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ORIGINAL ARTICLE

Bone Marrow Mesenchymal Stem Cells Alleviate Acute Severe Pancreatitis and Promote Lung Repair via Inhibiting NLRP3 Inflammasome in Rat

Jian Gong[1] · Zhaoming Xiong[1] · Weidi Yu[2] · Zhenshun Song[2]

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Abstract

Background Acute severe pancreatitis (SAP) is a severe acute abdominal disease, which can lead to pancreatic infection and necrosis as well as distant organ damage. Bone marrow mesenchymal stem cells (BMSCs) can exert anti-inflammatory effect on SAP, while NLRP3 inflammasomes play an important role in the inflammatory response. This study aimed to investigate whether BMSCs exert anti-inflammatory effect on SAP by inhibiting NLRP3 inflammasome.

Methods The rat SAP model was established. Serum amylase, lipase and inflammatory factor levels were measured by ELISA, and the level of tissue injury was assessed by HE staining. The expression of NLRP3 inflammasome was detected by PCR, Western Blot and immunohistochemistry. ML385 was used to block Nrf2 pathway, aiming to investigate whether Nrf2 pathway was involved in the therapeutic effect of BMSCs on SAP by regulating NLRP3 inflammasome expression.

Results In SAP rats, NLRP3 inflammasome was activated, which became more evident over time. After transplantation of BMSCs, the NLRP3 inflammasome expression decreased at both mRNA and protein levels, the serum levels of amylase, lipase and inflammatory factors decreased, and the pathological scores of the pancreas and lung were both improved. After blocking the Nrf2 pathway, the NLRP3 inflammasome expression increased in the injured pancreas and lung, and the inflammation deteriorated, which inhibited the therapeutic effects of BMSCs on SAP.

Conclusion The therapeutic effect of BMSC on SAP is at least partially ascribed to the inhibition of NLRP3 inflammasome, and Nrf2 pathway mediates the therapeutic effect of BMSC on SAP by inhibiting NLRP3 inflammasome.

Keywords BMSCs · SAP · NLRP3 · Inflammasome · Nrf2

Introduction

Acute pancreatitis (AP) is a common acute abdominal disease. The annual incidence of AP worldwide is about 15–45 /100,000 [1], and AP in about 20% of patients will progress into severe acute pancreatitis (SAP). Severe acute pancreatitis is characterised by persistent organ failure. The mortality of SAP patients is as high as 36–50% [2–4]. At present, conventional clinical treatments for SAP include analgesia, spasmolysis, fluid resuscitation and, nutritional support, etc. [5], but they fail to significantly halt the progression of SAP [6].

Bone marrow mesenchymal stem cells (BMSCs) are pluripotent stem cells with low immunogenicity. In recent years, other groups and our group have revealed that mesenchymal stem cells (MSCs) have the potential to promote the repair of injured pancreatic tissues, and MSCs may become promising therapeutic strategy for SAP. Our previous study has shown the therapeutic effect of BMSCs on SAP, which is related to the inhibition of inflammation and oxidative stress, paracrine, and inhibition of autophagy [7–9]. While all of these contributions can be used to explain the therapeutic effects of MSCs, but how MSCs put the brakes on the early stages of inflammation

Jian Gong and Zhaoming Xiong have contributed equally to this work.

✉ Zhenshun Song
zs_song@tongji.edu.cn

[1] Department of General Surgery, Shanghai Tenth People's Hospital, Tongji University School of Medicine, 200072 Shanghai, China

[2] Department of General Surgery, Shanghai Fourth People's Hospital, Tongji University School of Medicine, 200072 Shanghai, China

in SAP needs to be further explored. In recent years there have been a number of views that the assembly of NLRP3 inflammasome is a key event in the early progression of SAP [10, 11].

NLRP3 inflammasome is a best characterized pattern recognition receptor (PRR) in the innate immune response, and composed of a sensor (NLRP3 protein), a linker (ASC protein) and an effector (pro-caspase-1) [12]. ROS, mitochondrial DNA, mitochondrial phospholipid cardiolipin production, potassium efflux, cell volume change, calcium ion signal transduction and lysosomal damage have been found as key factors activating NLRP3 inflammasome [13]. Active NLRP3 inflammasome can recruit pro-caspase-1, leading to the its cleavage into active caspase-1. The activated Caspase-1 converts IL-1β and IL-18 precursors into mature IL-1β and IL-18, finally increasing the inflammatory response and causing injury [13].

It has been reported that Apocynin can inhibit NF-κB pathway and NLRP3 inflammasome to alleviate the pancreatic and lung injury in SAP [14]. In addition, indomethacin has also been proved to inhibit NLRP3 inflammasome, exerting therapeutic effect on SAP [15]. However, few study has been undertaken to investigate whether BMSCs exert therapeutic effect on SAP and promote the repair of pancreatic and lung tissues by inhibiting NLRP3 inflammasome.

Materials and Methods

Animals

SD rats used in this study were purchased from Charles River Experimental Animal Co., Ltd. and housed in a specific pathogen free environment of the Science and Technology Innovation Center of Shanghai Tenth People's Hospital. BMSCs were collected from male SD rats aged 2–3 weeks. Male SD rats weighing about 200 g and aged 6–8 weeks were used to establish an animal model of SAP. This study was approved by the Experimental Animal Ethics Committee of Shanghai 10th People's Hospital.

Separation, Culture and Identification of BMSCs

The separation, culture and identification of BMSCs were performed as previously reported [16]. The surface markers of BMSCs (CD34, CD90 and CD105) were detected by flow cytometry and cell differentiation potentials (adipose differentiation, osteogenic differentiation) were detected to identify BMSCs. Cells of the 3rd to 5th generations were used in the experiment.

SAP Animal Model and Experimental Design

As previously reported [7, 9], SAP animal model was established by retrograde injection of sodium taurocholate into the pancreatic duct. MCC950 (B7946, APExBIO) and ML385 (GC19254, GLPBIO) were dissolved in PBS to concentrations of 5 mg/mL and 1 mg/mL, respectively, and used to specifically block NLRP3 inflammasome and Nrf2 [17, 18]. SD rats were randomly divided into 8 groups: (1) normal control group (NC, n = 6): rats were routinely fed without any treatment; (2) Sham group (Sham, n = 6): rats received laparotomy, and the wound was immediately closed without other treatment; (3) SAP group (n = 18): rats received retrograde injection of sodium taurocholate into the pancreaticobiliary duct to induce SAP, but no other treatments were administered. (4) PBS group (SAP + PBS, n = 6): SAP rats were injected with 1 × PBS solution (the volume was equal to that of BMSC suspension) through the tail vein about 6 h after the establishment of animal model; (5) MSC treatment group (SAP + BMSCs, n = 6): SAP rats were injected with BMSCs (1×10^7 cells/rat) in 200 µl of 1× PBS; (6) MCC950 group (SAP + MCC950, n = 6): SAP rats were intraperitoneally injected with MCC950 (50 mg/kg) 1 h before the establishment of animal model. (7) ML385 group (SAP + ML385, n = 6): rats received intraperitoneal injection of ML385 (5 mg/kg) 2 h before the establishment of animal model; (8) ML385 and BMSCs group (SAP + BMSCs + ML385, n = 6): rats were pretreated with ML385, and then animal model was established, followed by treatment with BMSCs (1×10^7 cells/rat). All rats were routinely housed after treatment. Six rats in SAP group were killed at 24 and 48 h after the establishment of animal model, and the pancreas tissues were collected. The remaining rats were killed at 3 days after treatment, and the pancreas, lung, small intestine and blood were collected for further analysis.

