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Maresin1 Inhibits Ferroptosis via the Nrf2/SLC7A11/GPX4 Pathway to Protect Against Sepsis-Induced Acute Liver Injury

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Purpose: Maresin 1 (MaR1) is a specialized pro-resolving mediator with anti-inflammatory properties that promotes tissue repair. This study aims to investigate the molecular involvement of MaR1 in protecting against sepsis-induced acute liver injury (SI-ALI).

Methods: In vivo, a murine SI-ALI model was established using the cecal ligation and puncture (CLP) paradigm, providing a system in which the mechanistic functions of MaR1 could be tested. These analyses were supplemented through in vitro assays in which Alpha mouse liver 12 (AML12) hepatocytes and RAW264.7 macrophages were co-cultured in a Transwell system, with lipopolysaccharide (LPS) stimulation being used to establish a sepsis model. These cells were treated with MaR1 and/or nuclear factor erythroid 2-related factor 2 (Nrf2)inhibitor, while lentiviral transduction was used to knock down Nrf2 within AML12 cells. Hepatic pathological damage was assessed through hematoxylin and eosin staining. Lipid peroxidation-related analyses were conducted through the use of thiobarbituric acid, ferrous ions, glutathione, and appropriate fluorescent probes for reactive oxygen species detection. Liver enzymes and inflammatory mediators were quantified using appropriate Enzyme-Linked Immunosorbent Assays (ELISAs). Protein concentrations were evaluated via Western blot analysis.

Results: The presence of ferroptosis in SI-ALI. MaR1 was found to proficiently suppress ferroptosis in SI-ALI. Mechanistically, MaR1 enhanced Nrf2 expression in AML12 hepatocytes, while the Nrf2 inhibitor ML-385 or Nrf2 siRNA mitigated MaR1's regulatory influence on ferroptosis. Meanwhile, the expressions of the downstream genes solute carrier family 7 member 11 (SLC7A11) and glutathione peroxidase 4 (GPX4) diminished, suggesting that MaR1 has a protective function via activating the Nrf2/SLC7A11/GPX4 pathway to mitigate ferroptosis in septic liver injury.

Conclusion: The results indicate that MaR1 mitigates SI-ALI via stimulating the Nrf2/SLC7A11/GPX4 pathway to suppress ferroptosis. Moreover, it offers significant potential as a new agent for the prevention of SI-ALI.

Keywords: Maresin1, sepsis-induced acute liver injury, ferroptosis, Nrf2, inflammation

Introduction

Sepsis is a serious condition characterized by unrestrained inflammation and immune dysregulation in response to infection, and it represents a persistent threat to public health owing to its ability to cause life-threatening organ dysfunction.[1] The unique anatomical position of the liver, its rich blood supply, and its important role in cytokine production, immunity, and bacterial clearance make it a central site for the coordination of inflammatory and immunological activity.[2] Inflammatory dysregulation associated with sepsis commonly impacts the liver,[3] with liver dysfunction being apparent in 34–46% of all sepsis patients.[4] SI-ALI can arise from various mechanisms, with increased oxidative stress linked to a systemic inflammatory response playing a crucial role in liver damage. In sepsis, the liver-resident Kupffer cells, a type of macrophage, can be activated and release substantial amounts of reactive oxygen species (ROS). While these active substances aid in eliminating pathogens, they can also inflict damage on hepatocytes.

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As a form of iron-dependent cell death distinct from apoptosis, ferroptosis entails dysregulated iron metabolism within cells that coincides with lipid peroxide accumulation.[5] Iron metabolism disorders and redox equilibrium disturbances during sepsis can cause ferroptosis in cells, which immune cells can then identify and initiate a cascade of inflammatory or targeted immunological responses.[6] The close relationship between sepsis and ferroptosis highlights the increasingly important role of ferroptosis in sepsis-related organ damage including the lungs,[7,8] kidneys,[9] heart,[10] and liver.[11] As a result, investigating ferroptosis as a novel aspect of cell death research, especially its involvement in sepsis-induced liver injury, may facilitate the identification of more therapeutic targets and biomarkers associated with sepsis, thereby enhancing patient outcomes.

The transcription factor Nrf2 is the central regulator of redox homeostasis within cells,[12] and the antioxidant responses that it can induce are closely tied to the suppression of ferroptotic cell death. Nrf2 is capable of shielding cells from toxic damage caused by ROS through the regulation of cytoprotective and antioxidant genes.[13] Nrf2, under basal conditions, is bound by Kelch-like ECH-associated protein 1 (Keap1) and subsequently undergoes degradation. Oxidative stress exposure and other stressors, however, can trigger the dissociation of Nrf2 from Keap1 whereupon it translocates into the nucleus and binds to antioxidant response element (ARE) sequences in gene promoters, resulting in the upregulation of appropriate target genes.[14]

MaR1 is an endogenous polyunsaturated fatty acid-derived pro-resolving lipid mediator with robust anti-inflammatory and antioxidant characteristics.[15] MaR1 has previously been shown to activate Nrf2-related signaling pathways to help protect against hepatic fibrosis, sepsis-induced cardiac injury, and ischemia-reperfusion injury.[16–18] Despite prior evidence of the hepatoprotective functions of MaR1,[19–21] its role in the context of SI-ALI has yet to be documented. Here, MaR1 was found to inhibit ferroptotic activity by activating the Nrf2/SLC7A11/GPX4 signaling axis, ultimately protecting against SI-ALI.

Material and Methods

Drugs and Chemicals

Ferrostatin-1 (Fer-1), Maresin1, and ML385 were obtained from MedChemExpress (NJ, USA), and LPS (L2880) was purchased from Sigma Aldrich (USA). Western immunoblotting was performed using anti-GPX4 (HY-P80450, MCE), anti-SLC7A11 (A13658, Abclonal), anti-PTGS2 (BA3708, BOSTER), anti-Nrf2 (80593-1-RR, Proteintech), anti-Keap1 (80744-1-RR, Proteintech), anti-F4/80 (ab300421, abcam), and anti-β-Actin (R1207-1, Huabio), HRP Conjugated Goat anti-Rabbit IgG polyclonal Antibody(HA1001, Huabio), iFluor™ 488 Conjugated Goat anti-rabbit IgG Goat Polyclonal Antibody (HA1121, Huabio). DMEM/F12 and DMEM were purchased from Gibco (USA), and fetal bovine serum from CLARKBIO (USA). The ITS (PWL084) was acquired from Meiluncell (China), and the Nrf2 knockdown (Nrf2 KD) clone lentiviral particle was obtained from Genechem Co. LTD (Shanghai, China).

