

VOLUME 30 NUMBER 4 October 2024

pISSN 2287-2728
eISSN 2387-285X

CLINICAL and MOLECULAR HEPATOLOGY

The forum for latest knowledge of hepatobiliary diseases

T-cell therapy for HBV-HCC

Mortality from HCC and biliary tract cancers

Liver fibrosis scores and viral load in CHB

Genomic biomarkers for atezolizumab+bevacizumab in HCC

Epigenetic alteration of complement genes in MASLD

Current status and perspective on molecular targets and therapeutic intervention strategy in hepatic ischemia-reperfusion injury

Jia Liu*, Ranyi Luo*, Yinhao Zhang, and Xiaojiaoyang Li

School of Life Sciences, Beijing University of Chinese Medicine, Beijing, China

Hepatic ischemia–reperfusion injury (HIRI) is a common and inevitable complication of hepatic trauma, liver resection, or liver transplantation. It contributes to postoperative organ failure or tissue rejection, eventually affecting patient prognosis and overall survival. The pathological mechanism of HIRI is highly complex and has not yet been fully elucidated. The proposed underlying mechanisms include mitochondrial damage, oxidative stress imbalance, abnormal cell death, immune cell hyperactivation, intracellular inflammatory disorders and other complex events. In addition to serious clinical limitations, available antagonistic drugs and specific treatment regimens are still lacking. Therefore, there is an urgent need to not only clarify the exact etiology of HIRI but also reveal the possible reactions and bottlenecks of existing drugs, helping to reduce morbidity and shorten hospitalizations. We analyzed the possible underlying mechanism of HIRI, discussed various outcomes among different animal models and explored neglected potential therapeutic strategies for HIRI treatment. By thoroughly reviewing and analyzing the literature on HIRI, we gained a comprehensive understanding of the current research status in related fields and identified valuable references for future clinical and scientific investigations. (*Clin Mol Hepatol* 2024;30:585-619)

Keywords: Hepatic ischemia-reperfusion injury; Liver diseases; Animal model; Potential drug development

INTRODUCTION

Hepatic ischemia–reperfusion injury (HIRI) refers to a reperfusion injury caused by the restoration of blood supply to the liver after insufficient blood supply or even occlusion for a period of time and is an inevitable complication during liver trauma, resection and liver transplantation.¹ The latest epidemiological data show that the incidence of malignant liver diseases (primary or secondary liver malignancies),

which can only be treated through hepatectomy or liver transplantation, continues to increase.² HIRI is a common event that occurs during this operation and can result in organ damage, failure or acute/chronic tissue rejection. Thus, HIRI seriously limits the indications for liver resection, the application of marginal liver donors and even the application and therapeutic effect of liver transplantation.³ HIRI is a two-stage phenomenon, namely, the ischemia stage and reperfusion stage. The ischemia stage usually lacks liver

Corresponding author : Xiaojiaoyang Li

School of Life Sciences, Beijing University of Chinese Medicine, 11 Bei San Huan Dong Lu, Beijing 100029, China
Tel: +8615711163102, Fax: +86-010-53912158, E-mail: xiaojiaoyang.li@bucm.edu.cn
<https://orcid.org/0000-0003-0291-5856>

*These authors contributed equally to this work.

Editor: Won-Il Jeong, Korea Advanced Institute of Science and Technology, Korea

Received : Apr. 3, 2024 / **Revised :** Jun. 25, 2024 / **Accepted :** Jun. 26, 2024

blood supplementation, generating tissue hypoxia and cell damage. The reperfusion stage is due to the restoration of blood flow that triggers the complement cascade reaction and subsequently causes secondary damage.^{4,5} By combining information from multiple clinical studies and basic research, the occurrence of HIRI is now believed to be related to a series of complex events, such as mitochondrial damage and oxidative stress disorders, and to involve other unidentified pathological processes.⁶ Currently, although the main clinical methods for alleviating HIRI include drug intervention, surgical preconditioning and gene therapy,⁷ patients suffer from adverse reactions caused by chemical therapy. Moreover, the high risks and costs of surgical or genetic interventions can lead to unsatisfactory treatment effects for HIRI.