Biochemical Examinations

Serum levels of amylase (starch-iodine colorimetric method) and lipase test (colorimetric method) were detected using the corresponding kits from Nanjing Jiancheng Company. EILSA kit (R&D Systems) was used to determine the serum or tissue levels of TNF-α, IL-1 β, IL-6, IL-10 and IL-18.

Pathological Scoring

Five sections were obtained from each rat, and independently by two pathologists under a light microscope. Three fields were randomly selected from each section.

Table 1 Criteria for pathological scoring of pancreatic tissues

Score	Tissue swelling	Cell necrosis	Inflammatory cell infiltration	Vacuolar degeneration
0	No	No	No	No
1	Diffused dilation of interlobular septum	1–5 cells/field	Around pancreatic duct	Around pancreatic duct, < 5%
2	Diffused dilation of interlobular septum	6–10 cells/field	Interlobular, < 50%	5–20%
3	Diffused dilation of acinar septum	11–15 cells/field	Interlobular, 50-75%	20–50%
4	Diffused dilation of cellular septum	> 15 cells/field	Interlobular, > 75%	> 50%

Table 2 Criteria for pathological scoring of lung tissues

Score	Criteria
0	Normal alveolar cavity without atelectasis and inflammatory cell infiltration
1	Area of atelectasis less than 15% of the field, area of alveolar space more than 85%, and infiltration of a small amount of inflammatory cells
2	Area of atelectasis between 25% and 50%, area of alveolar space between 50% and 75%, and infiltration of a small amount of inflammatory cells
3	Area of atelectasis between 50% and 75%, area of alveolar space between 25% and 50%, and infiltration of some inflammatory cells
4	Area of atelectasis larger than 75%, area of alveolar space less than 25%, and infiltration of a lot of inflammatory cells

Table 3 Criteria for pathological scoring of intestine tissues

Score	Criteria
0	Normal intestinal mucosal villi
1	Intestinal villous oedema
2	Separation of the intestinal epithelium from the lamina propria
3	Accompanied by apical partial exfoliation of the intestinal villi on a 2-point basis
4	Exfoliation of the intestinal villi exposing the lamina propria with capillary dilatation
5	Loss of intestinal lamina propria with intestinal bleeding and ulceration

Pathological Scoring of Pancreatic Tissues

The pancreatic tissues were scored as follows (Table 1): total score = cell necrosis (0–4) + tissue swelling (0–4) + inflammatory cell infiltration (0–4) and tissue vacuolar degeneration (0–4).

Pathological Scoring of Lung and Intetissues

The lung injury was evaluated by alveolar and interstitial edema, inflammatory cell infiltration, alveolar hemorrhage and atelectasis. Intestinal injury evaluated by the degree of intestinal villi and lamina propria damage. The specific criteria are shown in Tables 2 and 3.

qRT-PCR

The mRNA sequence of target genes were searched in Gene Bank, and their specificity was validated through blast detection. Primers used for PCR were synthesized in Shanghai Sangon Biotech (Table 4), and GAPDH was used as the internal reference.

Table 4 Primers used in qRT-PCR

Gene	Sequence (sense, antisense:5′-3′)
NLRP3	GGAGTGGATAGGTTTGCTGG
	GGTGTAGGGTCTGTTGAGGT
Caspase1	TTTCCGCAAGGTTCGATTTTCA
	GGCATCTGCGCTCTACCATC
Pro-caspase1	GTGGAGAGAAAGAAGGAGTGGT
	GATGAGTGACTGAATGAAGAGG
ASC	GGAGGGGTATGGCTTGGAG
	TGAGTGCTTGCCTGTGTTGGT
GAPDH	CGCTAACATCAAATGGGGTG
	TTGCTGACAATCTTGAGGGAG

