehyde, embedded in paraffin, and stained with hematoxylin and eosin (H&E). Two experienced pathologists scored the pancreatic tissue samples on a scale from 0 to 4 for the degrees of edema, inflammation, hemorrhage, and necrosis. We applied the scoring system as shown in Table 1, and the final scores of each histological examination were added together [23].

2.10. Immunohistochemistry (IHC) analysis

IHC analysis was performed to examine protein expression levels of TNF- α , IL-1 β , MIF and MCP-1 in pancreatic tissue samples according to our previous report [24].

2.11. Transmission electron microscopy (TEM)

TEM was performed according to the previous report [25].

2.12. Statistical analysis

Data are expressed as the mean $\pm$ standard deviation (SD). The significance of differences in histopathological scores was assessed by the Kruskal-Wallis test. Other continuous data were analyzed by Student's *t* test and factorial design ANOVA. All statistical analyses were carried out using GraphPad Prism 7 (La Jolla, CA, USA) with statistical significance set at $P < 0.05$.

3. Results

3.1. p38 α KO in macrophages or SB203580 improves pancreatic and pulmonary injury in experimental SAP

We used caerulein plus LPS to establish a mouse SAP model and found that the mortality in the SAP group at 24 h was 33.3% (5/15). All mice in the control, SB203580 group and KO group survived at 24 h. Pancreatic injury, as determined by the histological scores, was significantly higher in SAP, SB and KO groups than that in the control group. However, the histological scores in the SB and KO groups were lower than those in the SAP group (Fig. 1A, B). Pulmonary injury, as

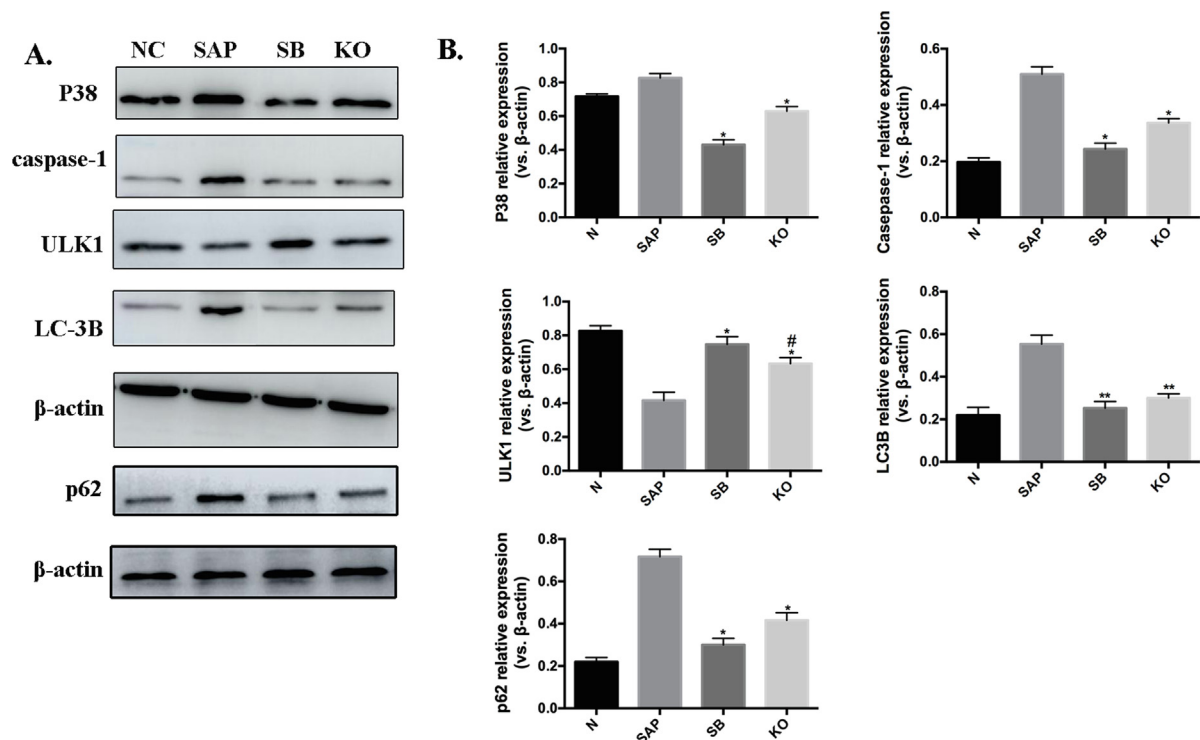


Fig. 6. p38 α KO in macrophages or SB203580 inhibits inflammation and regulates autophagy in experimental SAP. (A, B) Pancreatic p38, caspase-1, ULK1, LC3B and p62 protein expression was determined by Western blotting. * $P < 0.05$ vs. the SAP group. ** $P < 0.01$ vs. the SAP group. # $P < 0.05$ vs. the SB group.

determined by the W/D ratio, was also higher in SAP, SB and KO groups than that in the control group. However, the W/D ratios in the SB and KO groups were significantly lower than that in the SAP group (Fig. 1C).