A few decades ago, in the field of HIRI research, researchers focused on cell death pathways⁸ and the disharmony of the liver sinusoidal milieu.⁵ In recent years, many new reports and opinions have emerged. We used three literature retrieval systems, namely, PubMed, the Wiley Online Library and Elsevier ScienceDirect, and set the time range to ten years. Finally, we included 94 articles. Then,

we comprehensively summarized the possible underlying mechanism of HIRI induction or inhibition in different animal models after surgery involving different durations of ischemia and reperfusion and focused on the therapeutic effects or current limitations of drugs used to treat HIRI under different conditions. Due to the disparities among previous reviews, in this study, we systematically analyzed the key regulatory roles and corresponding therapeutic approaches of popular research targets. Additionally, the different mechanisms involved in autophagy and mitochondrial autophagy in HIRI were identified. We utilized many HIRI modules for analysis and investigated whether different animal modeling methods mimicking HIRI can be applied to explore the pathogenesis of different clinical diseases and whether these methods can assist in the direction of potential drug development. Moreover, we propose several potential research directions that are either omitted or emerging in the HIRI field, such as the effects of metabolic substrate consumption on mitochondrial function, the complex crosstalk mechanism between hepatocytes and immune cells, emerging techniques (hypothermic oxygenated perfusion [HOPE] and alternative splicing), and the mecha-

Abbreviations:

A2BAR, A2B adenosine receptor; Abx, antibiotics; ACT, acteoside; AIF, apoptosis-inducing factor; AKT, protein kinase B; ALR, augments of liver regeneration; ALT, alanine aminotransferase; ALXR, lipoxin A4 receptor; AMPK, adenosine monophosphate-activated protein kinase; ANT, adenine nucleotide translocator; ARE, antioxidant response element; ARRB2, β -Arrestin-2; ASC, apoptosis-associated speck-like protein; ASK1, apoptosis-regulating kinase 1; AST, aspartate aminotransferase; ATG5, autophagy-related protein 5; ATP, adenosine triphosphate; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma-2; BMs, bone marrow-derived macrophages; cAMP, cyclic adenosine monophosphate; CAST, calpastatin; CAT, catalase; CC1, CEACAM1; CG, celiac ganglion; CGA, chlorogenic acid; C-NP, carbohydrate-derived nanoparticle; CREB, cAMP-responsive element-binding protein; CRYAB, alpha B-crystallin; CSF3, colony-stimulating factor 3; CXCR, chemokine (C-X-C motif) receptor; CypD, cyclophilin-D; Cyt C, cytochrome C; DAMP, damage-associated molecular pattern; DCD, dermactin; DCs, dendritic cells; DEX, dexmedetomidine; dis-HMGB1, disulfide-HMGB1; DPP-4, dipeptidyl peptidase-4; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EMCN, endomucin; EMT, epithelial-mesenchymal transition; eNOS, endothelial NOS; ERK, extracellular signal-regulated kinase; ExT, exercise training; FGF18, fibrocyte growth factor 18; FMN, flavin mononucleotide; FTO, fat-mass-and-obesity-associated protein; FXR, farnesoid X receptor; GALNT4, N-acetylgalactosaminyltransferase-4; GPCRs, G protein-coupled receptors; GSDMD, Gasdermin-D; GSK, glycogen synthase kinase; GSTD, gastrin; H/R, hypoxia/reoxygenation; HCC, hydroxychloroquine; HDAC, histone deacetylase; HFD, high-fat diet; HGF, hepatocyte growth factor; HIF2A, hypoxia inducible factor 2 alpha subunit; HIRI, hepatic ischemia-reperfusion injury; HMGB1, high mobility group box-1 protein; HNF4 α , hepatocyte nuclear factor 4 α ; HO-1, heme oxygenase-1; HSF1, heat shock transcription factor 1; I/R injury, ischemia-reperfusion injury; IFN, interferon; iKC, inflammation-associated Kupffer cell subtype; IL, interleukin; iNOS, inducible NOS; IRF1, interferon regulatory factor 1; IRG1, immune-responsive gene 1; JAG1, Jagged1; JAM, junctional adhesion molecule; JNK, c-Jun N-terminal kinase; KAE, kaempferol; KC, Kupffer cells; KCNQ1OT1, potassium voltage-gated channel subfamily Q member 1 opposite strand/antisense transcript 1; KEAP1, kelch-like ECH-associated protein 1; KLF4, Kruppel-like factor 4; KO, knockout; LFA-1, lymphocyte function-associated antigen 1; LPS, lipopolysaccharide; LSEC, liver sinusoidal endothelial cell; m6A, N6-methyladenosine; MAPK, mitogen-activated protein kinases; MaR1, maresin 1; MARCKS, myristoylated alanine-rich C-kinase substrate; MCD, methioninecholine-deficient diet; MCJ, Methylation-controlled J protein; MDA, malondialdehyde; ME, methyl eugenol; Mfn2, mitofusin 2; MLKL, mixed lineage kinase-like protein; MMP, membrane potential; mPT, mitochondrial permeability transition; MR, mannose receptor; MSC, mesenchymal stem cell; mTOR, mammalian target of rapamycin; mTORC1, mammalian target of rapamycin complex 1; NADH, nicotinamide adenine dinucleotide; NET, neutrophil extracellular trap; NFATc1, nuclear factor of activated T cells 1; NF- κ B, nuclear factor kappa B; NICD, Notch1 intracellular domain; NLRP3, nucleotide-binding oligomerization domain (Nod)-like receptor family pyrin domain-containing 3; nNOS, neuronal NOS; NOS, nitric oxide synthase; Nox, NADPH oxidase; NQO, NADPH quinone oxidoreductase; Nr2f, nuclear factor erythroid 2-related factor 2; OLT, orthotopic liver transplant; PA, propionic acid; PAK4, p21-activated kinase 4; PBMC, peripheral blood mononuclear cells; PCK1, phosphoenolpyruvate carboxykinase; PEG35, polyethylene glycol 35 kDa; PGAM5, phosphoglycerate mutase family member 5; PIAS1, protein inhibitor of activated STAT1; PINK1, putative kinase 1; Pt NPs, platinum nanoparticles; Ptpb1, polypyrimidine track-binding protein-1; PTEN, phosphatase and tensin homolog; RGS14, regulator of G-protein signaling 14; RGS14-TG, RGS14 transgenic; RNF5, ring finger protein 5; ROS, reactive oxygen species; rTMD1, domain 1 of recombinant thrombomodulin; SCFAs, short-chain fatty acids; SDF1, stromal-derived factor 1; SEV, sevoflurane; SFN, sulforaphane; SIRPG, signal regulatory protein gamma; SIRT1, sirtuin1; SMAD, small mothers against decapentaplegic; SOD, superoxide dismutase; Steap3, six-transmembrane epithelial antigen of the prostate 3; STING, stimulator of interferon genes; SUMO, small ubiquitin-like modifier; SUMOylation, small ubiquitin-like modification; TAK1, transforming growth factor-beta-activated kinase 1; Timp3, tissue inhibitor of metalloproteinase 3; TLR4, toll-like receptor 4; TM, thrombomodulin; TNF- α , tumor necrosis factor-alpha; TXNIP, thioredoxin-interacting protein; UBA2, ubiquitin-like modifier-activating enzyme 2; USP16, ubiquitin carboxyl-terminal hydrolase 16

nism of action of the emerging biomarker CEACAM1 in HIRI. Collectively, under the guidance of clinical, translational and basic research requirements, we aim to provide strategic directions for future HIRI treatment, particularly by exploring neglected targets, mechanisms and drug research directions, to provide a reference for clinical and scientific research.