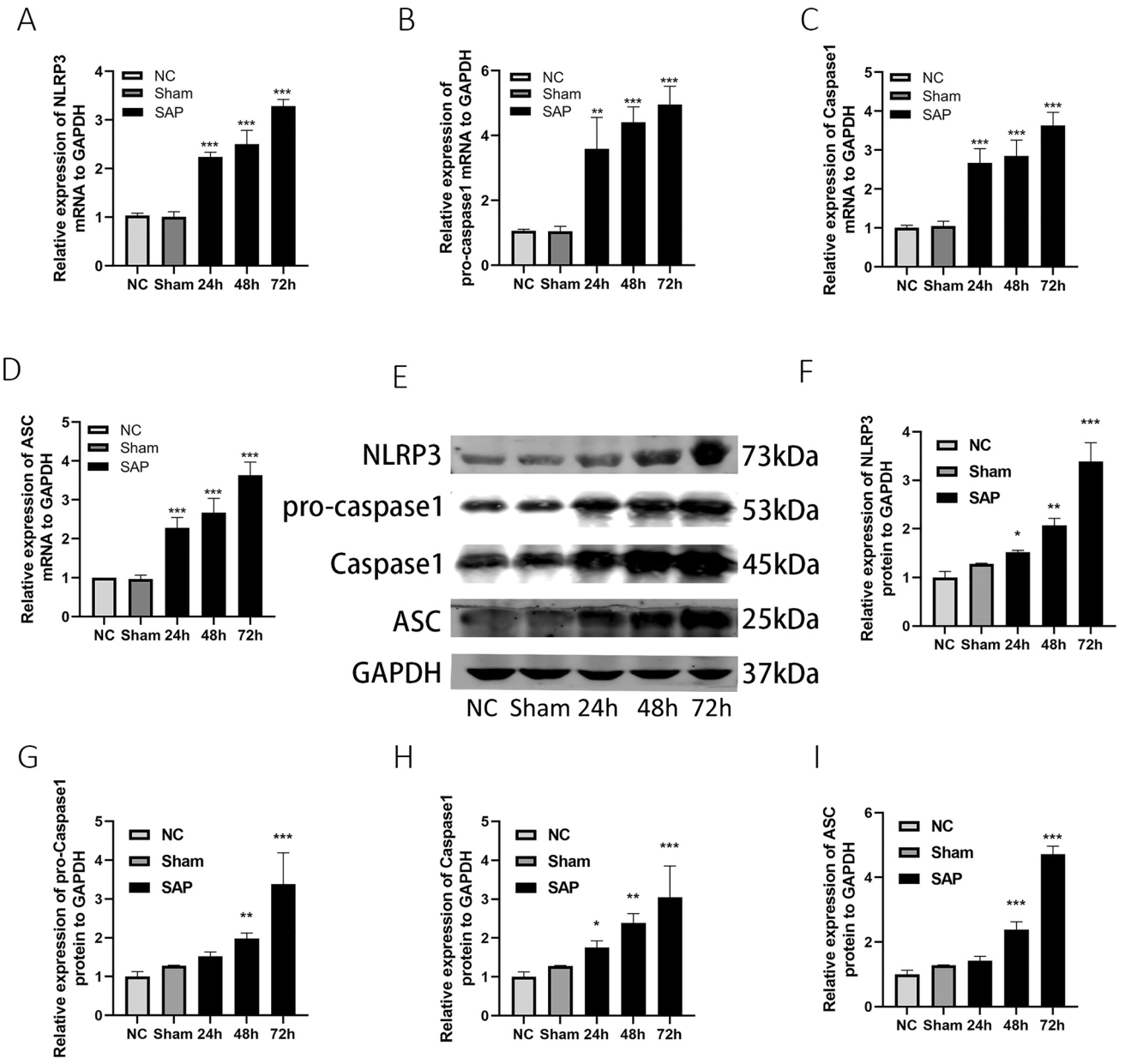

Fig. 1 NLRP3 inflammasome was activated in pancreatic tissues of SAP rats. **A–D** mRNA expression of NLRP3, Pro-caspase1, Caspase1 and ASC **E–I** Protein expression of NLRP3, Pro-caspase1, Caspase1 and ASC. *p < 0.05, **p < 0.01 and ***p < 0.001(vs NC group and Sham group)

Immunohistochemistry

The deparaffinized sections were incubated with 3% hydrogen peroxide for 30 min, and then with citric acid buffer. After antigen retrieval in boiling water for 10 min, sections were treated with anti-NLRP3 (1:100), anti-Caspase1 (1:100) or anti-Nrf2 antibody (1:100) overnight at 4 °C. Sections were rinsed with PBS and incubated with peroxidase-labeled secondary antibody at room temperature for 1 h. Finally, sections were subjected to staining with 3,3′- diaminobenzidine tetrahydrochloride (DAB) and hematoxylin. After mounting, sections were scored by two pathologists independently under a light microscope. Any discrepancy was resolved by consulting the third party.

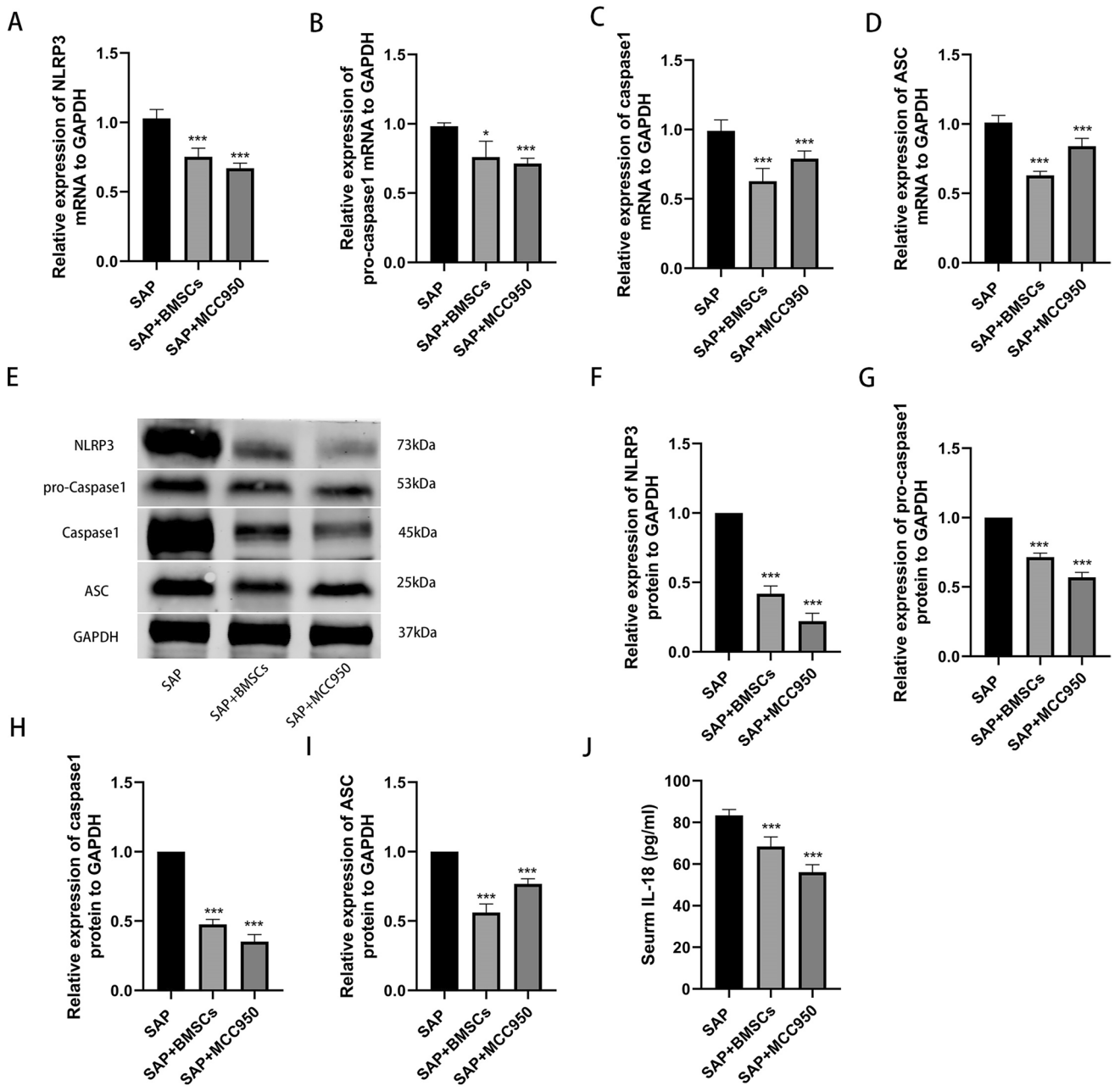

Fig. 2 MCC950 treatment and BMSC transplantation inhibited NLRP3 inflammasome in the pancreatic tissues of SAP rat. **A–D** mRNA expression of NLRP3, Pro-caspase1, Caspase1 and ASC **E–I** Protein expression of NLRP3, Pro-caspase1, Caspase1 and ASC **J** Expression of IL-18, a production of NLRP3 inflammasome. *p < 0.05, **p < 0.01 and ***p < 0.001 (*vs* SAP group)