Experimental Animals

Male C57BL/6J mice (20–25 g, 8–10 weeks old) from SiPeiFu (Beijing) Bio-Tech Co., Ltd. (Animal License No. SCXK (Jing) 2019–0010) were housed under controlled conditions (21±2°C, 60±5% humidity, 12 h light/dark cycle) with free access to standard chow and water. This study and the included experimental procedures were approved by the Animal Welfare Ethics Committee of the School of Public Health at Jilin University (approval no. 2024-09-004). All animal housing and experiments were conducted in strict accordance with the Laboratory animal—Guideline for ethical review of animal welfare (GB/T 35892–2018).

Experimental Design

The mice were randomized into six groups (n=6/group): (1) Sham+DMSO (200 μL i.p., 24 h before surgery), (2) CLP +DMSO (200 μL i.p., 24 h before surgery), (3) CLP+Fer-1 (5 mg/kg i.p., 24 h before surgery), (4) CLP+MaR1 (100 ng/mouse i.p., 1 h after surgery), (5) Sham+Fer-1 (5 mg/kg i.p., 24 h before surgery), and (6) Sham+MaR1 (100 ng/mouse i.p., 1 h after surgery) groups.

In vivo Modeling of Sepsis

CLP was used to model sepsis. Briefly, 2% pentobarbital sodium (50 mg/kg) was intraperitoneally injected for anesthesia, after which a 1–1.5 cm longitudinal incision was made along the abdominal wall midline. The cecum was then dissected layer-by-layer, after which it was gently pulled out of the abdominal cavity, feces were moved to the distal cecum, and a 3–0 surgical suture was used for ligation of the proximal half of the cecum. Then, a 21G needle was used to generate two bloodless punctures in the distal cecum, and forceps were then used to compress the cecum such that feces was expelled through the site of the puncture. The cecum was then replaced in the abdominal cavity, and 3–0 sutures were used to close the muscle and skin layers. Mice were then injected into the scruff with 1.0 mL of warm physiological saline. Mice in the sham group underwent laparotomy but not cecal ligation or puncture. After this procedure, mice in the CLP group exhibited reduced activity, lethargy, dull furr, diarrhea, and the impairment of liver function, consistent with successful modeling.

Cells Culture

AML12 hepatocytes and RAW264.7 macrophages purchased from Shanghai Fuheng Biotechnology Co., Ltd. were used for this study. AML12 cells were cultured in DMEM/F12 with 10% FBS and 1% insulin-transferrin-selenium (ITS), while RAW264.7 macrophages were cultured in high-glucose DMEM containing 10% FBS.

Co-Culture System

AML12 cells (5×10^5 per mL) were added to 6- or 24-well plates, while RAW264.7 macrophages (1×10^5 per mL) were added to transwell inserts (24 or 6.5 mm in diameter and 0.4-μm pore size; Corning Inc., NY).

Serum Assays

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), TNF-α, and IL-6 levels were assessed with ELISA kits (Shanghai Enzyme Linked, China) used as directed.

Haematoxylin–Eosin (H&E) Staining

The left liver lobe was harvested, fixed using 4% paraformaldehyde, dehydrated with an ethanol gradient, paraffin-embedded, and cut to produce 5 μm sections. Hematoxylin and eosin (H&E) staining of liver tissue sections was performed, after which they were imaged under an optical microscope (Nikon, Japan). The extent of liver damage was assessed by two independent technicians who were unaware of the experimental group's protocol. Samples were randomly assigned to different evaluators for blind scoring.

Immunohistochemical (IHC) Staining

An UltraSensitive™ SP immunohistochemistry kit (KIT-9720, Fuzhou Maixin Biotechnology Co., Ltd.) was utilized as directed for the F4/80, Nrf2 and Keap1 IHC staining of liver tissue samples, after which they were assessed with a fluorescence microscope.

Immunofluorescence

Fix the cells with 4% paraformaldehyde and block with 5% BSA, then incubate overnight at 4 degrees Celsius with Keap1 (1:500) and Nrf2 (1:200). Subsequently, incubate with the secondary antibody (1:500) at room temperature for 1 hour. Stain the cell nuclei with DAPI. Finally, capture images using a fluorescence microscope.

ROS Analyses

DCFH-DA was used as a fluorescent probe to detect ROS. Cells in 6-well Transwell systems were treated for 24 h using LPS and/or MaR1, rinsed three times using serum-free media, and incubated in the dark for 30 min with DCFS-DA at 37°C. After three additional washes, they were visualized with a live cell imaging system.

BODIPY 581/591 C11 Staining

Lipid peroxidation level was assessed using BODIPY 581/591 C11 staining (L267; Dojindo Laboratories, Japan). The stained cells were analyzed using a microscope.

FerroOrange

To detect cellular Fe2+, FerroOrange (F374; Dojindo Laboratories, Japan) was used according to the manufacturer's protocol. AML12 cells were treated with chemicals and then stained with a final concentration of 1 μmol/L FerroOrange for 30min at 37 °C. Images were acquired using a microscope.

Measurement of Malondialdehyde (MDA), Reduced Glutathione (GSH), and Oxidized Glutathione Disulfide (GSSG)

According to the manufacturer's instructions, the levels of MDA in liver tissues and AML12 cells were detected using a lipid peroxidation MDA assay kit (BC0025; Solarbio, Beijing, China). GSH and GSSG levels in liver tissues and AML12 cells were measured using a commercially available GSH and GSSG assay kit (S0053; Beyotime, Shanghai, China).