THE MECHANISMS AND POTENTIAL THERAPEUTICS OF HIRI

Usually, HIRI is mainly an injury event occurring during hepatectomy, liver transplantation and other operations. Most current studies on HIRI tend to consider how damaged liver cells cause hepatic cell death and the subsequent pathological intercellular communication and immune cascade reaction in the local tissue microenvironment. Mechanistically, hypoxia usually first attracts and exerts significant negative impacts on hepatocytes, causing mitochondrial permeability changes and mitochondrial metabolic disorders, such as respiratory chain breaks, intracellular nicotinamide adenine dinucleotide (NADH)/NAD⁺ increase, depletion of adenosine triphosphate (ATP), dysregulation of Ca²⁺ plasma or significant increase of cyclic adenosine monophosphate (cAMP) which regulates glucose metabolism.⁹ Secondly, excessive reactive oxygen species (ROS) mainly derive from liver cells after reperfusion injury to cause oxidative stress, which can lead to DNA damage, protein oxidation, dysfunctional mitochondria and lipid peroxidation, ultimately leading to different kinds of liver cell death including apoptosis, pyroptosis and ferroptosis.^{10,11} Simultaneously, cell death resulted in the mass release of damage-associated molecular patterns (DAMPs), like high mobility group box-1 protein (HMGB1) or interleukin-33 (IL-33), altered other normal hepatocyte metabolism, activated immune cells and even affected the cell fate of other cells in the hepatic sinusoidal microenvironment, corporately forming feedforward to further accelerate HIRI progression.¹² Subsequently, we will delve into the pathogenesis and drug discovery efforts targeting different pathological stages based on the sequence of injury events in HIRI.

Roles of mitochondrial damage played in HIRI and positive impacts of therapeutic strategies

Admittedly, mitochondrial damage and oxidative stress as mentioned above are closely related and even can influence each other. However, for clarifying the associations more clearly in this section, we will specifically focus on exploring the involvement of mitochondrial-related pathological changes in HIRI initiation and progression. Mitochondria produce ATP through various mitochondrial respiratory chain complexes, which are essential for the proper functioning of hepatic cells. However, during HIRI, mitochondria are the first to be affected by ATP synthesis impairments, leading to their own heterogeneous lesions. This imbalance in ATP production not only disrupts the normal metabolic processes in liver cells but also triggers the generation of high levels of ROS, thereby exacerbating HIRI.¹³ Therefore, the factors that affect the function of mitochondria including disturbing ATP synthesis, ROS balance and coenzyme (coenzyme NAD⁺, NADH) dysfunction may be involved in the pathogenesis of HIRI. Methylation-controlled J protein (MCJ), an endogenous negative regulator of mitochondrial respiration, is reported to reduce ATP synthesis by restraining complex I activity and supercomplex formation.¹⁴ On the contrary, the knockdown of MCJ could increase ATP levels to promote the early activation of Kupffer cells (KCs) accompanied by increased production of tumor necrosis factor- α (TNF- α), IL-6 and heparin-binding epidermal growth factor (EGF), which contribute to liver regeneration and prevent cell damage during liver regeneration in murine models of HIRI.¹⁵ As we said at the beginning, this abnormality in ATP is often accompanied by an abnormal imbalance of ROS in mitochondria, leading to mitochondrial dysfunction. This dysfunction can manifest as abnormal mitochondrial membrane potential, changes in redox potential, and disruptions in mitochondrial quality control. In a study by Teixeira et al.¹⁶, it was found that preconditioning with polyethylene glycol 35 kDa (PEG35) could mitigate hepatic hypoxia/reoxygenation-induced mitochondrial dysfunction by inhibiting the toxicity of ROS and the exhaustion of ATP, as well as restoring the mitochondrial membrane potential (MMP). Similarly, SS-31, a peptide with strong antioxidant and anti-inflammatory effects, also restrained lipopolysaccharide (LPS)-induced mitochondrial dysfunction by reducing the production of

mtROS, increasing the production of ATP in mitochondria and restoring the MMP in Raw 264.7 cells.¹⁷