Western Blotting

Total protein and nuclear protein were extracted with corresponding kits (Nanjing Jiancheng Company). Protein concentration was determined with BCA method according to the manufacturer's instructions (Yamei Company). The gel was cut according to the molecular weight of markers. LaminB1 was used as the internal reference for nuclear protein and GAPDH as the internal reference for other proteins. Proteins were separated with a constant current at 400 mA and then transferred to the NC membrane, which was then blocked in 5% non-fat milk for 1 h. The membrane was incubated with primary antibody at 4 °C overnight, rinsed with TBST for 3 times, and incubated with the second antibody at room temperature for 1 h. After rinsing with TBST for 3 times, the protein bands were visualized with Odyssey

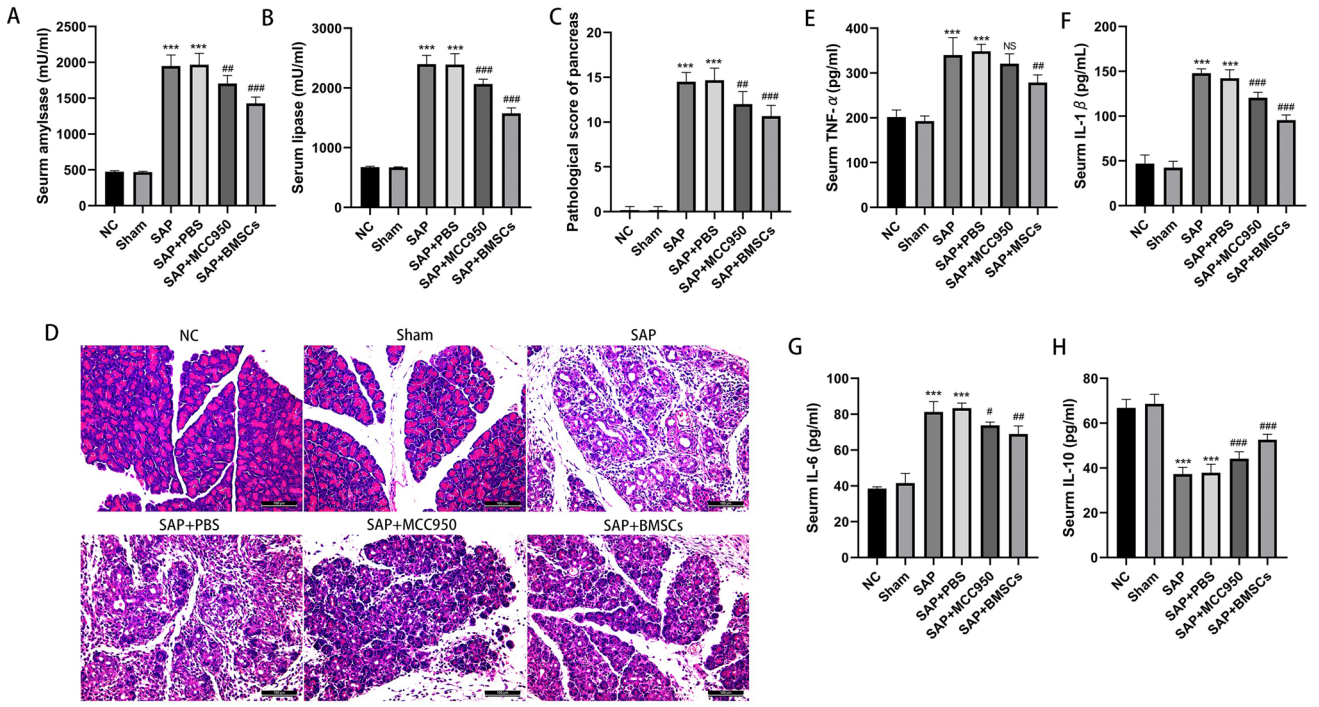

Fig. 3 MCC950 treatment and BMSC transplantation attenuated pancreatic injury and inflammation in SAP rats. **A**, **B** serum levels of amylase and lipase **C** pathological score of pancreatic tissues **D** HE staining of pancreatic tissues (200×) **E–H** expression of TNF-α, IL-6, IL-1β and IL10. ***p < 0.001 (*vs* NC group and Sham group);$^{\#}$p < 0.05, $^{\#\#}$p < 0.01 and $^{\#\#\#}$p < 0.001 (*vs* SAP group and SAP+PBS group); NS, no significant difference

XL scanner and the optical density was determined for each band.

Statistical Analysis

Data from at least three detections were used for analysis. Data are expressed as mean ± standard deviation (SD). Statistical analysis was performed with GraphPad 8.0. Comparisons were done with one way analysis of variance among groups. Data without homogeneity of variance were compared with nonparametric test. Student t test was used for the comparisons between two groups. A value of $p < 0.05$ was considered statistically significant.

Results

NLRP3 Inflammasome Was Activated in Pancreatic Tissues of SAP rats

Rats were killed at 24 h, 48h and 72 h after the establishment of SAP rat model, and pancreatic tissues were collected for analysis. qRT-PCR showed that the mRNA expression of NLRP3, Pro-caspase1, Caspase1 and ASC in the pancreatic tissue of SAP group increased significantly as compared to the NC group and Sham group, and their expression increased gradually over time (Fig. 1a–d). The protein expression of NLRP3, Pro-caspase1, Caspase1 and ASC in the pancreatic tissues was similar to that in qRT-PCR (Fig. 1e–i).

MCC950 and BMSCs Transplantation Reduced NLRP3 Inflammasome in the Pancreatic Tissues of SAP rats

As compared to SAP group, MCC950 treatment or BMSCs transplantation significantly reduced the mRNA expression of NLRP3, Pro-caspase1, Caspase1 and ASC in the pancreatic tissues of SAP rats (Fig. 2a–d). Western Blotting showed the protein expression of NLRP3, Pro-caspase1, Caspase1 and ASC in the pancreatic tissues of SAP + MCC950 group and SAP + BMSCs group was markedly lower than in the SAP group (Fig. 2e–i). In addition, both MCC950 treatment and BMSCs transplantation could effectively reduce the expression of IL-18, a product of inflammasome (Fig. 2j).