Western Blotting

RIPA buffer with protease and phosphatase inhibitors was used to extract proteins from cells or tissues, with a BCA assay then being used for quantification. Following dilution using 5× protein loading buffer, samples were boiled for 5 min, separated by SDS-PAGE, and transferred onto PVDF membranes. These blots were next blocked using 5% skim milk, probed with primary antibodies at 4°C overnight, rinsed with TBST, incubated for 2 h with secondary antibodies, and visualized with an ECL reagent kit. An imaging system was employed to assess band intensity.

CCK-8 Assay

Cellular viability was analyzed with a Cell Counting Kit-8 assay (MCE, China) as directed by the manufacturer.

Cells Transfection Assay

AML12 hepatocytes were cultured in DMEM/F12 (Gibco, USA) supplemented with 10% fetal bovine serum and incubated at 37 °C in a 5% CO_2 environment. Lentiviral particles for Nrf2 knockdown (Nrf2 KD) were transfected into AML12 hepatocytes to establish stable Nrf2 KD cell lines. The transfection of Nrf2 was executed according to the provided protocols, with cells transfected with a scramble sequence serving as negative controls.

Statistical Analysis

GraphPad Prism (9.0; GraphPad Software, CA, USA) was used for statistical analyses. Results are presented as means ± SD. Three or more replicates were required for all results. We used one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to compare multiple groups of data. $P < 0.05$ and $P < 0.01$ were respectively considered significant and highly significant.

Results

A CLP-Induced Murine SI-ALI Model is Characterized by Ferroptosis

Initially, liver damage following CLP modeling was assessed. H&E staining of liver tissue samples confirmed the CLP-induced liver damage in these mice, as evidenced by the swelling, disorganization, and necrotic death of hepatocytes (Figure 1A). Immunohistochemical F4/80 staining revealed significant macrophage accumulation in the liver (Figure 1A). Serum analyses also indicated high levels of AST and ALT elevation in the CLP group relative to sham control mice (Figure 1B). CLP is thus capable of inducing significant inflammatory damage. Liver tissues were then collected from these mice for ferroptosis-related analyses, revealing that at 24 h post-CLP modeling, ferroptosis-related

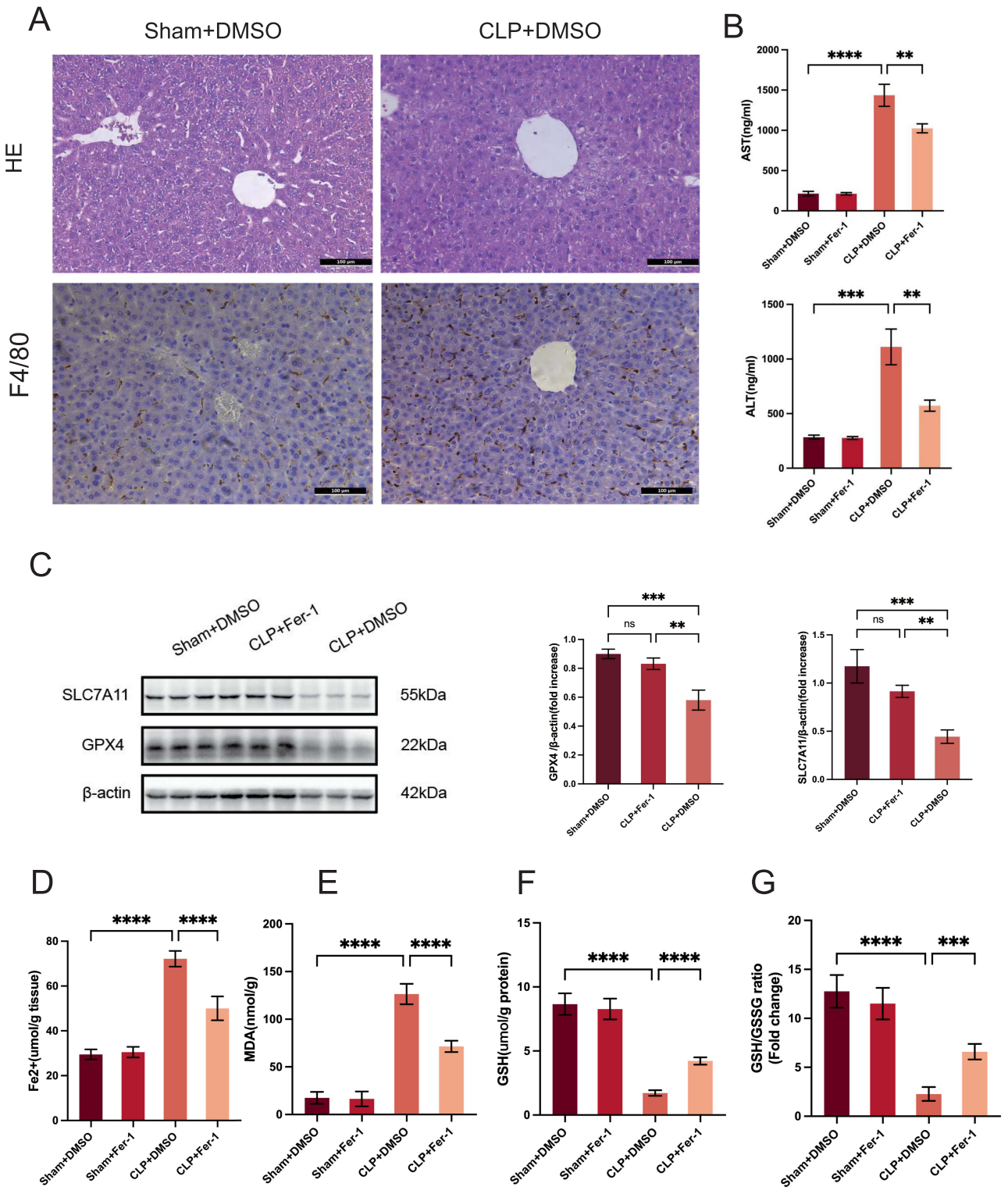

Figure 1 CLP induces ferroptosis in a septic acute liver injury in mice. (**A**) Liver tissue samples were stained with H&E or via IHC to detect F4/80 in the livers of mice that were or were not subjected to CLP modeling. (Original magnification, ×200, Scale bar, 100 μm). (**B**) Serum ALT and AST levels, n = 6. (**C**) GPX4 and SLC7A11 levels were detected by Western immunoblotting, n = 3. Data are means ± SD from three replicates. (**D**) Tissue iron levels. (**E–G**) Hepatic MDA, GSH, and GSH/GSSG levels. ns means no significant, **p < 0.01, ***p < 0.001, ****p< 0.0001.

