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Salidroside promotes liver regeneration after partial hepatectomy in mice by modulating NLRP3 inflammasome-mediated pyroptosis pathway

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ABSTRACT

Insufficient residual liver tissue after partial hepatectomy (PH) may lead to serious complications such as hepatic failure and small-for-size syndrome. Salidroside (SAL) is obtained from Rhodiola rosea through modernized separation and extraction and has been validated for treating various liver diseases. It's yet unknown, nevertheless, how SAL affects liver regeneration after PH. This study aimed to determine whether SAL could promote liver regeneration after PH in mice. We demonstrated that SAL could attenuate liver injury after PH and promote hepatocyte proliferation and liver mass recovery. Mechanistically, SAL inhibited the NOD-like receptor pyrin domain containing 3 (NLRP3) inflammasome, attenuating pyroptosis. RNA-seq analysis indicated that SAL downregulated the transcription of NLRP3 and GSDMD genes and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed that the NOD-like receptor signaling pathway was significantly enriched in down-regulated signaling pathways. Notably, SAL in combination with the NLRP3 inhibitor MCC950 did not further inhibit NLRP3 inflammasome and promote liver mass recovery. In summary, our findings proved that SAL could be a potential agent for improving liver function and promoting liver regeneration after PH.

1. Introduction

For malignant liver tumors, partial hepatectomy (PH) is the main course of treatment [1]. Following PH and other liver damage, liver regeneration is crucial to recovery. However, insufficient postoperative residual liver tissue results in the inability to adapt to the body's normal metabolic needs, and regeneration of the residual liver will be compromised, which in turn may lead to serious postoperative complications such as hepatic failure and small-for-size syndrome, which becomes an important cause of perioperative recovery and postoperative survival of patients [2]. Thus, developing strategies to avoid and manage postoperative liver failure is very crucial. Hepatocellular regeneration is the process of liver repair after partial loss of the liver caused by surgery, trauma, toxicity, infection, necrosis, or liver transplantation [3]. The remaining healthy liver tissue can regenerate and revert to a state that is similar to its initial size and function, even in cases where a significant section of the liver is removed. In mice after 2/3 PH, most of the liver recovers within 7–8 days and complete recovery within 3 weeks [4]. Liver injury is a complex process that negatively affects all hepatocytes, while other cells are recruited outside the liver to initiate and induce inflammatory and regenerative responses to injury [5]. Liver regeneration has a deep background in research and its mechanisms remain a hot research topic in medicine.

Rhodiola rosea is the dried root and stem of Rhodiola rosea grandiflora of the genus Sedum in the family Sedum, which is a traditional Tibetan medicine mainly grown at high altitudes of 3000–4000 m [6]. SAL is obtained from Rhodiola rosea through modernization separation and extraction and has anti-aging, anti-inflammatory, analgesic, immunomodulatory, and other pharmacological effects [7]. SAL promotes angiogenesis, thereby repairing the blood-brain barrier and treating Cerebral Small Vascular Disease (CSVD) [8]. SAL also increases osteogenesis and angiogenesis, which stops bone loss [9]. In addition, it has been demonstrated that SAL increases hepatocyte regeneration while reducing inflammatory reactions and abnormalities in hepatic lipid metabolism [10]. It's yet unknown, nevertheless, how SAL affects liver regeneration after PH.

Pyroptosis is mediated by the Gasdermin family proteins that facilitate the formation of cellular pores. Pyroptosis has the effect of causing

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cells to swell and rupture, releasing pro-inflammatory mediators, and triggering inflammation [11]. Typically, there are two phases in the inflammasome pathway: First, there is an initiation phase that depends on NF-κB activation. Secondly, the NLRP3 inflammasome forms, consisting of NLRP3, the ASC junction, and the effector cysteine asparaginase-1 (Caspase-1). NLRP3 inflammasome activates downstream Caspase-1, and activated Caspase-1 causes cleavage of Gasdermin D to form the N-terminal active structural domain of Gasdermin D (GSDMD-N), where IL-1β and IL-18 are secreted, causing an inflammatory response [12,13]. It has been reported that PH triggers Caspase-11/GSDMD-mediated pyroptosis, and that inhibition of GSDMD contributes to the recovery of liver mass [14]. However, whether SAL promotes liver regeneration after PH by inhibiting the NLRP3 inflammasome-mediated pyroptosis pathway remains uncertain.

Accordingly, this study aimed to determine whether SAL could promote liver regeneration after PH, and after determining its promoting effect, the possible mechanisms of action were further explored.

2. Materials and methods

2.1. Reagents

SAL with 98 % purity was from Nanjing Jinyibai Biological Technology Co., Ltd. And MCC950 was purchased from Shanghai Topscience Co., Ltd. Antibodies against PCNA, CCND1, pro Caspase-1, and cleaved Caspase-1 were obtained from ABclonal Technology Co., Ltd. Antibodies against GAPDH, β-actin, NLRP3, ASC, and GSDMD were provided by Proteintech Group, Inc. Anti-GSDMD-N was obtained from Cell Signaling Technology. IL-18, and IL-1β ELISA Kits were provided by YoBiBiotech Co., Ltd. Primers for RT-qPCR were from Shanghai Generay Biotech Co., Ltd.

2.2. Animals

Male C57BL/6 mice (6–8 weeks, 18–23 g) were acquired from SPF (Beijing) Biotechnology Co., Ltd (Beijing, China, SCXK (Jing) 2019–0010). Mice underwent a one-week acclimatization period before administration of the drug or surgery. All operations involving animals in this study have been authorized by the Animal Ethics Committee in the Affiliated Hospital of Nanjing University of Chinese Medicine (No.2023DW-073-01). Humanitarian care was given in line with the 3R principle during the experiment.

2.3. Partial hepatectomy (PH)

As previously reported [15], 70 % partial hepatectomy (PH) was carried out on mice. Anesthesia was induced in mice using 3–5% isoflurane. After proximal ligation, a midline incision was made in the upper abdomen to expose the liver fully. The left lateral, left median, right median liver lobes, and gallbladder were subsequently removed. Finally, the surgical incision was sutured.