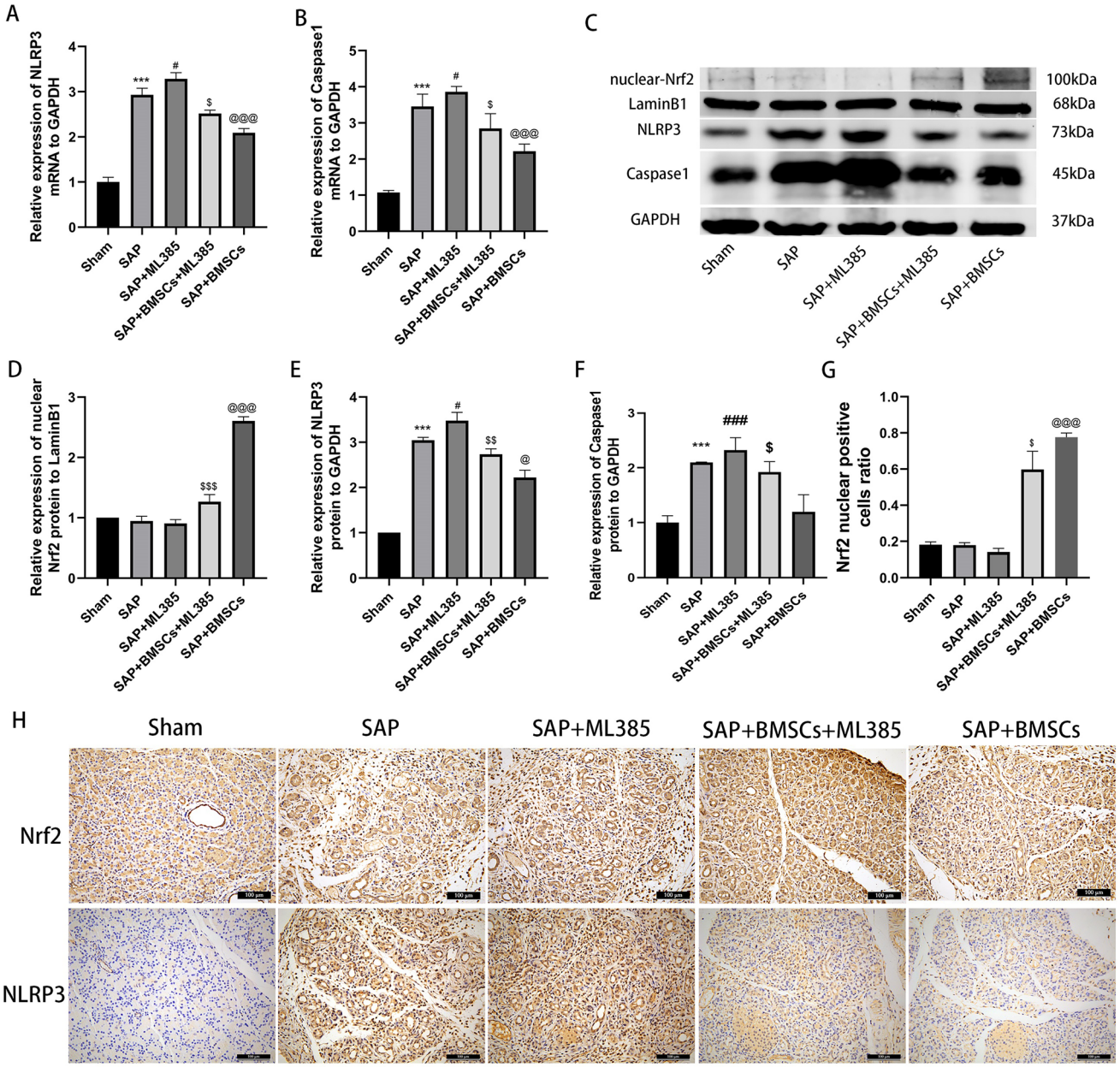

Fig. 4 Blocking Nrf2 attenuated the inhibition of NLRP3 inflammasome in the pancreatic tissues of SAP after BMSC transplantation. **A**, **B** mRNA expression of NLRP3 and Caspase1. **C–F** Protein expression of nuclear-Nrf2, NLRP3 and Caspase1 in the pancreatic tissues **G** Nuclear Nrf2 positive rate **H** Immunohistochemistry for Nrf2 and NLRP3. ***$p < 0.001$ *vs* Sham group; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ *vs* SAP; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$ *vs* SAP+BMSCs group; $^{@}p < 0.05$; $^{@@@}p < 0.001$ *vs* SAP group

MCC950 Treatment and BMSC Transplantation Attenuated Pancreatic Injury and Inflammation

The serum levels of amylase and lipase in the SAP rats were significantly higher than in the NC and Sham groups. As compared to the SAP group, MCC950 treatment and BMSCs transplantation effectively reduce the serum levels of amylase and lipase (Fig. 3a and b). Pathological examination of pancreatic tissues showed that BMSCs transplantation effectively reduced the tissue edema, inflammatory cell infiltration, cell necrosis and other pathology caused by SAP, and MCC950 treatment achieved similar effects (Fig. 3c and d). Compared with NC and Sham groups, the serum levels of pro-inflammatory factors TNF-α, IL-6 and IL-1β increased significantly in the SAP group, but IL-10 level reduced

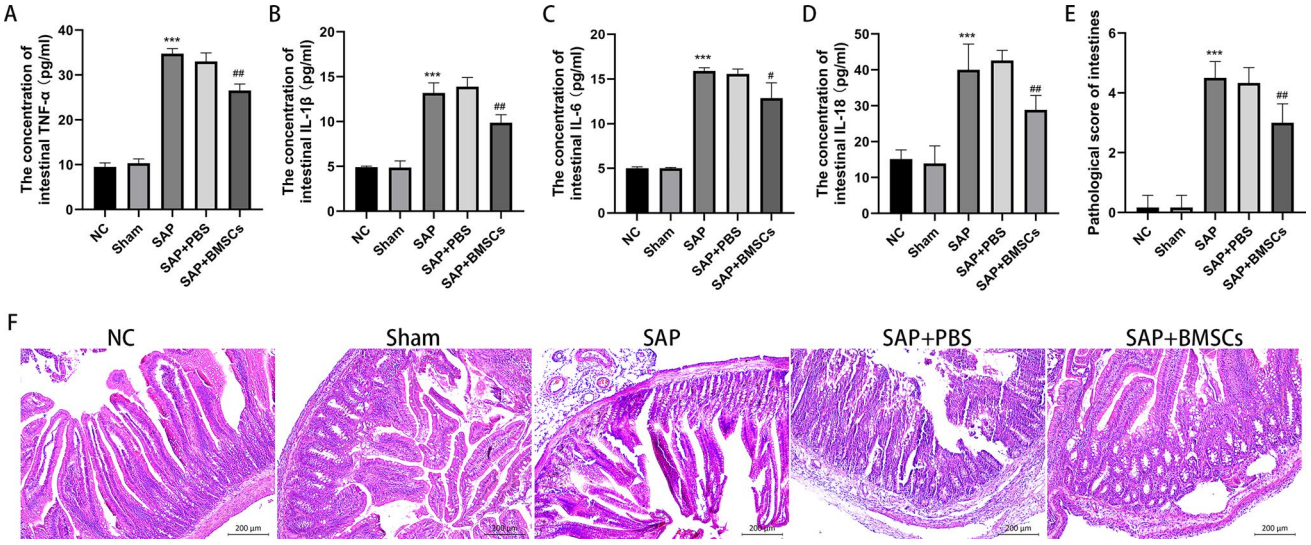

Fig. 5 BMSC transplantation attenuated intestine injury and inflammation of SAP rats. **A–D** expression of TNF-α, IL-1β, IL-6, and IL-18 **E** small intestine pathological score **F** HE staining of intestine tissues. ***p < 0.001 *vs* Sham group; $^{\#}$p < 0.05, $^{\#\#}$p < 0.01, $^{\#\#\#}$p < 0.001 *vs* SAP group; $^{@}$p < 0.05; $^{@@}$p < 0.01; $^{@@@}$p < 0.001 *vs* SAP group; $^{\$}$p < 0.05, $^{\$\$}$p < 0.01 *vs* SAP+BMSCs group