3.2. p38 α KO in macrophages or SB203580 ameliorates experimental SAP

We then found that p38 α KO in macrophages or SB203580 dramatically suppressed the elevation of serum amylase and LDH levels as compared with the SAP group (Fig. 2A, B). In addition, p38 α KO in macrophages or SB203580 protected against pancreatic injury and pancreatitis-associated lung injury, as shown by decreased pancreatic and lung MPO levels as compared with the SAP group (Fig. 2C, D).

3.3. p38 α KO in macrophages or SB203580 suppresses the production of proinflammatory cytokines in experimental SAP

We found that p38 α KO in macrophages or SB203580 reduced the mRNA expression of proinflammatory cytokines TNF- α , IL-1 β and IL-18 in the pancreas as compared with the SAP group (Fig. 3A–C). In addition, SB203580 decreased the levels of the proinflammatory cytokines IL-17, MIF and MCP-1 as compared with the SAP group (Fig. 3D–F). We also found that p38 α KO in macrophages or SB203580 lowered the serum levels of the proinflammatory cytokines TNF- α and IL-1 β as compared with the SAP group (Fig. 3G, H).

In addition, we assessed the protein expression of TNF- α , IL-1 β , MIF and MCP-1 by IHC and found that p38 α KO in macrophages or SB203580 also decreased the protein levels of TNF- α and IL-1 β , but only SB203580 inhibited the protein expression of MIF and MCP-1 as compared with the SAP group in pancreatic tissues (Fig. 4).

3.4. p38 α KO in macrophages or SB203580 inhibits inflammation and facilitates autophagy in experimental SAP

As demonstrated in Fig. 5, ultra-structurally, as compared with the control group, SAP group showed severe pancreatic acinar cell damage,

for example, the nucleus was pyknotic, most of mitochondrial cristae were disappeared, rough endoplasmic reticulum (RER) was severely expanded, the size of zymogen granules (ZG) was varied and impaired autophagy was increased, such as abnormal aggregation of autophagosomes. However, SB and KO groups showed relatively mild acinar cell damage, normal nuclear structure, obvious swelling of mitochondria, slight RER expansion, uniform size of ZG, and a small amount of damaged autophagy.

To further evaluate the potential mechanisms of SAP, the protein expression levels of p38, caspase-1, ULK1, LC3B and p62 were detected by Western blotting analysis. We found that p38, caspase-1, LC3B and p62 levels were increased, but the ULK1 level was decreased in the SAP group than those in the control group (Fig. 6A, B). Intriguingly, p38 α KO or SB203580 reduced the activity of caspase-1, LC3B and p62, but increased ULK1 expression as compared with the SAP group (Fig. 6A, B).

4. Discussion

Patients with SAP suffer from severe pancreatic hemorrhage, necrosis, and systemic inflammatory response syndrome, and these features are accompanied by lung injury, acute renal injury and intestinal mucosal injury. However, little is known about the pathogenesis of SAP and its effective treatment [20,26]. In the present study, we found that SB203580 and p38 α KO in macrophages reduced pancreatic and lung injury (Figs. 1 and 2) and the release of proinflammatory cytokines TNF- α , IL-1 β and IL-18 (Figs. 3 and 4). Caspase-1 is an effector protein involved in inflammatory responses. Inhibition of caspase-1 and its subsequent proinflammatory cytokines IL-1 β and IL-18 alleviates lung injury, acute renal injury and intestinal mucosal injury in SAP [27–29]. In our study, SB203580 and p38 α KO in macrophages downregulated caspase-1 expression, and in turn inhibited the production of inflammatory cytokines TNF- α , IL-1 β and IL-18.

Autophagy and inflammation are highly intertwined cellular processes [30]. Autophagy is a key protective mechanism that enables cell survival and adaptation to fluctuations in environmental conditions

[31]. Impaired autophagy is recognized as a major pathogenic event in pancreatitis [32,33], increases the accumulation of reactive oxygen species (ROS) and results in defective clearance of damaged mitochondria and activate oxidative stress in AP [31–34]. A recent study suggested that autophagy is activated in experimental AP, but its completion (lysosomal degradation) is inhibited [35]. However, the understanding of the role of autophagy in pancreatitis appears to be incomplete [36]. ULK1 is known as autophagy-related gene 1 (ATG1) in yeast. Autophagy initiation is controlled by the ULK1/ATG1-mediated complex [37]. p38 MAPK can phosphorylate ULK1 and destroy ULK1/ATG13 complex, causing disrupted autophagy in response to proinflammatory signals [21]. p38 MAPK inhibitor SB203580 can alleviate pancreatitis [38], but the effect of p38 α in macrophages on SAP has not been reported. In the present study, we found that autophagy was impaired in SAP, but B203580 and p38 α KO in macrophages reduced damaged autophagy by upregulating ULK1 and downregulating caspase-1, LC3B and p62 in SAP.