2.4. Experimental design

Experiment I: Mice were randomly divided into 5 groups (n = 6 per group): Sham, PH, PH + SAL (100 mg/kg), PH + SAL (200 mg/kg), and PH + SAL (300 mg/kg) groups. Mice in the PH and PH + SAL groups were subjected to PH while mice in the sham group were only made a midline incision in the upper abdomen and then sutured. Mice in PH + SAL groups received intragastric administration of different doses of SAL once daily for 4 consecutive days before PH and 2 consecutive days after PH, while equal volumes of saline were gavaged into the mice in the PH and sham groups.

Experiment II: Mice were randomized into 3 groups (n = 6 per group): Sham, PH, and SAL groups. The SAL dose was the optimal dose from the conclusion in experiment I. The modeling method, dose and duration of administration were the same as in the previous experiment, and pyroptosis-related indexes were detected.

Experiment III: Mice were randomly assigned to five groups of six animals each in the experimental trial employing the NLRP3 inflammasome inhibitor MCC950 (MC): Sham, PH, SAL, MC, and SAL + MC groups. The SAL dose was the optimal dose as in the conclusion of experiment I. MCC950 was dissolved in saline, and the administration dose of 10 mg/kg was initiated 2 days before the surgery and continued for 2 days after the surgery. The other groups were treated as in experiment I.

2.5. Liver/body weight ratio

Two days after surgery, the mice were anesthetized, weighed, and then sacrificed. Residual livers were removed and weighed. The ratio of the weight of the residual liver to the body weight was the liver/body weight ratio.

2.6. Liver function

Blood taken from mouse eyeballs was centrifuged to obtain the supernatant. An auto-analyzer (Chemray-240, Rayto, China) was used to measure the levels of serum ALT and AST.

2.7. Immunohistochemistry staining

After paraffin-embedded liver tissue was divided into sections, it was dewaxed, antigenically repaired, and then it was incubated with antibodies against NLRP3 and Ki67 for an entire night at 4 °C. After that, it was rinsed with PBS and sealed at the normal temperature. After adding goat anti-rabbit/mouse secondary antibody that had been HRP-labeled, DAB staining was applied.

2.8. Quantitative real-time PCR

Similar to the previous study [10], RT-qPCR tests were conducted. Analysis of mRNA expression of target genes by ABI 7500 system. The primer sequences used are in Table 1.

2.9. Western blotting

Liver tissues were collected, thawed, homogenized with a tissue homogenizer, separated by centrifugation (12000 rpm, 4 °C, 15 min), and the BCA assay was employed to measure protein content. Proteins were separated by electrophoresis and transferred to PVDF membranes. Following a 5 % BSA blocking step, the membranes were treated for a whole night at 4 °C with various primary antibodies. After that, the membrane was treated for 1 h at room temperature with secondary antibodies after several washes. The membrane was observed by the chemiluminescent substrate.

Table 1
Primer sequences.

Primer name	Primer type	Sequence(5′-3′)
NLRP3	Forward	ATTACCCGCCCGAGAAAGG
	Reverse	TCGCAGCAAAGATCCACACAG
PYCARD	Forward	CTTGTCAGGGGATGAACTCAAAA
	Reverse	GCCATACGACTCCAGATAGTAGC
Caspase-1	Forward	CCAATAATGAATACAACCACTCGTACAC
	Reverse	CAGATCCTCCAGCAGCAACTTC
IL-1β	Forward	TCGCAGCAGCACATCAACAAG
	Reverse	TCCACGGGAAAGACACAGGTAG
β-actin	Forward	GGCTGTATTCCCCTCCATCG
	Reverse	CCAGTTGGTAACAATGCCATGT

2.10. Histopathological examination

Mouse liver tissues were embedded, sectioned, and stained with hematoxylin and eosin (H&E). To conduct observations, an optical microscope was used.

2.11. Enzyme-linked immunosorbent assay (ELISA)

Concentrations of IL-1β and IL-18 in mouse serum were determined using the corresponding kits. The procedure followed the kit instructions.

2.12. Transcriptomic sequencing analysis of liver tissue

Mouse liver samples were collected for RNA-seq analysis, and detailed experimental procedures were described in the previous study [16].

2.13. Statistical analysis

Measurements were consistent with normal distribution and displayed as mean ± SEM. GraphPad Prism 10 was used to conduct the statistical analysis. The one-way analysis of variance (ANOVA) and the Bonferroni post-hoc test were used to statistically examine the data. A difference that is statistically significant was determined to be $P < 0.05$.

3. Results

3.1. SAL promoted hepatocyte proliferation and contributed to liver mass recovery after PH

To evaluate the hepatic recovery of SAL after PH, we monitored the residual liver weight and body weight of mice 2 days after surgery. Among the different doses of SAL tested, the liver/body weight ratio was significantly higher in the 300 mg/kg dose group compared with the PH group. However, there was no statistically significant difference in liver/body weight ratio in the PH + SAL (100 mg/kg) and PH + SAL (200 mg/kg) groups as compared to the PH group (Fig. 1A). Consequently, further investigations to explore the mechanism of action of SAL were conducted using the administered dose of 300 mg/kg.

All phases of the process of cell proliferation involve the expression of Ki-67, whereas the quiescence phase does not [17]. Quantification of Ki-67 positive staining showed that hepatic expression of Ki-67 was significantly increased in the PH group compared with the Sham group. Of the different SAL concentrations tested, the 300 mg/kg dose was the most effective (Fig. 1B–C). These results revealed that the proliferation of hepatocytes was enhanced 2 days after PH under SAL intervention.