GPX4 and SLC7A11 levels were markedly reduced (Figure 1C), while iron ion levels were substantially elevated as were malondialdehyde levels indicative of lipid peroxidation. GSH levels and the GSH/GSSG ratio were also reduced in these animals (Figure 1D–G). Ferroptosis thus occurs in the livers of these mice following CLP modeling.

MaR1 and Fer-1 Protect Against CLP-Induced Acute Liver Injury in Mice

Fer-1 is an established ferroptosis inhibitor that is widely used in research settings. In this study (Figure 2A), the treatment of mice with both Fer-1 and MaR1 was sufficient to protect against CLP-induced liver damage, as determined based on histological staining and the quantification of serum AST and ALT levels (Figure 2B and C).

MaR1 Inhibits Ferroptosis in a Murine Model of CLP-Induced Liver Injury

The effect of MaR1 on CLP-induced hepatic ferroptosis was next evaluated. While CLP-induced mice presented with reduced hepatic GPX4 and SLC7A11 expression, pretreatment with MaR1 reversed these changes in ferroptosis-related protein expression (Figure 3A). MaR1 also reduced hepatic iron ion and MDA levels in these CLP mice while increasing the levels of GSH and GSH/GSSG (Figure 3B–E). MaR1 is thus capable of significantly inhibiting hepatic ferroptosis in this CLP mouse model system. To further investigate the potential mechanisms through which MaR1 inhibits liver ferroptosis, we performed an analysis of Keap1 and Nrf2 in liver tissue using IHC. The results showed that MaR1 treatment resulted in a decrease in Keap1 expression and an increase in Nrf2 expression in the livers of CLP mice (Figure 3F). Thus, MaR1 may exert a hepatoprotective effect by activating Nrf2 and subsequently inhibiting ferroptosis.

MaR1 protects against the release of in vitro inflammatory mediators and prevents LPS-induced hepatocyte ferroptosis

Lipopolysaccharide (LPS) is a primary driver of sepsis-related inflammation and organ damage. To test the effects of MaR1 on hepatocyte ferroptosis in vitro in the context of sepsis, murine macrophages were stimulated with LPS and then co-cultured with murine hepatocytes, thereby simulating an inflammatory microenvironment (Figure 4A). An LPS dose of 1.0 μg/mL was selected for this model based on prior publications, and the effects of various LPS and MaR1 doses on RAW264.7 and AML12 cell viability were also confirmed via CCK-8 assay (Figure 4B and D). At the 1.0 μg/mL LPS dose level, AML12 viability was not significantly impacted. However, when AML12 and RAW264.7 cells were co-cultured, LPS was sufficient to significantly reduce AML12 cell viability (Figure 4C). Supernatant TNF-α and IL-6 levels were notably elevated under these conditions (Figure 4E and F), supporting a key role for macrophages as mediators of inflammatory injury to hepatocytes culminating in sepsis-induced liver damage. We further examined the ferroptosis phenotypes, and a decrease in the expression of SLC7A11 and GPX4 was detected (Figure 4G–I). Meanwhile, LPS led to an increase in the accumulation of iron ions (Figure 4J), and also increased the MDA and ROS levels in AML12 cells (Figure 4K and M), reduced the GSH/GSSG ratio (Figure 4L), and When BDP 581/591 C11 was used as a probe to

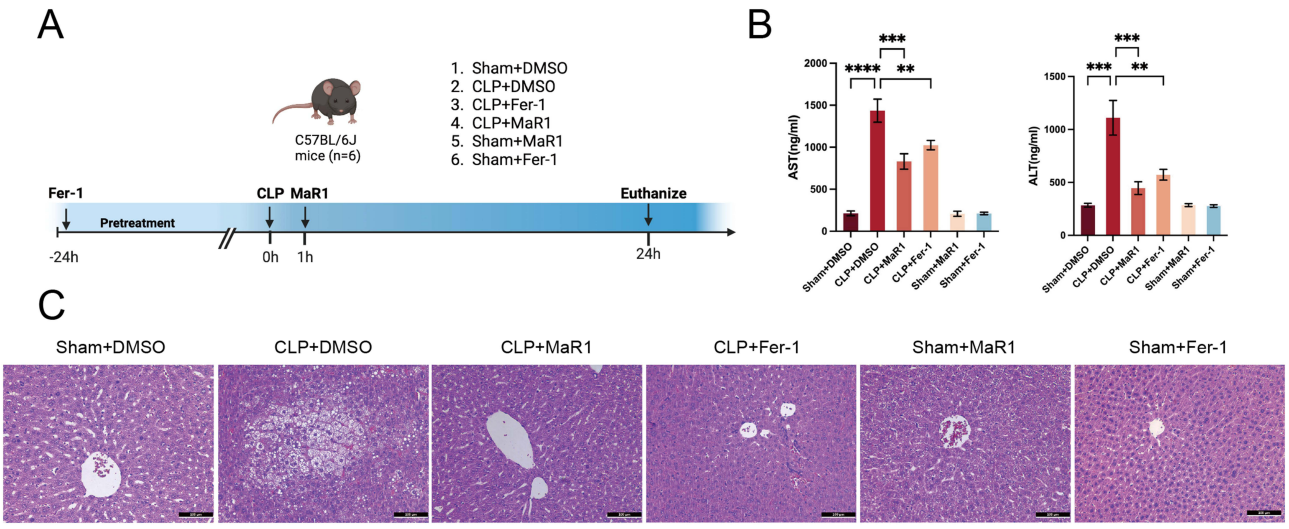

Figure 2 MaR1 and Fer-1 protect against CLP-induced liver injury in mice. (**A**) Schematic overview of the experimental design. Created in BioRender. Guo, Y. (2024) BioRender.com/r38m990. (**B**) ALT and AST levels, n = 6. (**C**) Histological images of H & E stained liver samples from mice in the indicated groups. (Original magnification, ×200, Scale bar, 100 μm). **p < 0.01, ***p < 0.001, ****p< 0.0001.