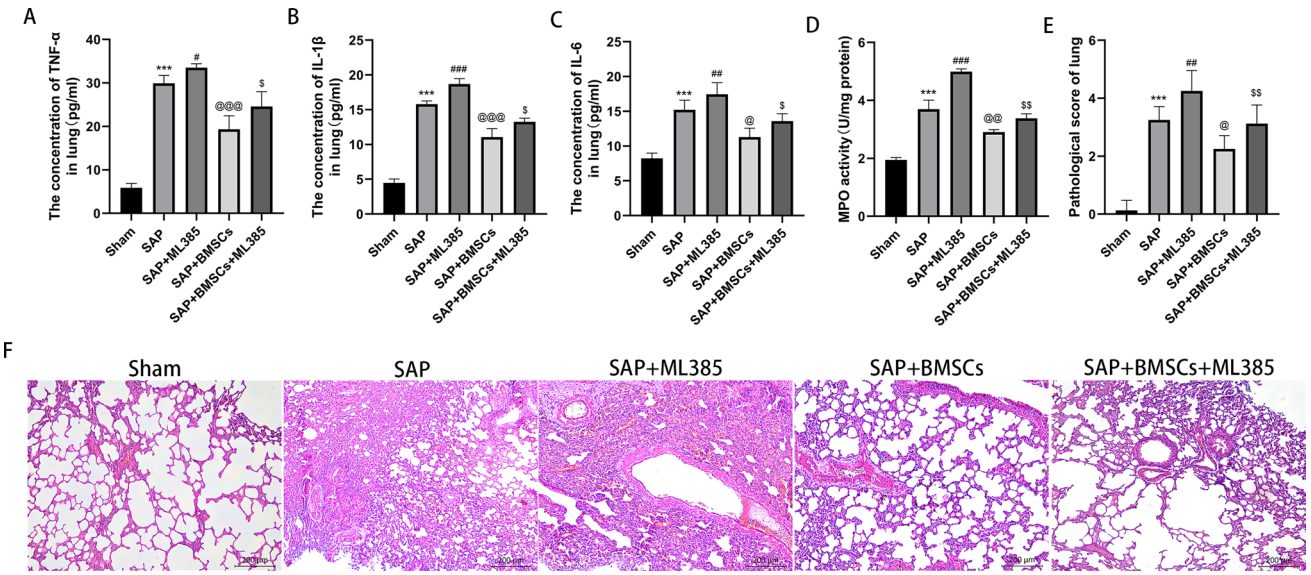

Fig. 6 BMSC transplantation attenuated lung injury and inflammation of SAP rats, but blocking Nrf2 compromised these protective effects of BMSC transplantation. **A–C** expression of TNF-α, IL-1β and IL-6 **D** MPO expression **E** lung pathological score **F** HE staining of lung tissues. ***p < 0.001 *vs* Sham group; $^{\#}$p < 0.05, $^{\#\#}$p < 0.01, $^{\#\#\#}$p < 0.001 *vs* SAP group; $^{@}$p<0.05; $^{@@}$p<0.01; $^{@@@}$p< 0.001 *vs* SAP group; $^{\$}$p < 0.05, $^{\$\$}$p < 0.01 *vs* SAP+BMSCs group

markedly. Transplantation of BMSCs significantly reduce inflammatory factors (TNF-α, IL-6 and IL-1β) and increase the IL-10 expression. In addition, MCC950 treatment also reduced IL-6 and IL-1β and increase the IL-10 expression, but had no influence on the TNF-αexpression (Fig. 3e–h).

Blocking Nrf2 Compromised the Inhibition of NLRP3 Inflammasome in the Pancreatic Tissues of SAP Rats After BMSC Transplantation

The Nrf2 was inhibited with ML385 to further investigate the possible pathway underlying the therapeutic effect of BMSCs transplantation on the inflammasome in SAP rats. Results showed that, compared with SAP