To observe hepatocyte proliferation and to ascertain the cell cycle of proliferating hepatocytes after PH, PCNA and cyclin D1 (CCND1) levels were evaluated among the various groups. In comparison to the PH group, PCNA and CCND1 expression were significantly elevated in the SAL dose groups, with no discernible changes observed in the Sham

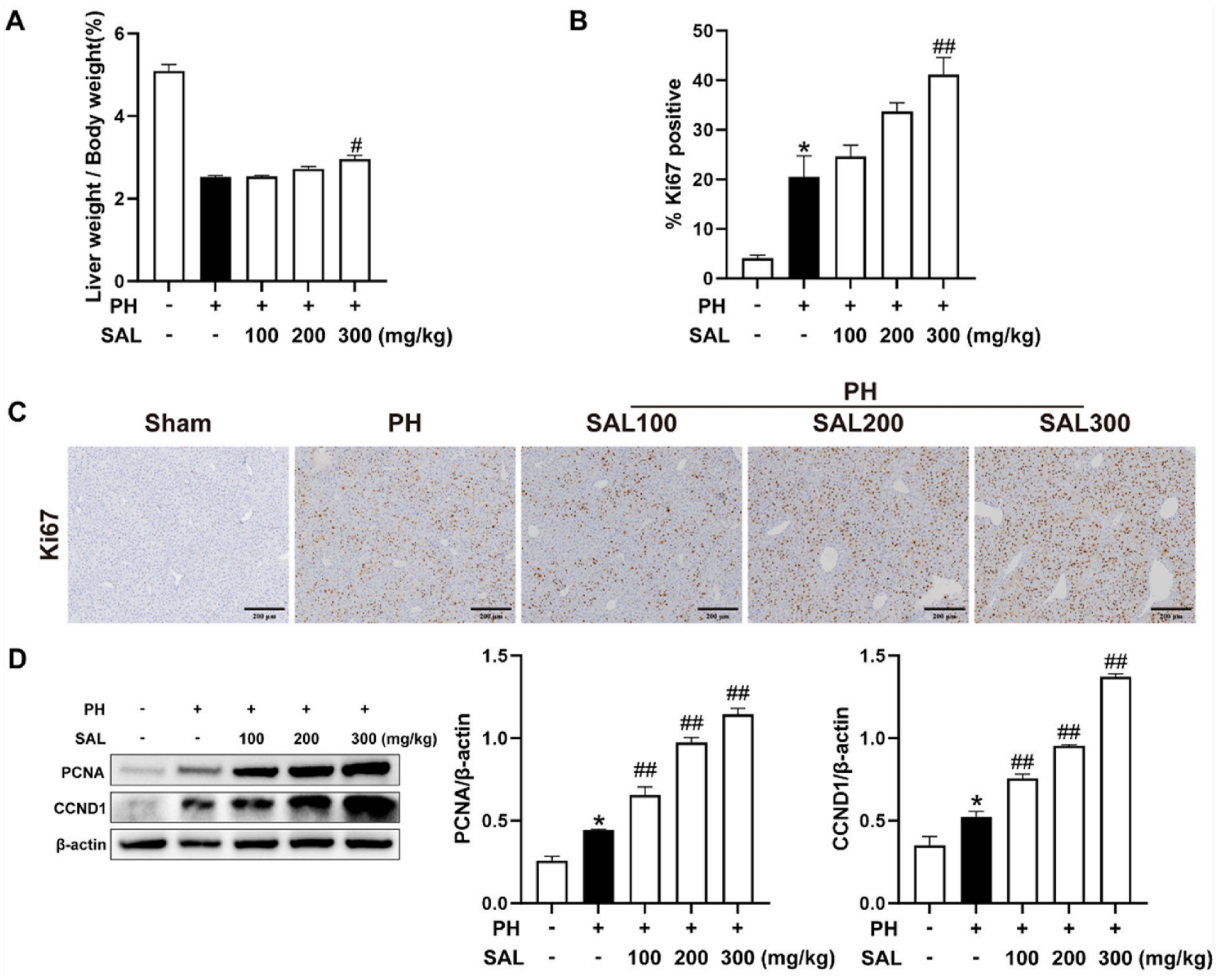

Fig. 1. SAL promoted hepatocyte proliferation and contributed to liver mass recovery after PH. (A) Liver/body weight ratio. (B–C) IHC staining of Ki67 and statistical analysis. Scale bar = 200 μm. (D) Western blot and statistical analysis of PCNA and CCND1. Values represent mean ± SEM (n = 3–6). *$P < 0.05$, **$P < 0.01$ vs. the Sham group; #$P < 0.05$, ##$P < 0.01$ vs. the PH group.

group. This indicates that SAL could facilitate the transition of hepatocytes from G0 to S phase and hepatocyte proliferation, with the most pronounced rise observed in the PH + SAL (300 mg/kg) group (Fig. 1D).

3.2. SAL attenuated postoperative liver injury in PH

When liver tissue is damaged, enzymes such as ALT and AST are released into the bloodstream. The detection of serum AST and AST can therefore be used to reflect the liver function. In our study, as expected, both ALT and AST levels were significantly increased in the PH group in comparison to the Sham group, whereas SAL suppressed the increase of ALT and AST in a dose-dependent manner. (Fig. 2A–B). H&E staining results demonstrated that liver tissues exhibited notable hepatocyte swelling, fatty degeneration, and nuclear consolidation after PH compared to the Sham group. Conversely, SAL application exhibited superior preservation of cytoplasmic and nuclear morphology, with the most pronounced effect observed at high doses, suggesting that SAL treatment may facilitate the repair of liver injury after PH (Fig. 2C).

3.3. SAL treatment inhibited the NLRP3 inflammasome-mediated pyroptosis pathway after PH

To examine the mechanism by which SAL facilitates hepatic regeneration, we conducted RNA sequencing analysis to examine SAL-mediated transcriptome profiles in PH-induced acute liver injury. A total of 1983 up-regulated genes and 2569 down-regulated genes were identified by differential gene expression analysis(Fig. 3A). Additionally, the heatmap indicated that the expression of NLRP3 and GSDMD was down-regulated following SAL treatment (Fig. 3B). GO enrichment analysis of the core genes showed that the biological processes of salidroside in attenuating acute liver injury and promoting liver regeneration after PH were related to “cellular metabolic process” and “metabolic process”, etc., the molecular functions were mainly related to “catalytic activity” and “binding”, etc., and the cellular component was mainly related to “intracellular” and “intracellular organelle”, etc. (Fig. 3C). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed that the NOD-like receptor signaling pathway was significantly enriched in down-regulated signaling pathways (Fig. 3D–E). As illustrated in Fig. 4, the NLRP3 inflammasome was shown to be activated after PH, as evidenced by a considerable increase in the protein levels of the inflammasome and the downstream effectors of the canonical NLRP3 pathway (pro Caspase-1, cleaved Caspase-1, and ASC) in the PH group as compared to the Sham group. Moreover, the mRNA levels of these indicators confirmed the results of western blot, and SAL supplementation reversed this increase. GSDMD and GSDMD-N protein levels were markedly elevated after PH, and SAL significantly attenuated their expression.