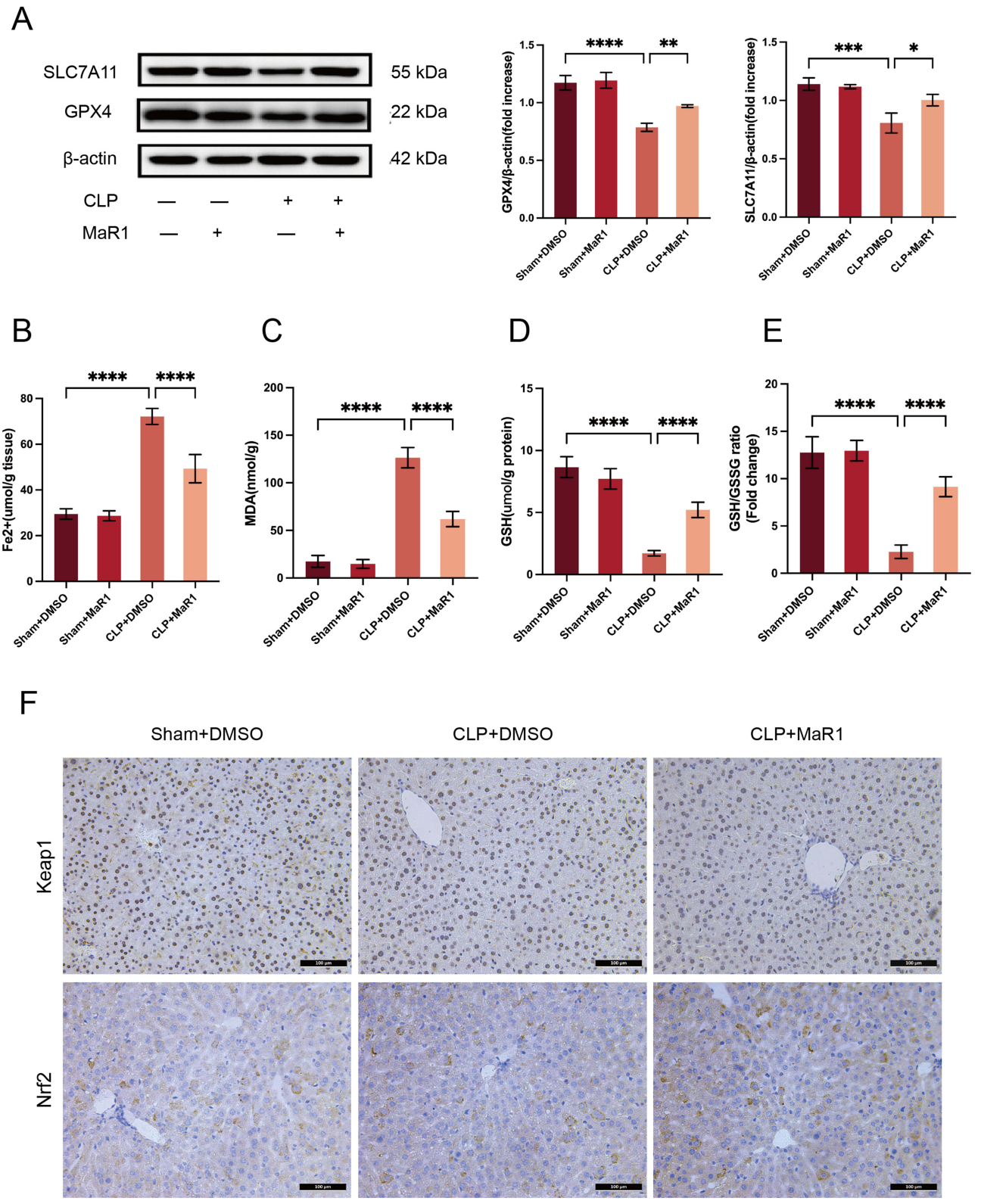

Figure 3 MaR1 inhibits hepatic ferroptosis in CLP model mice. (**A**) GPX4 and SLC7A11 were detected by Western blotting, n = 3. Data are means ± SD from three replicates. (**B**) Hepatic iron levels. (**C**–**E**) Hepatic MDA, GSH, and GSH/GSSG levels. (**F**) Liver tissue samples were examined for the expression of Keap1 and Nrf2 using IHC staining (Original magnification, ×200, Scale bar, 100 μm). *p < 0.05, **p < 0.01, ***p < 0.001, ****p< 0.0001.