In addition, mitochondrial dysfunction and consequent energy deficit may be causally related to pancreatic cell injury. We found that SB203580 and p38 α KO ameliorated the morphological injury of pancreatic cells and facilitated the autophagy in SAP. Thus, p38 α -ULK1 axis might be involved in regulating the inflammatory responses in SAP, but the precise regulatory mechanisms between them in SAP need be further explored.

In conclusion, our findings demonstrated that, macrophage derived p38 α promoted the experimental severe acute pancreatitis by regulating inflammation responses and autophagy.

Acknowledgment

This study was supported by grants from the National Natural Science Foundation of China (81573747 & 81873143).

Declaration of Competing Interest

The authors declare that they have no competing interests.

References

- [1] D.L. Clemens, M.A. Wells, K.J. Schneider, et al., Molecular mechanisms of alcohol associated pancreatitis, *World J. Gastrointest. Pathophysiol.* 5 (2014) 147–157.
- [2] G.I. Papachristou, Prediction of severe acute pancreatitis: current knowledge and novel insights, *World J. Gastroenterol.* 14 (41) (2008) 6273–6275.
- [3] R.K. Jha, Q. Ma, H. Sha, et al., Acute pancreatitis: a literature review, *Med. Sci. Monit.* 15 (2009) RA147e156.
- [4] S.J. Pandol, A.K. Saluja, C.W. Imrie, et al., Acute pancreatitis: bench to the bedside, *Gastroenterology* 132 (2007) 1127e1151.
- [5] R. Pezzilli, L. Bellacosa, C. Feliciani, Lung injury in acute pancreatitis, *JOP* 10 (2009) 481–484.
- [6] V. Mylona, V. Koussoulas, D. Tzivras, et al., Changes in adaptive and innate immunity in patients with acute pancreatitis and systemic inflammatory response syndrome, *Pancreatol.* 11 (2011) 475–481.
- [7] J. Norman, The role of cytokines in the pathogenesis of acute pancreatitis, *Am. J. Surg.* 175 (1998) 76–83.
- [8] J. Han, R.J. Ulevitch, Limiting inflammatory responses during activation of innate immunity, *Nat. Immunol.* 6 (2005) 1198–1205.
- [9] C. Widmann, S. Gibson, M.B. Jarpe, et al., Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human, *Physiol. Rev.* 79 (1999) 143–180.
- [10] Y.J. Kang, J. Chen, M. Otsuka, et al., Macrophage deletion of p38 α partially impairs lipopolysaccharide-induced cellular activation, *J. Immunol.* 180 (7) (2008) 5075–5082.
- [11] K. Ono, J. Han, The p38 signal transduction pathway: activation and function, *Cell Sign.* 12 (2000) 1–13.
- [12] A. Cuenda, S. Rousseau, p38 MAP-kinases pathway regulation, function and role in human diseases, *BBA* 1773 (2007) 1358–1375.
- [13] J.C. Lee, J.T. Laydon, P.C. McDonnell, et al., A protein kinase involved in the regulation of inflammatory cytokine biosynthesis, *Nature* 372 (1994) 739–746.
- [14] S.H. Kim, J. Kim, R.P. Sharma, Inhibition of p38 and ERK MAP kinases blocks endotoxin-induced nitric oxide production and differentially modulates cytokine expression, *Pharmacol. Res.* 49 (2004) 433–439.
- [15] W. Zhu, J.S. Downey, J. Gu, et al., Regulation of TNF expression by multiple mitogen-activated protein kinase pathways, *J. Immunol.* 164 (2000) 6349–6358.
- [16] D.G. Perregaux, D. Dean, M. Cronan, et al., Inhibition of interleukin-1 production by SKF86002: evidence of two sites of in vitro activity and of a time and system dependence, *Mol. Pharmacol.* 48 (1995) 433–442.
- [17] V. Deretic, T. Saitoh, S. Akira, Autophagy in infection, inflammation and immunity, *Nat. Rev. Immunol.* 13 (2013) 722–737.
- [18] L. Antonucci, J.B. Fagman, J.Y. Kim, et al., Basal autophagy maintains pancreatic acinar cell homeostasis and protein synthesis and prevents ER stress, *PNAS* 112 (2015) E6166–E6174.