3.4. SAL inhibited the NLRP3 inflammasome-mediated pyroptosis pathway and promoted liver regeneration after PH, which imitated the effect of the NLRP3 inhibitor MCC950

Previous RNA sequencing revealed that salidroside may promote liver regeneration by inhibiting the NLRP3 inflammasome-mediated pyroptosis pathway. To further validate our speculation, we verified this effect using the NLRP3 inhibitor MCC950 and observed the effect of MCC950 on acute liver injury and liver regeneration after PH by inhibiting NLRP3 inflammasome. The results showed that the protein expression levels of GSDMD, GSDMD-N, NLRP3, ASC, pro Caspase-1 and cleaved Caspase-1 were significantly reduced in the SAL, MC, and SAL + MC groups compared with the PH group (Fig. 5A–C-H). NLRP3 IHC staining further confirmed the outcome (Fig. 5B). Compared to the SAL group, the GSDMD protein level was observed to be significantly decreased in the SAL + MC group(Fig. 5A–H). The hepatic NLRP3, PYCARD, Caspase-1, and IL-1β mRNA levels were significantly lower in the SAL and MC groups as compared to the PH group, according to the results of RT-qPCR(Fig. 5I–L). Furthermore, the combination of SAL and MC exhibited a further reduction in mRNA levels of these indices, yet no statistically significant differences were observed between the SAL, MC, and SAL + MC groups, which was consistent with the results of western blot analysis. ELISA results showed that the activity of serum IL-1β and IL-18 in the PH group was significantly increased compared with the Sham group. The SAL, MC, and SAL + MC groups inhibited the secretion of IL-1β and IL-18, but there was no significant difference among the three groups (Fig. 5M – N). After that, we analyzed liver regeneration in mice following the administration of SAL and the NLRP3 inhibitor

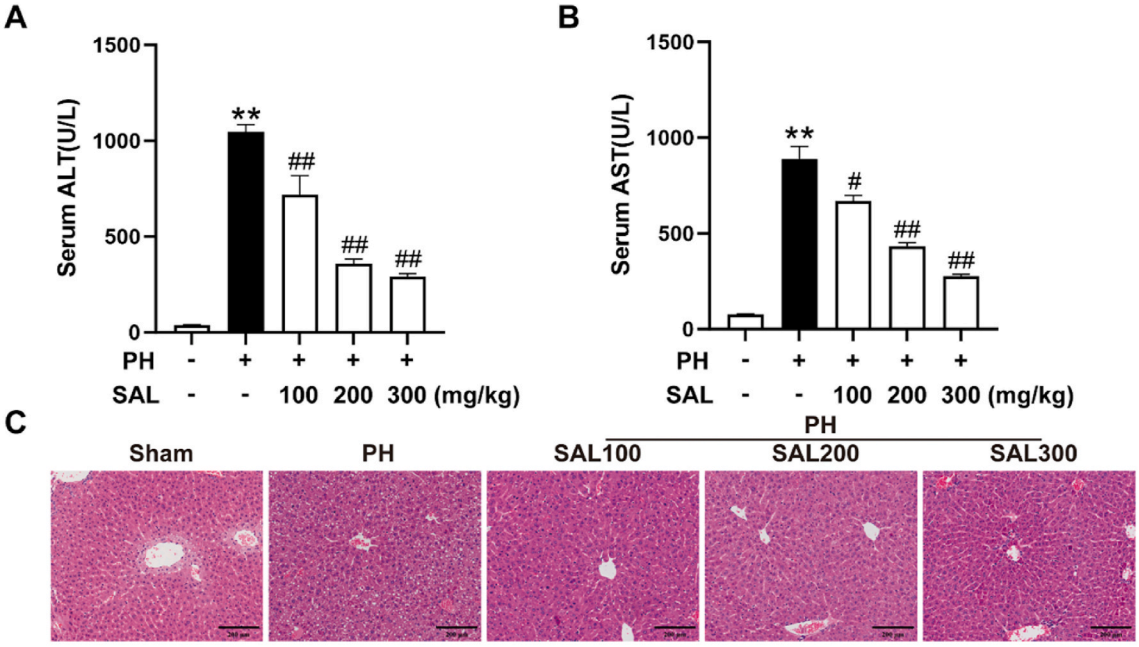

Fig. 2. SAL attenuated liver injury after PH. (A) Serum ALT activity. (B) Serum AST activity. (C) H&E stained liver sections. Scale bar = 200 μm. Values represent mean ± SEM (n = 3). $*P < 0.05$, $**P < 0.01$ vs. the Sham group; $\#P < 0.05$, $\#\#P < 0.01$ vs. the PH group.