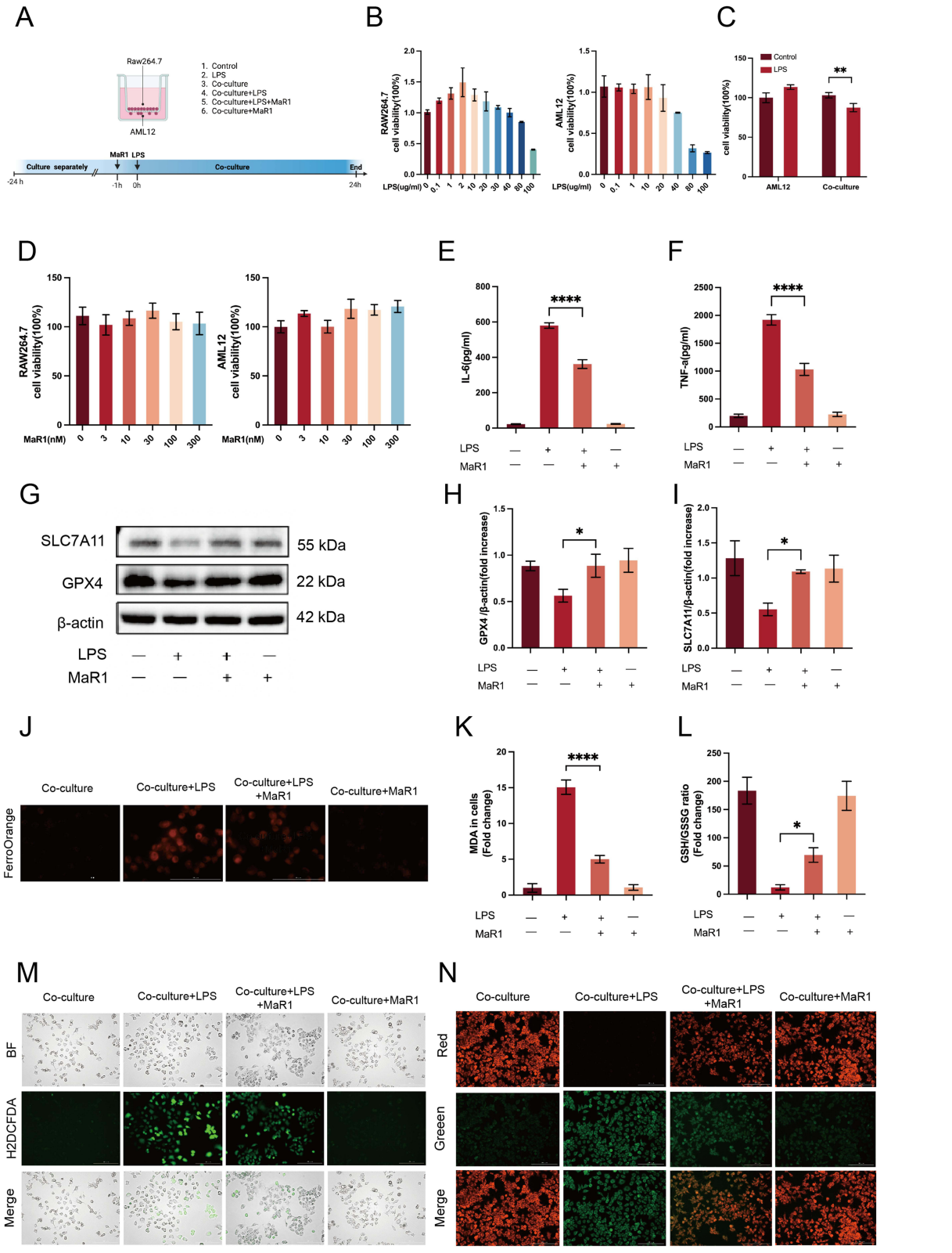

Figure 4 MaR1 prevents inflammatory mediator release and LPS-induced hepatocyte ferroptosis. (**A**) The cell treatment strategy. Created in BioRender. Guo, Y. (2024) https://BioRender.com/a15y848. (**B** and **C**) The viability of LPS-stimulated cells. (**D**) The viability of MaR1-pretreated cells. (**E** and **F**) MaR1 can significantly decrease LPS-induced increases in supernatant IL-6 and TNF-α levels. (**G–I**) GPX4 and SLC7A11 were detected via Western immunoblotting, n = 3. Data are means ± SD for three replicates. (**J**) FerroOrange was used to detect iron ions in AML12 cells. (**K–M**) MDA, GSH/GSSG, and ROS levels. (**N**) BDP 581/591 C11- was used to detect lipid peroxidation. *p < 0.05,**p < 0.01, ****p< 0.0001.

detect lipid peroxidation, elevated levels thereof were observed following LPS treatment (Figure 4N). Treatment with MaR1, however, was sufficient to reverse these changes, indicating that MaR1 is capable of inhibiting the LPS-driven release of inflammatory mediators in vitro, thereby protecting against hepatocyte ferroptosis.

MaR1 Activates Nrf2 to Suppress Ferroptosis

To test the potential of MaR1 in protecting against ALI by activating Nrf2, immunofluorescence staining was performed on LPS-stimulated cells. The results showed that in the control group, AML12 cells exhibited high expression of Keap1, which inhibited Nrf2 activity. After LPS stimulation, Keap1 expression decreased while Nrf2 was activated. Treatment with MaR1 further suppressed Keap1 expression and enhanced Nrf2 expression as well as its translocation to the nucleus (Figure 5A and B). When AML12 cells were pre-treated with ML385 (1 μM), an inhibitor of Nrf2, for 1 h, a significant drop in the protective effects of MaR1 was observed. Relative to control cells, enhanced lipid peroxidation and iron ion accumulation were observed in the ML385-treated cells (Figure 5C–E). The lentiviral knockdown of Nrf2 in these AML12 cells additionally led to a drop in SLC7A11 and GPX4 expression (Figure 5F), together with a concomitant rise in the levels of MDA consistent with extensive lipid peroxidation (Figure 5G). Based on these results, MaR1 appears to protect against hepatocyte ferroptosis in response to LPS stimulation through the activation of the Nrf2/SLC7A11/GPX4 axis.

Discussion

One of the leading causes of death for patients admitted to the intensive care unit (ICU) is sepsis, a dysregulated systemic inflammatory response to infection.[4,22] Multiple organ malfunctions can result from this potentially fatal condition.[1] Septic liver injury, one of the fatal sepsis repercussions has a major negative effect on the prognosis of the patient.[23,24] The mechanisms underlying liver failure due to sepsis are highly complex, with elevated oxidative stress associated with a systemic inflammatory response playing a key role in liver damage. Therefore, there is a critical need to investigate novel therapeutic agents that possess both antioxidant and anti-inflammatory properties to treat SI-ALI.

In vivo, a CLP modeling approach was implemented in mice as this strategy, which is the most widely used model of sepsis, can induce multi-organ dysfunction and severe peritonitis, ultimately recapitulating certain features of sepsis that are evident in humans.[25] Significant liver damage was observed in the mice in this study, including elevated serum ALT and AST, indicating that successful SI-ALI modeling had been achieved. In vitro, murine RAW264.7 macrophages were co-cultured with AML12 hepatocytes and treated with LPS as a model of hepatocyte injury. In this system, LPS stimulation resulted in an increase in supernatant IL-6 and TNF-α levels together with reduced AML12 cell viability, successfully establishing an in vitro model of septic liver injury.