Considering the inseparable relationship between ATP and mitochondrial respiratory chain, subsequent studies paid more attention to the effect of local respiratory chain complex on mitochondrial damage and HIRI. From a structural point of view, ATP is the product of the fifth complex of mitochondrial respiration and its production is simultaneously affected by different respiratory complexes along the electron transport chain, including respiratory chain complex I (NADH dehydrogenase, specifically at the change of NADH/NAD⁺), complex II (succinic acid-Q oxidoreductase), complex III (UQ-cytochrome C oxidoreductase), complex IV (cytochrome C oxidase) and complex V (ATP synthase).¹⁸ Coenzyme Q₁₀ (CoQ₁₀) is the only non-protein REDOX carrier in the respiratory chain. It can move rapidly through the membrane to transfer electrons from NADH dehydrogenase in the respiratory chain, promoting MMP maintenance and recovery of superoxide dismutase (SOD) vitality to alleviate hepatic damage in ischemic surgery-induced HIRI mouse model. Meanwhile, the use of a CoQ₁₀-loaded virus-mimicking liposomal system (VMLs), which employs dendritic lipopeptides to mimic the viral envelope, may offer a more effective therapeutic approach compared to using CoQ₁₀ alone for treating HIRI.¹⁹ Additionally, NAD⁺ serves as a vital coenzyme for dehydrogenase in biocatalytic reactions, enhancing the physiological function of CoQ₁₀ and acting as a co-substrate for various enzymes, such as sirtuins.²⁰ It has been suggested that NAD⁺, short for coenzyme I, protects the kidney from acute stress damage through sirtuin1 (SIRT1, an NAD-consuming deacetylase)/glycogen synthase kinase 3 β (GSK-3 β).²¹ Specifically, NAD supplementation reduced damage in a SIRT1-dependent manner that was associated with the decrease in GSK-3 β levels and activity and an increase in Nrf2 expression.²¹ In liver, pretreatment with indirubin-30-oxime (a GSK-3 β inhibitor) prevented cyclophilin-D (CypD) phosphorylation located in the mitochondrial matrix to maintain the content and synthesis of ATP by inhibiting GSK-3 β activity; on the other hand, NAD⁺ pretreatment promoted the deacetylation of CypD accompanied by decreased mitochondrial efficiency and ROS generation by increasing SIRT3 activity, leading to the decline in the binding of CypD to adenine nucleotide translocator (ANT) and mitochondrial permeability transition (mPT) induction, thus protected

against ischemic surgery-induced HIRI.²² Furthermore, mitochondrial metabolic reprogramming can have a direct impact on glucose metabolism and the mitochondrial respiratory chain, particularly complex I (NADH dehydrogenase) which plays a crucial role in regulating the HIRI process. A separate study showed that HOPE treatment induced mitochondrial reprogramming by elevating ATP levels in liver tissue and reducing mitochondrial succinate accumulation, as well as releasing flavin mononucleotide (FMN) and altering the NADH/NAD ratio. This reprogramming was found to offer protection against mitochondrial injury and HIRI in rat livers donated after circulatory death prior to liver transplantation.²³

In addition, the normal operation of mitochondrial function can maintain the energy balance of cells through the regulation of the dynamic balance between mitochondrial dynamics (fission/fusion) and quality control (biogenesis/mitochondrial autophagy).²⁴ Originally, mitochondrial fusion and fission are natural protective mechanisms employed by the body to eliminate damaged, aging and excessive mitochondria, thereby maintaining overall health and normal immune system function. However, in cases of abnormal damage, these processes can become disrupted, leading to mitochondrial fragmentation with abnormal division or prolonged mitochondrial morphology with abnormal fusion. Gradually, more and more evidence has found that HIRI can affect the self-renewal and repair process of the above mitochondria, thus causing devastating damage to the liver. It has been reported that HIRI caused fragmented mitochondria and downregulated the expression of N6-methyladenosine (m6A) demethylase (FTO), a fat mass and obesity-associated protein. But, adeno-associated virus-mediated liver-specific overexpression of FTO (AAV8-TBG-FTO) attenuated HIRI and mitochondrial fragmentation *via* inhibiting the expression of dynamin-related protein 1 (drp1) that could regulate the mitochondrial fission process in cell survival.²⁵ Similarly, previous evidence pointed out that augmenter of liver regeneration (ALR) was involved in maintaining physiological operation of mitochondria and preventing mitochondrial fission by inhibiting drp1 phosphorylation to alleviate HIRI-induced apoptosis.²⁶ With the deepening of the research, it was found that ALR also could interact with transcription factor Yin Yang-1 (YY1) and suppress its nuclear import. This interaction contributed to decreasing ubiquitin-like modifier-activating enzyme