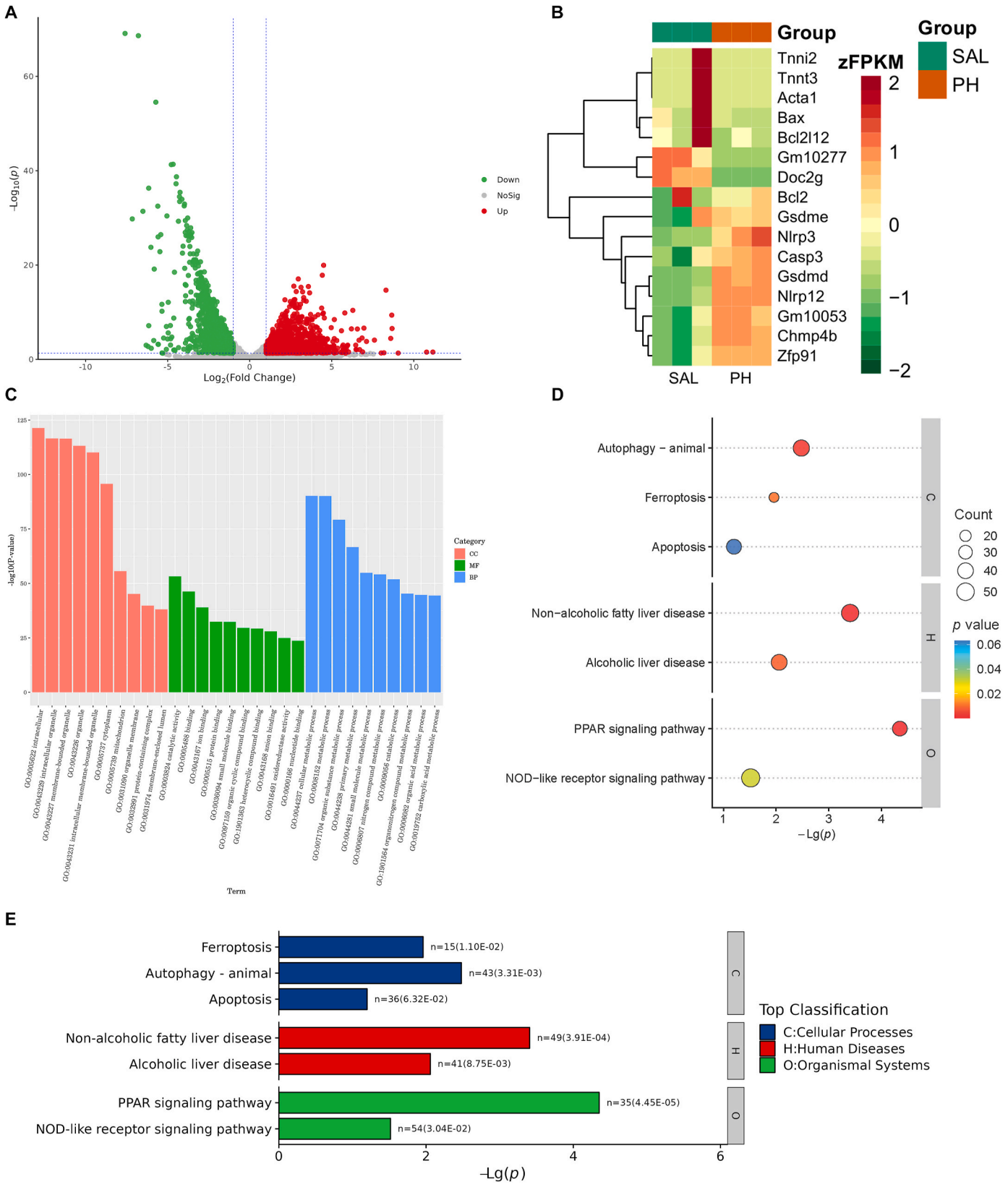

Fig. 3. Transcriptomics results showed that the NOD-like receptor signaling pathway was significantly enriched in down-regulated signaling pathways and SAL downregulated the transcription of NLRP3 and GSDMD genes in mice. (A) Volcano plot of differentially expressed genes. Red dots represent significantly up-regulated genes, green dots represent significantly down-regulated genes, and gray dots represent genes with no significant difference. (B) Hierarchical clustering heatmap of significant differentially expressed genes in the PH and the PH + SAL (300 mg/kg) groups of mice. (C) Bar graph of top 10 enrichment analysis of GO-CC, GO-MF, and GO-BP in the PH and the PH + SAL (300 mg/kg) groups of mice. CC: Cellular component; MF: Molecular function; BP: Biological process. (D) Bubble map for KEGG enrichment analysis in the PH and the PH + SAL (300 mg/kg) groups of mice. (E) Bar graph of KEGG enrichment analysis in the PH and the PH + SAL (300 mg/kg) groups of mice. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