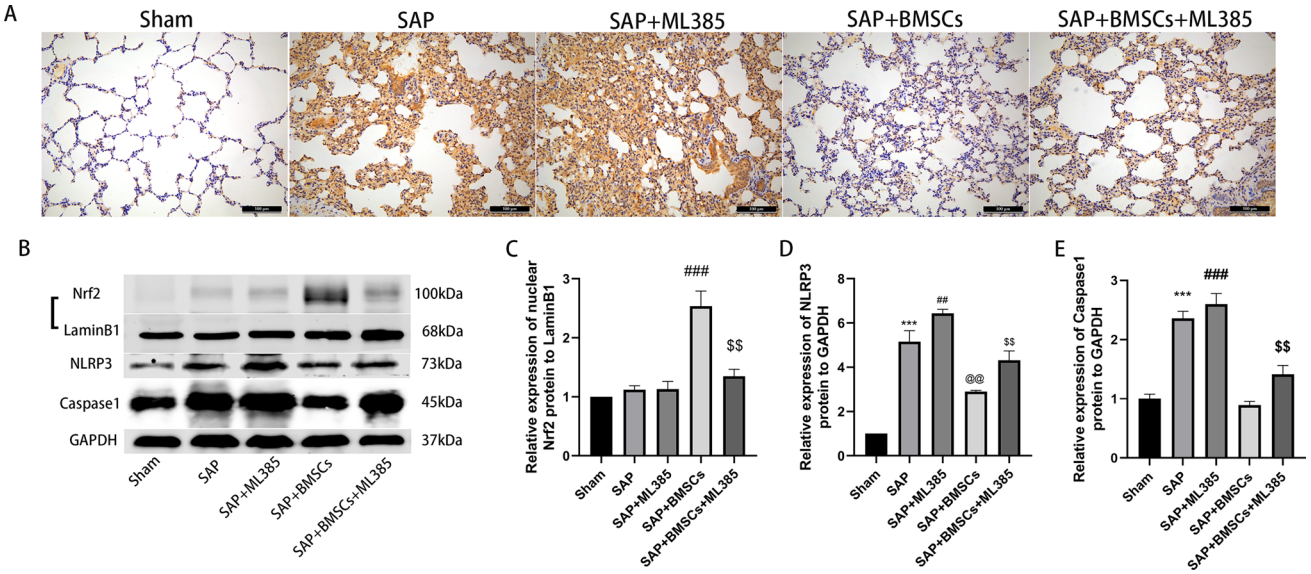

Fig. 7 BMSCs transplantation reduced NLRP3 inflammasome in the lung of SAP rats, but blocking Nrf2 compromised the protective effects of BMSCs transplantation. **A** Immunohistochemistry for NLRP3 **B–E** protein expression of nuclear Nrf2, NLRP3 and Caspase-1 in the lung. ***$p < 0.001$ *vs* Sham group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ *vs* SAP group; $^{@}p < 0.05$; $^{@@}p < 0.01$; $^{@@@}p < 0.001$ *vs* SAP group; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ *vs* SAP+BMSCs group

group and SAP + BMSCs group, the mRNA expression of NLRP3 and Caspase-1 in the pancreatic tissues increased significantly in the SAP + ML385 group and SAP + BMSCs + ML385 group after blocking Nrf2 (Fig. 4a and b). Western blotting and immunohistochemistry also showed that the expression of NLRP3 and Caspase-1 in the pancreatic tissues increased after blocking Nrf2 (Fig. 4c and e, f, h). In addition, nuclear protein detection and immunohistochemistry revealed that the Nrf2 expression in the acinar nuclei significantly increased after BMSCs transplantation, while ML385 attenuated the effect of BMSCs (Fig. 4c, d and g, h).

BMSCs Transplantation Attenuated Lung and Intestine Injury of SAP Rats, but Blocking Nrf2 Compromised the Protective Effects of BMSCs Transplantation

ELISA was employed to detect the inflammatory cytokines in the lung and small intestine. Results showed the expression of TNF-α, IL-1β and IL-6 in the lung and intestine increased significantly in the SAP group, but was reduced after BMSCs transplantation (Figs. 5a–c and 6a–c). In addition, BMSCs transplantation could effectively reduce the expression of IL-18 in small intestine tissue (Fig. 5d). MPO measurement was used to assess the infiltration of neutrophils in the lung. Results showed the MPO expression in the lung of SAP group and SAP + BMSCs group reduced significantly as compared to SAP group (Fig. 6d). As compared to SAP group, BMSC transplantation could markedly reduce the lung and intestine pathological score in SAP rats (Figs. 5e and f and 6e and f). In addition, ML385 deteriorated the lung inflammation in the SAP rats and compromised the protective effects of BMSC transplantation on the lung injury secondary to SAP (Fig. 6).

BMSCs Transplantation Reduced NLRP3 Inflammasome in the Lung of SAP Rats, but Blocking Nrf2Compromised the Protective Effects of BMSC Transplantation

As compared to SAP group, the protein expression of NLRP3 and Caspase-1 reduced significantly in the SAP + BMSCs. In the presence of Nrf2 blocking with ML385, the protein expression of NLRP3 and Caspase-1 increased in SAP rats receiving BMSCs transplantation (Fig. 7a, b, d and e). In addition, BMSCs transplantation induced the nuclear Nrf2 expression, but ML385 treatment reduced the nuclear Nrf2 expression (Fig. 7b, c).

Discussion

Inflammatory cytokines play an important role in the occurrence and development of SAP [19–21]. Reducing the release of inflammatory cytokines is key to the SAP treatment. Numerous studies have confirmed the anti-inflammatory effects of BMSCs on the SAP. Our previous study [22] also confirmed that transplantation of mesenchymal

stem cells significantly reduced the inflammatory cytokines (TNF-α, IL-1 β and IL-6) in SAP rats, but increased anti-inflammatory cytokines (IL-4 and IL-10), exerting therapeutic effects on the SAP. In a study of Zhao et al. [23], MSCs transplantation significantly reduced the expression of TNF-α and IL-1βin the pancreas and lung. However, the specific anti-inflammatory mechanism underlying the protective effects of BMSCs transplantation on the SAP remains to be further studied. Studies have shown that [24, 25], NLRP3 inflammasome plays a crucial role in the SAP induced inflammation, and the degree of NLRP3 inflammasome activation is closely related to the outcome of SAP. Hou et al. [26] found that T-614 exert protective effects on the experimental SAP through anti-inflammatory activity, which is manifested as inhibition of NLRP3 inflammasome and NF-κB. Sheng et al. [27] found that procyanidin could reduce ROS, inhibit NLRP3 inflammasome and suppress M1 macrophage polarization to exert protective effects on mouse SAP. This study was undertaken to investigate whether BMSCs transplantation could exert anti-inflammatory effect on SAP via inhibiting NLRP3 inflammasome.

Our results showed that NLRP3 inflammasome was activated in SAP rat model, and the degree of NLRP3 inflammasome activation increased over time. As a specific inhibitor of NLRP3 inflammasome, MCC950 has definite inhibitory effect on NLRP3 inflammasome [28]. Results confirmed that MCC950 could effectively alleviate the pancreatic injury and inflammation of SAP rats, and thus we speculate that NLRP3 inflammasome plays an important role in the occurrence and development of SAP. In addition, BMSCs transplantation exerted effects similar to MCC950 in SAP: BMSCs transplantation inhibited the expression of NLRP3 inflammasome and related factors, and the expression of IL-18 and IL-1β (end product of NLRP3 inflammasome) reduced significantly. However, the results of pancreatic injury suggest that simply inhibiting the assembly of NLRP3 inflammasome early in SAP does not rival the therapeutic effect of MSCs. This finding indicated that BMSC transplantation improves SAP at least partially through inhibiting NLRP3 inflammasome. Moreover, our results showed MCC950 pretreatment failed to significantly reduce TNF- α expression, which might be ascribed as that MCC950 specifically inhibits the NLRP3 inflammasome and has no influence on the AIM2, NLRC4 and NLRP1 inflammasome activation. Our results were consistent with previously reported [17].