2 (UBA2, YY1 downstream target gene) transcription levels. Consequently, this led to a reduction in drp1 and small ubiquitin-like modification (SUMOylation)-mediated mitochondrial fission in a rat model of hepatic IRI.²⁶ Currently, the majority of studies focus on probing the impact of mitochondrial division on the HIRI process. Nevertheless, there is a noticeable dearth of research concerning mitochondrial fusion and its associated interventional drugs.

Additionally, emerging evidence indicates that mitochondrial autophagy plays a role in selectively degrading dysfunctional mitochondria, thereby mitigating damage and preventing the accumulation of impaired mitochondria in the liver. Previous studies have shown that lysosomal Ca^{2+} signaling is the vital part of the traditional mucolipin 1 (MTORC1, a master regulator of autophagy)-dependent autophagy pathway, and induces autophagy.²⁷ Calpastatin

(CAST) is an endogenous calpain inhibitor, which can maintain the stability of intracellular calmodulin in the liver.²⁸ After HIRI stimulation, the aged liver showed a rapidly decreasing CAST resulting in the activation of calmodulin, defective autophagy, cleaving autophagy-related protein 5 (ATG5), mPT onset, mitochondrial depolarization and hepatocyte death. However, CAST overexpression restored autophagy for eliminating abnormal mitochondria and prevented mitochondrial depolarization to protect hepatocytes against HIRI.²⁹ Similarly, PEG35 preconditioning enhanced autophagy and mitochondrial quality by increasing the levels of autophagy-related proteins (light chain 3 II [LC3II] and protein sequestosome 1/p62 [p62]) and genes involved in mitochondrial biogenesis (peroxisome proliferator-activated receptor gamma, coactivator 1 alpha [*ppargc1a*], *nrf1*, mitochondrial fission 1 [*fis1*], dynamin 1-like gene [*dn-*