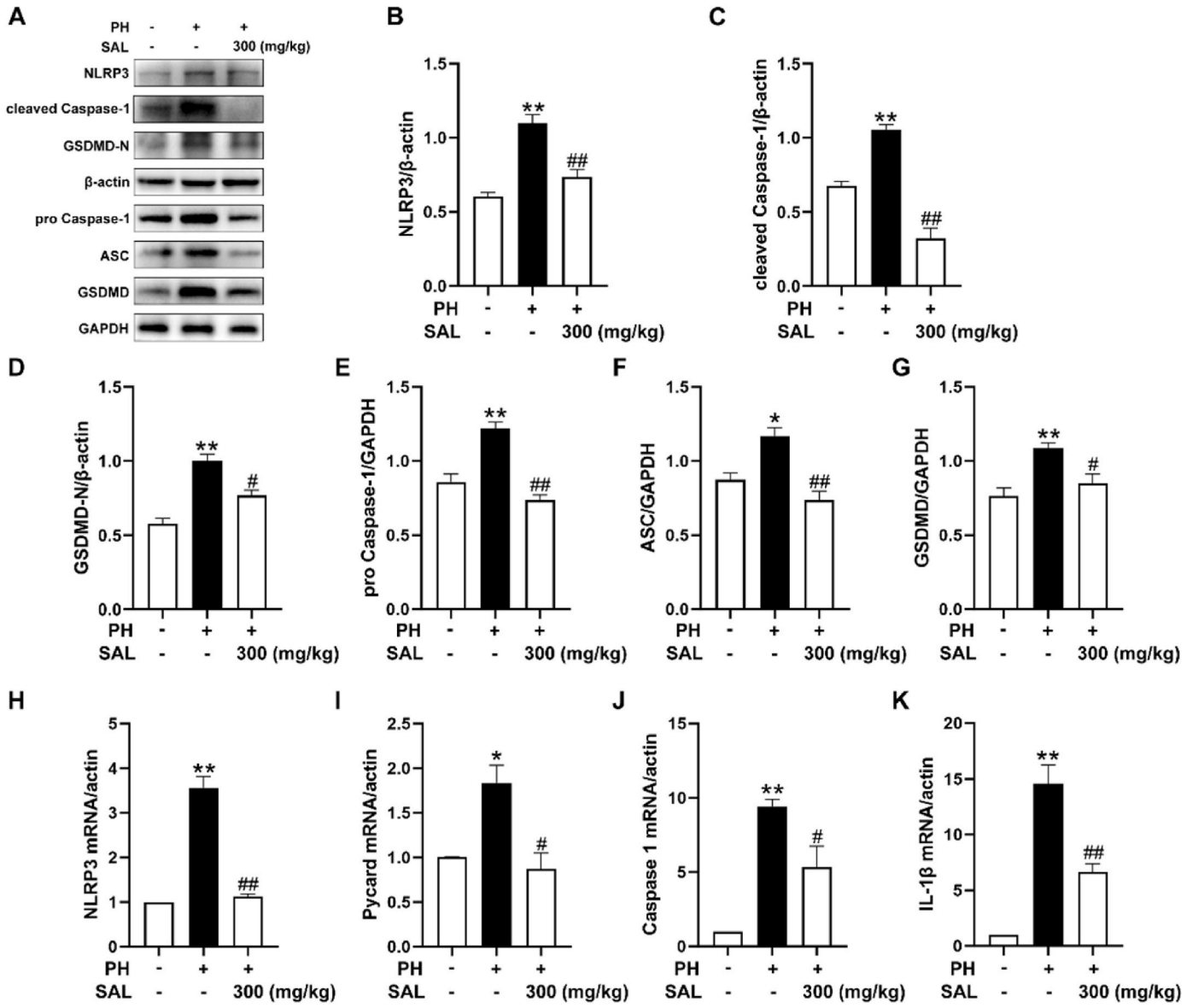

Fig. 4. SAL treatment inhibited the NLRP3 inflammasome-mediated pyroptosis pathway after PH. (A–G) Western blot and statistical analysis of NLRP3, pro Caspase-1, cleaved Caspase-1, ASC, GSDMD, and GSDMD-N. (H–K) RT-qPCR and statistical analysis of NLRP3, PYCARD, Caspase-1, and IL-1β. Values represent mean ± SEM (n = 3–5). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the Sham group; $\#P < 0.05$, $\#\#P < 0.01$ vs. the PH group.

MCC950 (Fig. 6). The SAL, MC, and SAL + MC groups exhibited significantly elevated liver/body weight ratios and Ki67-positive cell ratios, accompanied by markedly elevated protein levels of PCNA and CCND1, and significantly reduced serum ALT and AST levels. However, there was no discernible statistical difference between the SAL, MC, and SAL + MC groups. According to these findings, SAL inhibited the NLRP3 inflammasome-mediated pyroptosis pathway and promoted liver regeneration after PH, which imitated the effect of the NLRP3 inhibitor MCC950.

4. Discussion

Effective liver regeneration is necessary for the clinical use of PH in treating primary or metastatic liver cancers [18]. In the absence of external stimuli, hepatocytes exhibit minimal mitotic activity. However, upon exposure to stressors such as surgical resection and pharmacological agents, hepatocytes promptly respond to mitogens, enter the mitotic phase, and initiate proliferation. After rapidly returning to a state that does not inconvenience survival, the residual liver undergoes a period of slowed regeneration, after which it gradually regains its original size and function [19]. In our report, the levels of ALT and AST were significantly elevated after PH, and this trend was corroborated from a histological perspective, with a notable increase in liver histopathologic alterations and morphological damage. Examining the liver regeneration 2 days post-surgery, we discovered that, in comparison to the Sham group, the proportion of Ki67-positive cells in immunohistochemistry and the protein level expression of PCNA and CCND1 were both significantly higher. These results suggest that the experimental modeling was successful, that severe acute liver injury occurs after partial hepatectomy, and that the liver could regenerate through the body's own regulation.

SAL has been reported to inhibit cardiomyocyte apoptosis, prevent myocardial fibrosis, and ameliorate myocardial ischemia [20,21], as well as antidepressant or ameliorate Parkinson's disease by inhibiting pyroptosis [22,23]. SAL also exerts a protective influence against a range of liver diseases, including those caused by non-alcoholic fatty liver disease, autoimmune hepatitis, chemicals and alcohol, hepatic fibrosis, and hepatic malignancies [24–29]. Here, we observed the impact of SAL