MaR1 is a bioactive molecule that is categorized as a lipid-mediated inflammatory regulator and is produced through the metabolism of omega-3 fatty acids.[26] It is important for regulating inflammatory reactions, antioxidant activity, and preserving immunological homeostasis.[27] Therefore, its significance in sepsis has been increasingly acknowledged,[21] particularly regarding sepsis-induced organ impairment.[28,29] Investigating the role of MaR1 in sepsis may offer novel approaches for addressing sepsis-induced liver injury. In our research, MaR1 was found to protect against SI-ALI through a mechanism linked to inhibits the release of pro-inflammatory cytokines during sepsis, reduce oxidative stress, and suppresse ferroptosis.

Ferroptosis is a non-apoptotic, iron-dependent cell death pathway driven by lipid peroxidation, ferroptosis is under the control of antioxidant and oxidative systems.[30] This process is primarily driven by oxidative stress-induced iron-dependent lipid hydroperoxide accumulation, while the inhibition of lipid peroxidation that can protect against ferroptotic induction is facilitated by SLC7A11-mediated cystine uptake mediated by SLC7A11 and the production of GSH.[31] In sepsis, the inflammatory response and cellular damage result in enhanced oxidative stress, while the generation of reactive oxygen species facilitates lipid peroxidation and triggers ferroptosis. A growing wealth of evidence supports the functional importance of ferroptosis in sepsis.[32–35] In contrast with apoptosis, which is regarded as immunologically silent, ferroptotic cell death can be immunogenic, releasing an array of alarmins and damage-associated molecular patterns (DAMPs) and alarm proteins that can initiate and amplify inflammatory and cell death pathway activity.[33] Relative to control mice, these model animals exhibited clear evidence of hepatic

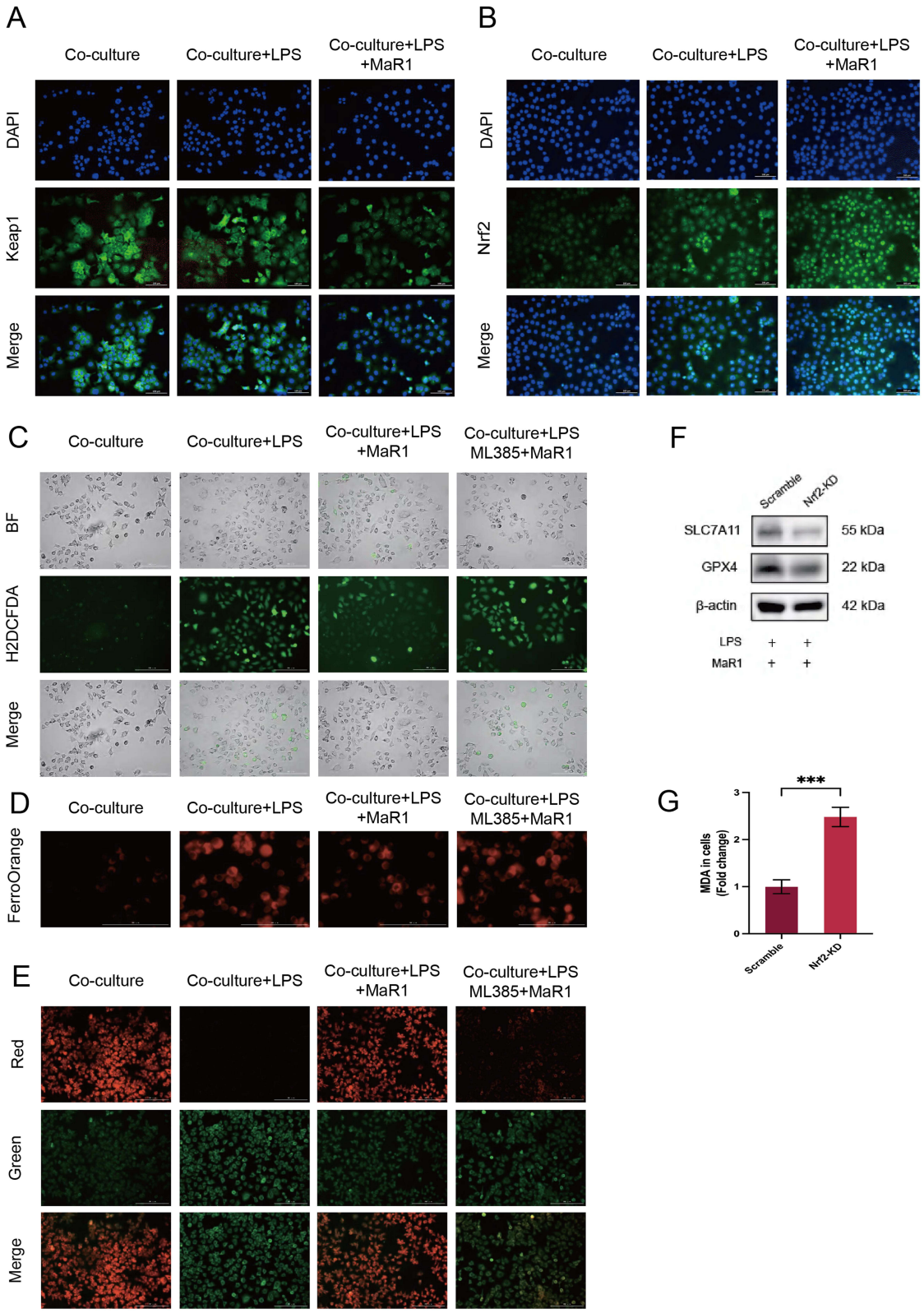

Figure 5 MaR1 activates Nrf2 to suppress ferroptosis. (**A** and **B**) Immunofluorescent detection of Nrf2 and Keap1. (**C**–**E**) Following ML385 treatment, ROS, iron ion, and lipid peroxide levels in cells were assessed. (**F**) GPX4 and SLC7A11 expression were detected by Western immunoblotting, n = 3. (**G**) MDA content. ***p < 0.001.

ferroptosis, significant macrophage accumulation in the liver, and elevated serum IL-6 and TNF-α levels. MaR1 and Fer-1 treatment, in contrast, protected against this liver damage, leading to a drop in serum ALT and AST. To test whether the protective benefits of MaR1 were linked to the inhibition of ferroptosis, ferroptosis-related protein levels were next analyzed, revealing that intraperitoneal MaR1 injection reversed the reduced ferroptosis-related protein (GPX4, SLC7A11) levels, iron ion accumulation, and evidence of lipid peroxidation in these mice. MaR1 thus appears to be capable of inhibiting hepatic ferroptosis in this CLP-induced sepsis model, thereby protecting against liver injury.