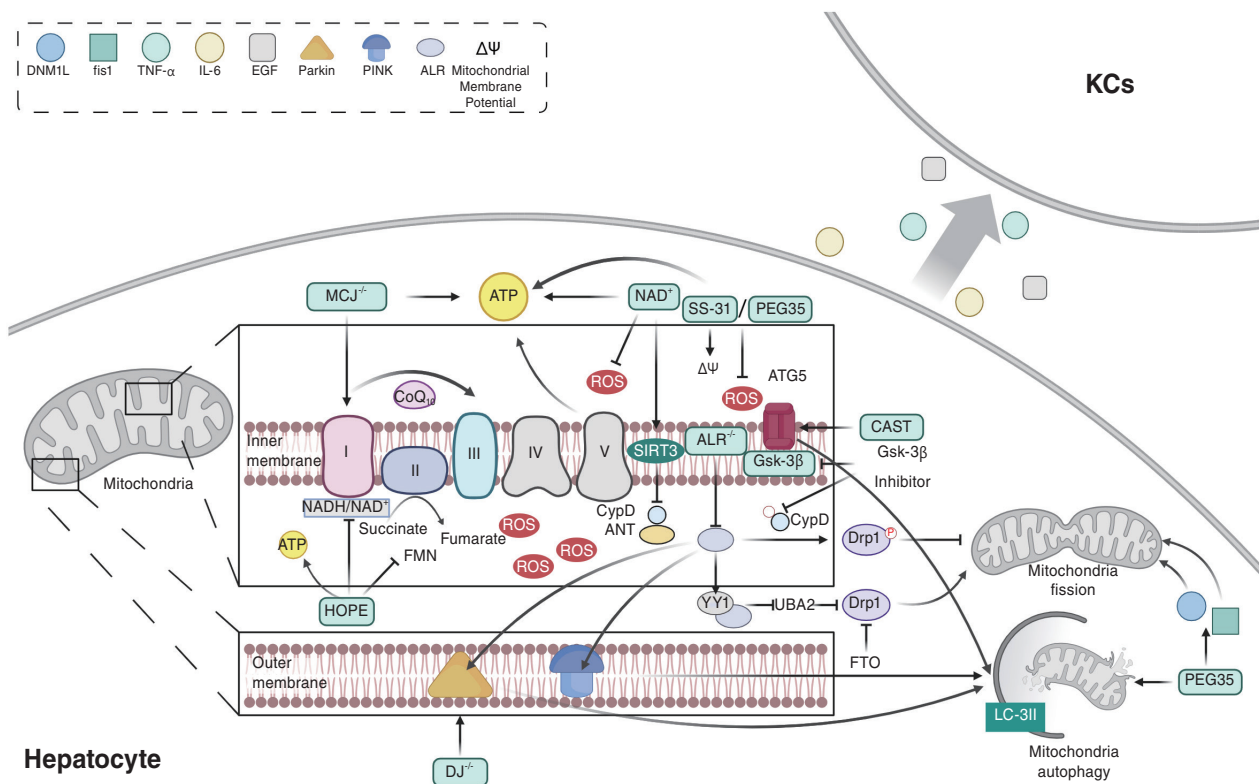


Figure 1. Mitochondrial damage and the crosstalk between Kupffer cells and mitochondria play crucial roles in progression of HIRI. ATP synthesis, ROS balance, membrane potential, quality control in mitochondria, mitophagy, mitochondrial fusion and fission may contribute to the regulation of I/R injury. KCs, Kupffer cells; DNM1L, dynamin 1-like gene; Fis1, mitochondrial fission 1; TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6; EGF, heparin-binding epidermal growth factor; ALR, augments liver regeneration; MCJ, methylation-controlled J protein; ATP, adenosine triphosphate; NADH, nicotinamide adenine dinucleotide; PEG35, polyethylene glycol 35 kDa; ATG5, cleaving autophagy-related protein 5; ROS, reactive oxygen species; CoQ₁₀, coenzyme Q₁₀; SIRT3, sirtuin3; GSK-3 β , glycogen synthase kinase 3 β ; CAST, calpastatin; HOPE, hypothermic oxygenated perfusion; FMN, flavin mononucleotide; CypD, cyclophilin-D; ANT, adenine nucleotide translocator; Drp1, dynamin related protein 1; YY1, Yin Yang-1; UBA2, ubiquitin-like modifier-activating enzyme 2; FTO, fat-mass-and-obesity-associated protein; LC3II, light chain 3 II.

m1l) and optic atrophy 1 [*opa1*]) to ameliorate HIRI.¹⁶ However, these studies only observed the simultaneous existence of autophagy and mitochondrial clearance or increase but still did not provide direct evidence of mitophagy, which has specific targets and markers such as phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1), mitofusin 2 (Mfn2) and Parkin, which were different from general autophagy. Other subsequent research has elucidated the direct evidence of mitophagy participating in HIRI pathogenesis in multiple gene-knockout animals. ALR-knockout (KO) mice exhibited more severe hepatocellular necrosis and inflammatory cell infiltration that were associated with decreased mitophagy *via* inhibiting PINK1 accumulation and PARKIN translocation to mitochondria, which could be reversed by transfecting Mfn2.³⁰ Meanwhile, DJ-1 is protein associated with mitochondrial function. In DJ-1 knockout (DJ-1^{-/-}) mice subjected to hepatic ischemia-reperfusion (I/R) injury, DJ-1 deficiency enhanced mitochondrial accumulation and protein stability of PARKIN, which in turn enhanced mitophagy, contributing to the clearance of damaged mitochondria.³¹ Collectively, in various models of apoptosis, there was observed a collective occurrence of mitochondrial fragmentation, disruption of reticular structure, remodeling of mitochondrial ridge, and a notable increase in mitochondrial number. Thus, it is suggested that mitophagy, mitochondrial fusion, and fission may serve as potential mechanisms for HIRI by influencing the quantity and composition of mitochondria (Fig. 1).