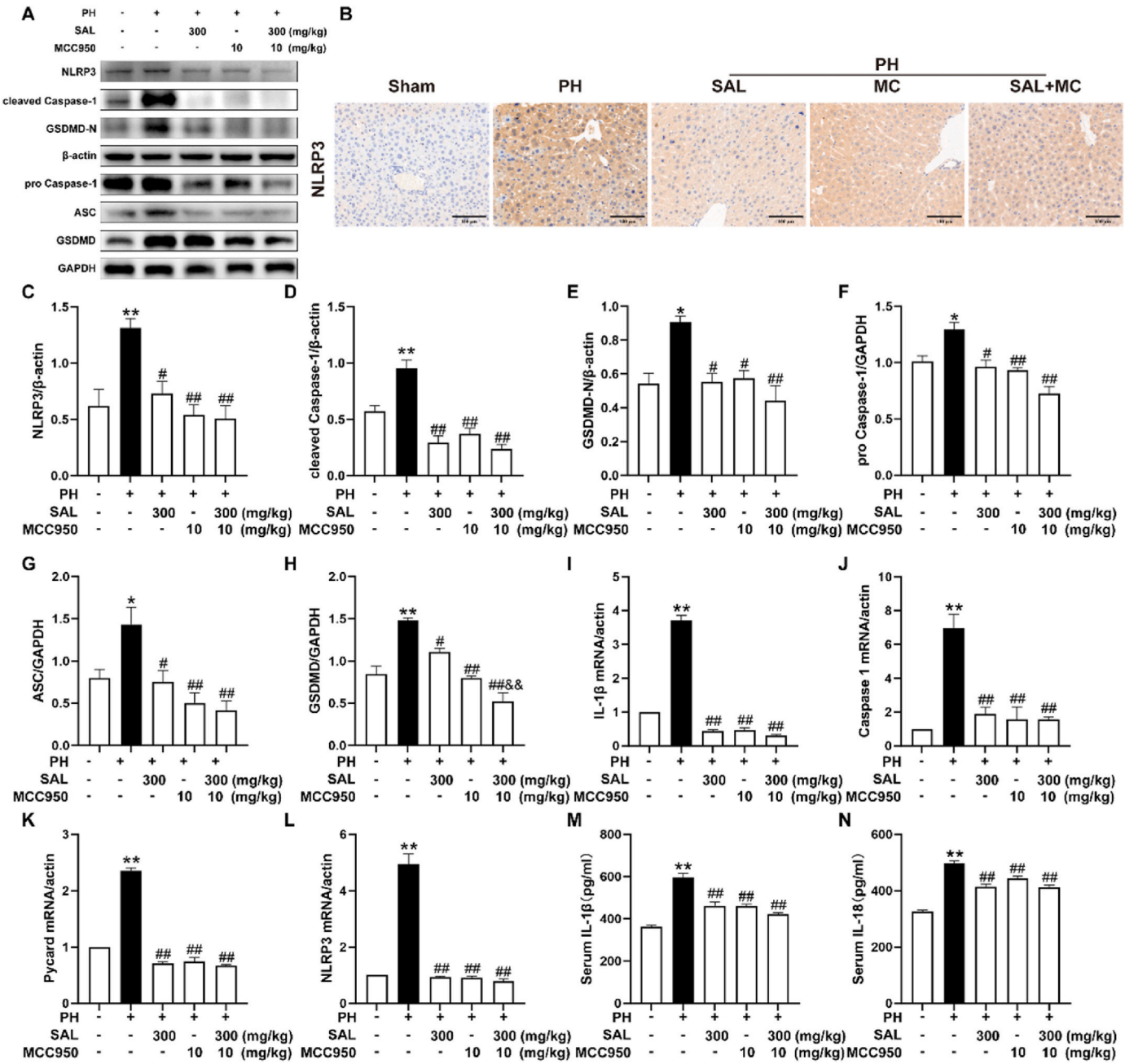

Fig. 5. The NLRP3 inhibitor MCC950 in combination with SAL inhibited the NLRP3 inflammasome-mediated pyroptosis pathway after PH. (A, C–H) Western blot and statistical analysis of NLRP3, pro Caspase-1, cleaved Caspase-1, ASC, GSDMD, and GSDMD-N. (B) IHC staining for NLRP3. Scale bar = 100 μm. (I–L) RT-qPCR and statistical analysis of IL-1β, Caspase-1, PYCARD, and NLRP3. (M − N) Levels of serum IL-1β and IL-18. Values represent mean ± SEM (n = 3–5). $*P < 0.05$, $**P < 0.01$ vs. the Sham group; $\#P < 0.05$, $\#\#P < 0.01$ vs. the PH group. $\&\&P < 0.01$ vs. the SAL group.

on liver regeneration 2 days after PH and provided evidence that SAL treatment attenuated acute liver injury after PH and promoted hepatocyte proliferation and liver mass recovery. It indicated that the liver/-body weight ratio notably increased following SAL treatment. Additionally, the levels of PCNA, CCND1, and the proportion of Ki67-positive cells exhibited a significant increase, indicating that SAL facilitated the proliferation of hepatocytes after PH, which was conducive to the recovery of liver weight. Furthermore, the superior maintenance of cytoplasm and nucleus morphology in liver pathological sections and the significant decline in ALT and AST levels indicated that SAL could effectively mitigate acute liver injury.

The mechanisms underlying liver regeneration after PH remain complex and not yet fully elucidated. A previous study has shown that apoptosis hinders liver regeneration, and it has also been suggested that pyroptosis and necrotic apoptosis are involved in this process, but it remains uncertain [19]. According to a recent study, dexmedetomidine inhibited NLRP3 inflammasome activation, which enhanced hepatic regeneration [30]. Similarly, dopamine may inhibit NLRP3 inflammasome and enhance hepatic regeneration in acute liver failure patients [31]. However, a study has also shown that NLRP3 could promote liver regeneration after PH [32]. Therefore, it is still debatable whether NLRP3 inflammasome promotes or prevents liver regeneration. It was also found that NLRP3 and ASC expression was downregulated, but Caspase-1 expression was upregulated at 6 h, 12 h, and 18 h after APAP-induced acute liver injury [33]. By contrast, our findings indicated that NLRP3 inflammasome pathways in the liver are significantly