Nrf2 is a basic transcription factor capable of enhancing the expression of roughly 200 genes through the binding of antioxidant response elements.[36] Under normal conditions, Nrf2 is located in the cytoplasm, where it is bound and degraded by the Keap1 protein. However, during oxidative stress, Nrf2 dissociates from Keap-1 and translocates to the nucleus, where it binds to antioxidant response elements in DNA, leading to the activation of various antioxidant and cytoprotective genes. Prolonged oxidative stress, however, can result in lipid peroxidation and the collapse of the antioxidant defense system, ultimately suppressing Nrf2 expression. Among the downstream target molecules of Nrf2, GPX4 and SLC7A11 are recognized as key markers of ferroptosis.[37–39] Therefore, it is reasonable to hypothesize that the inhibitory effect of MaR1 on ferroptosis is mediated through the Nrf2 activation and the upregulation of GPX4 and SLC7A11 expression.

To further validate our inference, an in vitro sepsis liver injury model was established. In this system, LPS stimulation resulted in an increase in supernatant IL-6 and TNF-α levels together with reduced AML12 cell viability, lower GPX4 and SLC7A11 levels, and more extensive lipid peroxidation consistent with ferroptotic induction. MaR1 administration protected against these changes, promoting GPX4 and SLC7A11 upregulation while lowering the MDA levels in cells. Immunofluorescent staining also revealed the ability of MaR1 to promote Nrf2 localization of the nucleus. When ML385 treatment or genetic silencing strategies were used to inhibit Nrf2 within target cells, this significantly abrogated the protective benefits of MaR1. Prior investigations concerning MaR1 in sepsis were limited and predominantly focused on its modulation of inflammation.[21] These results thus offer the first evidence in favor of MaR1 being capable of protecting against SI-ALI through the activation of Nrf2/SLC7A11/GPX4 axis signaling and the consequent inhibition of ferroptosis.

It is important to acknowledge several limitations of this study. First, the absence of lipidomics data on phospholipid levels and the quantitative assessment of ferroptosis in mitochondria limits a comprehensive understanding of the process. Additionally, ferroptosis is not the only cell death mechanism involved in SI-ALI; apoptosis and necroptosis have also been observed and may be linked to the pharmacological effects of MaR1. Further investigation is required to

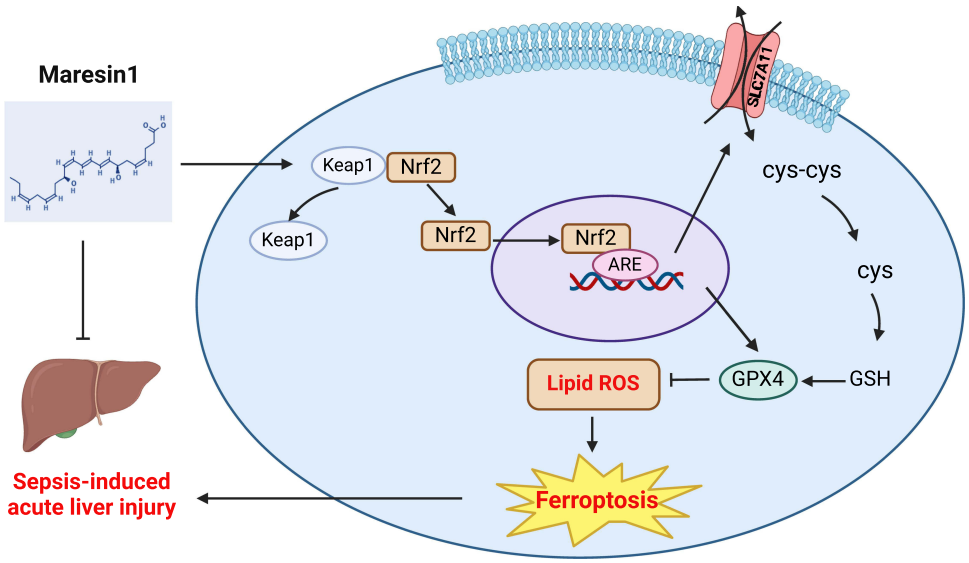

Figure 6 Schematic diagram for the mechanism of MaR1.
Notes: Created in BioRender. Guo, Y. (2024) BioRender.com/s34u427.

elucidate how MaR1 activates Nrf2 expression and to determine the extent to which MaR1 mitigates SI-ALI by inhibiting ferroptosis.

Conclusion

In summary, these results provide new insights into the mechanism of SI-ALI, while revealing that MaR1 protects against SI-ALI by inhibiting ferroptosis signaling pathways (see Figure 6). Mechanistically, our study found that MaR1 can activate the Nrf2/SLC7A11/GPX4 signaling pathway to reduce the occurrence of ferroptosis in hepatocytes, ultimately protecting the liver from damage caused by sepsis. Therefore, MaR1 may serve as a promising therapeutic agent, holding significant value in managing cases of septic liver injury, warranting further investigation.

Abbreviations

ALT, alanine aminotransferase; ARE, antioxidant response element; AST, aspartate aminotransferase; CLP, cecal ligation and puncture; DAMPs, damage-associated molecular patterns; GSH, glutathione; GSSG, oxidized glutathione; Keap1, Kelch-like ECH-associated protein 1; LPS, lipopolysaccharide; MaR1, Maresin 1; MDA, Measurement of malondialdehyde; Nrf2, nuclear factor erythroid 2-related factor 2; ROS, reactive oxygen species; SI-ALI, sepsis-induced acute liver injury.

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Disclosure

The authors report no conflicts of interest in this work.

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