- [19] O.A. Mareninova, K. Hermann, S.W. French, et al., Impaired autophagic flux mediates acinar cell vacuole formation and trypsinogen activation in rodent models of acute pancreatitis, *J. Clin. Invest.* 119 (2009) 3340.
- [20] A.S. Gukovskaya, I. Gukovsky, H. Algül, et al., Autophagy, inflammation, and immune dysfunction in the pathogenesis of pancreatitis, *Gastroenterology* 153 (2) (2017) 1212–1226.
- [21] Y. He, H. She, T. Zhang, et al., p38 MAPK inhibits autophagy and promotes microglial inflammatory responses by phosphorylating ULK1, *J. Cell Biol.* 217 (1) (2018) 315–328.
- [22] F.B. Engel, M. Schebesta, M.T. Duong, et al., p38 MAP kinase inhibition enables proliferation of adult mammalian cardiomyocytes, *Genes Dev.* 19 (2005) 1175–1187.
- [23] A.M. Kusske, A.J. Rongione, S.W. Ashley, et al., Interleukin-10 prevents death in lethal necrotizing pancreatitis in mice, *Surgery* 120 (2) (1996) 284–288.
- [24] W. Chen, W. Da, C. Li, et al., Network pharmacology-based identification of the protective mechanisms of taraxasterol in experimental colitis, *Int. Immunopharmacol.* 71 (2019) 259–266.
- [25] T.G. Jacob, V.I. Sreekumar, T.S. Roy, et al., Electron-microscopic evidence of mitochondria containing macroautophagy in experimental acute pancreatitis: implications for cell death, *Pancreatol.* 14 (6) (2014) 454–458.
- [26] C.E. Forsmark, S.S. Vege, C.M. Wilcox, Acute pancreatitis, *N. Engl. J. Med.* 375 (20) (2016) 1972–1981.
- [27] J. Yu, L. Ni, X. Zhang, et al., Surfactant protein D dampens lung injury by suppression NLRP3 inflammasome activation and NF- κ B signaling in acute pancreatitis, *Shock* 51 (5) (2019) 557–568.
- [28] J.W. Ning, Y. Zhang, M.S. Yu, et al., Emodin alleviates intestinal mucosal injury in rats with severe acute pancreatitis via the caspase-1 inhibition, *Hepatobil. Pancr. Dis. Int.* 16 (4) (2017) 431–436.
- [29] X.H. Zhang, M.L. Li, B. Wang, et al., Caspase-1 inhibition alleviates acute renal injury in rats with severe acute pancreatitis, *World J. Gastroenterol.* 20 (30) (2014) 10457–10463.
- [30] B. Levine, N. Mizushima, H.W. Virgin, Autophagy in immunity and inflammation, *Nature* 469 (2011) 323–335.
- [31] R. Mukherjee, O.A. Mareninova, I.V. Odinkova, et al., Mechanism of mitochondrial permeability transition pore induction and damage in the pancreas: inhibition prevents acute pancreatitis by protecting production of ATP, *Gut* 65 (2016) 1333–1346.
- [32] N. Li, X. Wu, R.G. Holzer, et al., Loss of acinar cell IKK α triggers spontaneous pancreatitis in mice, *J. Clin. Invest.* 123 (2013) 2231–2243.
- [33] K.N. Diakopoulos, M. Lesina, S. Wormann, et al., Impaired autophagy induces chronic atrophic pancreatitis in mice via sex- and nutrition-dependent processes, *Gastroenterology* 148 (2015) 626–638.e17.
- [34] A.S. Gukovskaya, I. Gukovsky, Autophagy and pancreatitis, *Am. J. Physiol. Gastrointest. Liver Physiol.* 303 (2012) G993–G1003.
- [35] G. Biczko, E.T. Vegh, N. Shalueva, et al., Mitochondrial dysfunction, through impaired autophagy, leads to endoplasmic reticulum stress, deregulated lipid metabolism, and pancreatitis in animal models, *Gastroenterology* 154 (3) (2018) 689–703.
- [36] D. Grasso, A. Ropolo, A. Lo Ré, et al., Zymophagy, a novel selective autophagy pathway mediated by VMP1-USP9x-p62, prevents pancreatic cell death, *J. Biol. Chem.* 286 (10) (2011) 8308–8324.
- [37] L.E. Gallagher, O.A. Radhi, M.O. Abdullah, et al., Lysosomotropism depends on glucose: a chloroquine resistance mechanism, *Cell Death Dis.* 8 (8) (2017) e3014.
- [38] M.H. Cao, J. Xu, H.D. Cai, et al., p38 MAPK inhibition alleviates experimental acute pancreatitis in mice, *Hepatobil. Pancr. Dis. Int.* 14 (1) (2015) 101–106.