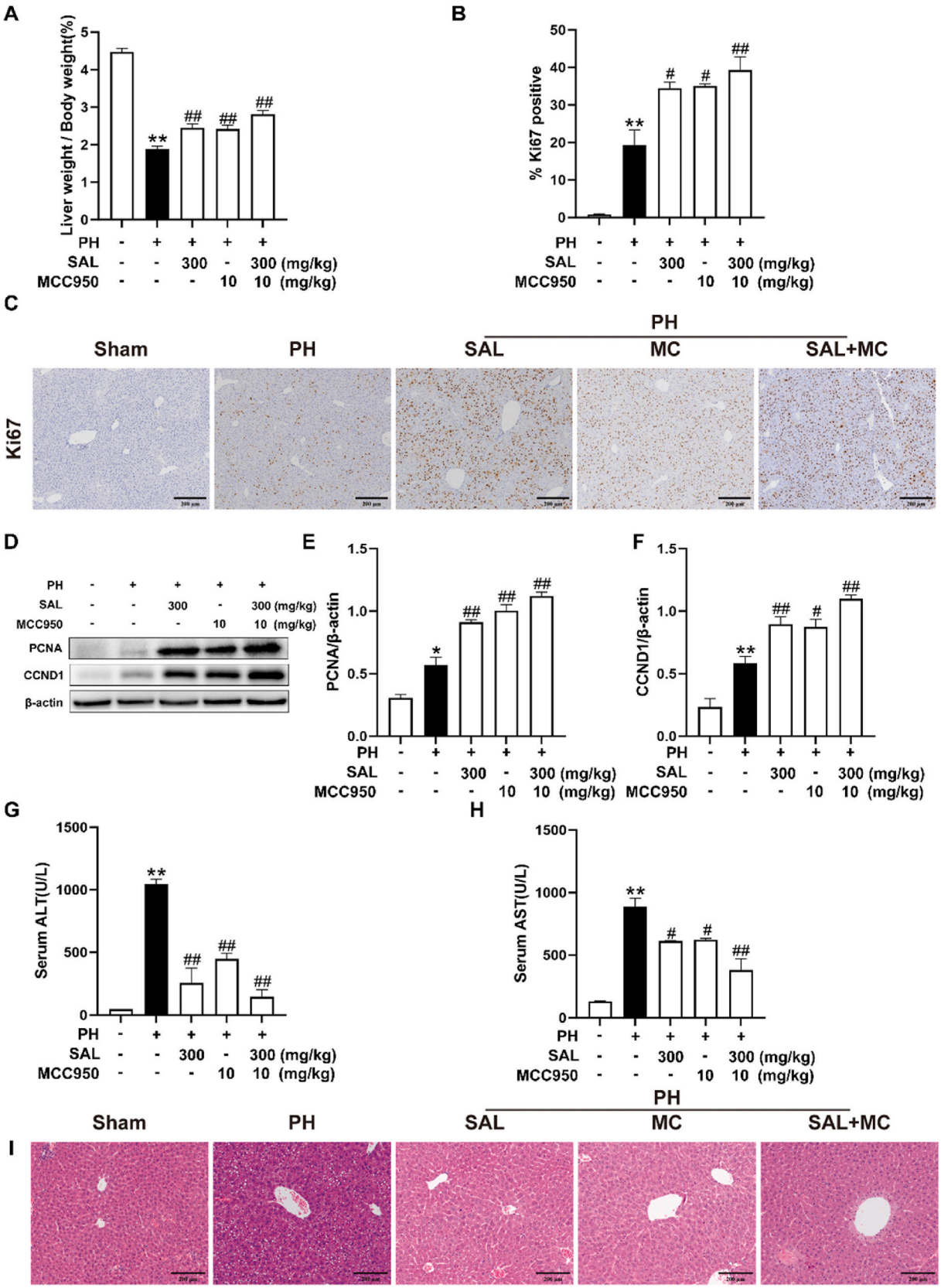

Fig. 6. The NLRP3 inhibitor MCC950 in combination with SAL promoted liver regeneration after PH. (A) Liver/body weight ratio. (B–C) IHC staining of Ki67 and statistical analysis. Scale bar = 200 μm. (D–F) Western blot and statistical analysis of PCNA and CCND1. (G) Serum ALT activity. (H) Serum AST activity. (I) H&E stained liver sections. Scale bar = 200 μm. Values represent mean ± SEM (n = 3–6). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the Sham group; $\#P < 0.05$, $\#\#P < 0.01$ vs. the PH group.

activated after PH, suggesting that suppression of NLRP3 inflammasome activation may be a useful tool to halt the progression of complications such as PH-induced liver failure. It is noteworthy that SAL was observed to significantly inhibit the inflammasome inflammatory cascade in the liver, which may be a component of its hepatoprotective mechanism. Consistent with the findings of this study, a prior study [34] employed SAL as a positive control drug and found that SAL significantly decreased the expression of pyroptosis-associated proteins after liver injury caused by high-altitude hypoxia. After applying the NLRP3 inhibitor MCC950, significant suppression of the NLRP3 inflammasome was observed in both MC and SAL + MC groups. It should be noted that the expression levels of NLRP3, ASC, GSDMD, Caspase-1, IL-1β, and IL-18 in the SAL + MC group were found to be lower than those in the SAL and MC groups. However, statistical significance was not achieved. MCC950 is the inhibitor of NLRP3, and SAL also acts primarily on NLRP3 inflammasome, so we speculated that the redundancy of effects when the two are combined would prevent a superimposed effect.

Moreover, several points about this study require clarification. Liver regeneration encompasses three distinct processes: the initiation phase, during which quiescent hepatocytes transition from the G0 phase to the G1 phase in response to various stimuli; the proliferation phase, during which hepatocytes progress from the G1 phase to undergo mitosis; and finally, they enter the termination phase under the modulation of some negative regulators, such as TGF-β [35]. In rats, the first peak in DNA replication occurs after 24 h of PH, the second peak occurs between 36 and 48 h, and the fastest cell division occurs at 36 h after PH. However, the peak in mice occurs 12–16 h later than in rats [36,37]. In addition, one study reported that Ki67 and PCNA expression peaked 2 days after PH and then began to decline [38]. Therefore, in this study, we chose to sacrifice mice 2 days after PH to collect liver samples. Secondly, three concentration gradients were selected for the dosage of SAL, with specific reference to previous studies [39–41]. Finally, there are numerous models of liver regeneration, which are mainly categorized into the 70 % hepatectomy model, repeated hepatectomy model, extreme hepatectomy model, chemical injury model, and portal vein branch ligation model. The 70 % hepatic resection model is the most classical hepatic resection model, also known as the 2/3 hepatic resection model. Inderbitzin et al. [42] realized the model of 26 %, 60 %, 75 %, and 83 % PH, and found that the best liver regeneration effect was achieved after 75 % PH. The 70 % PH model was employed in numerous experiments investigating liver regeneration [38,43,44], and thus was also used in our study.

5. Conclusion

In conclusion, our data indicated that SAL may promote liver regeneration after PH by suppressing NLRP3 inflammasome and thereby attenuating pyroptosis, and may serve as a potential agent to mitigate acute liver injury.

CRediT authorship contribution statement

Saiya Zhang: Writing – original draft, Methodology. **Meilu Yu:** Methodology, Data curation. **Fen Wang:** Methodology. **Sha Li:** Data curation. **Xuefei Li:** Software. **Hongyu Hu:** Software. **Zhen Zhang:** Validation. **Xiangpeng Zhu:** Validation. **Weiqian Tian:** Supervision, Resources, Methodology.

Declaration of competing interest

The authors declare that there are no financial conflicts of interest.

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