Nrf2 (nuclear factor erghroid 2-like 2) is a major transcription factor that can regulates the expression of a variety of cytoprotective genes and involved in the regulation and control of expression of metabolic enzymes, antioxidant stress and detoxification [29]. ROS is one of the important pathways influenced by NLRP3 inflammasome activation [30]. Nrf2 pathway has been proved to be closely related to the production and accumulation of ROS in SAP [31]. Therefore, we further explored whether Nrf2 related pathway was involved in the NLRP3 inflammasome activation. ML385 is a new and specific Nrf2 inhibitor, and can inhibit the expression of downstream gene of Nrf2. In our experiment, blocking Nrf2 with ML385 was found to up-regulate the expression of inflammasome-related factors in the pancreatic tissues of SAP rats, suggesting that Nrf2 pathway is involved in the regulation of inflammasome activation. BMSCs transplantation could induce the nuclear translocation of Nrf2. After blocking Nrf2, the inhibitory effect of BMSCs transplantation on the inflammasome was compromised. This indicates that Nrf2 related pathway mediates the anti-inflammatory effect of BMSCs transplantation. In the animal model of cerebral ischemia-reperfusion injury, results showed Nrf2 could inhibit the activation of NLRP3 inflammasome by regulating Trx3/TXNIP complex [32]. Nrf2/ARE pathway can inhibit the activation of NLRP3 inflammasome in BV2 cells induced by inhibiting ROS [33]. The specific role of Nrf2 pathway in the inhibitory effect of BMSCs transplantation needs to be further studied (Fig. 7).

It has been reported that, in the early stage of SAP, inflammation can induce SIRS, further leading to distant organ injury and MODS [34], the incidence of MODS in early stage of SAP is as high as 50% [35], and MODS is a major cause of death. The most common and serious SIRS is SAP-related lung injury, which is mainly characterized by the increased permeability of endothelial and epithelial barrier, leakage of exudates into alveolar space and interstitial lung, and the resultant pulmonary edema and gas exchange dysfunction [36]. Inflammatory cytokines (TNF-α, IL-1 βand IL-6) are key to the lung injury. TNF-α and IL-6 can recruit white blood cells into lung and intestine tissues, and IL-1β It can induce the aggregation of monocytes and macrophages and accelerate the process of lung and intestine injury. MPO is a heme protein rich in neutrophils and has been used as a marker of neutrophil infiltration [37]. Our results showed BMSCs transplantation reduced the expression of pro-inflammatory cytokines in the lung, and inhibited the infiltration of neutrophils, exerting inhibitory effect on lung inflammation. In addition, blocking Nrf2 deteriorated lung inflammation and compromised the therapeutic effect of BMSCs transplantation. This implies that the therapeutic effect of BMSCs transplantation is related to Nrf2 pathway.

Taken together, our study indicates that BMSCs can alleviate pancreatic injury and promote the repair of injured lung by inhibiting NLRP3 inflammasome in SAP rats. Moreover, Nrf2 pathway may be one of the regulatory pathway mediating the anti-inflamamtory effect of BMSCs transplantation on the inflammasome in SAP rats (Fig. 8).

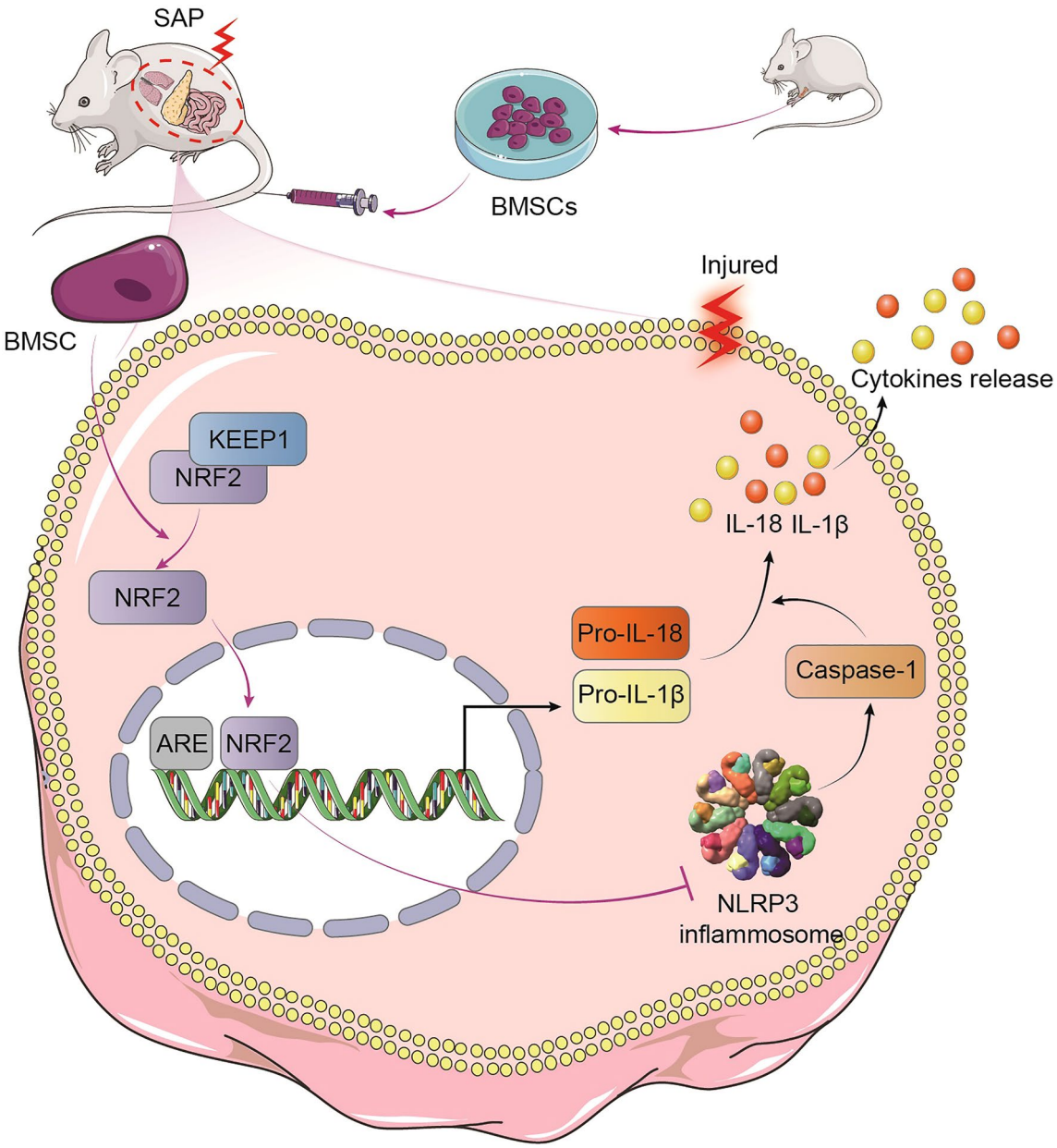

Fig. 8 Mechanism figure: BMSCs may exert anti-inflammatory effects on SAP rats by inhibiting inflammasome assembly via Nrf2

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Author's contribution ZS and ZX: Conceptualization and supervision; JG and WY: Data curation, Writing—original draft, formal analysis, methodology and software.

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Data availability The data that supports the findings of this study are available in the article and in the Supporting Information.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval All animal experiments are approved by the Animal Ethics Committee of Shanghai Tenth People's Hospital (SHDSYY-2022-2330).

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