The dual role of oxidative stress signaling during HIRI and potential interventions

After reperfusion, the restoration of blood flow led to the release of large amounts of oxidative intermediates, exacerbating the imbalance of oxidative and antioxidant homeostasis in the liver microenvironment. Among these oxidative intermediates, ROS is the most widely known damage agent, secreted by mitochondria and can be eliminated by antioxidant substances. ROS impacts the expression of downstream genes or proteins and regulates different oxidative stress-related signaling pathways, affecting the process of HIRI.¹¹ In Wistar male rats suffering from I/R, ROS increased significantly as the injury marker, meanwhile the levels of aspartate aminotransfer-

ase (AST), alanine aminotransferase (ALT), malondialdehyde (MDA) and TNF- α also raised in serum, which could be diminished by metformin administration.³² At the same time, the increase of ROS also involves a variety of immune reactions and the intervention of immune cells, such as mediating the aggregation of neutrophils and macrophages, and the adhesion of leukocytes, thus exacerbating this oxidative stress imbalance and HIRI damage. With the rise of nanomaterials in medical treatment, Zhang et al.³³ designed the Ceria@Apt synthesized by nanoceria and anti-fifth component of complement (C5a) aptamers to inhibit the formation of ROS. The Ceria@Apt alleviated C5a-mediated inflammation *via* switching valence states to scavenged ROS, restraining neutrophil recruitment and inflammation in the murine model of HIRI.³³

Except for ROS, nitric oxide (NO), as small molecular free radicals, can regulate oxidative stress through regulating various signaling pathways. NO could interact with ROS to derive reactive nitrogen species (RNS) or nitro compounds with high oxidation activity for inducing oxidative and nitrosative stress.³⁴ Ferreira-Silva et al.³⁵ found that in addition to ROS and TNF- α , RNS production was also raised in the rat model subjected to hepatic IR by surgery, which could be improved by administration of liposomal quercetin nanoformulation. Another research demonstrated that simvastatin pretreatment could reverse oxidative and nitrosative stress in liver by preventing the increase of NO and nitrotyrosine.³⁶ Li et al. further interpreted that pretreatment with dietary nitrate significantly decreased oxidative stress by increasing the contents of nitrate, nitrite and NO in liver. Meanwhile, it improved inflammatory responses by decreasing Ly6G⁺ neutrophil infiltration and levels of proinflammatory factors (TNF- α , interferon-gamma [IFN- γ], IL-1 β , IL-1 α , IL-17A, IL-27), while increasing the levels of IL-10 in liver to ameliorate HIRI.³⁷ Interestingly, investigators also found that NO played a double-edged role in HIRI. Generally, NO is catalyzed through the enzymatic action of nitric oxide synthase (NOS) family including inducible NOS (iNOS), endothelial NOS (eNOS) and neuronal NOS (nNOS).³⁸ A zeolitic imidazolate framework-8 (ZIF-8)-based hybrid nanoreactor contains iNOS and platinum nanoparticles (Pt NPs) that act as SOD and catalase (CAT) mimics. This nanoreactor generates NO *via* the catalysis of iNOS enzyme and eliminates ROS by converting O₂⁻ and H₂O₂ to O₂, accompanied by the in-

hibition of macrophage activation and neutrophil accumulation.³⁹ Current evidence suggests that these functional enzymes responsible for the regulation of NO are also involved in the regulation of multiple signaling pathways and thus affect HIRI in multiple ways; for instance, eNOS is also involved in the regulation of mitogen-activated protein kinases (MAPK) family-associated signaling pathway. In the mice with HIRI by surgery, pretreatment of myricitrin, a flavonoid with hepatoprotective property, could raise the NO production and phosphorylation of eNOS expression through activating the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway to ameliorate liver I/R injury.⁴⁰

Besides, several regulatory factors that govern cellular redox reactions and contribute to the development of HIRI to varying degrees have also been identified. These factors include nuclear factor erythroid 2-related factor 2 (Nrf2), heme oxygenase-1 (HO-1), nuclear factor kappa B (NF- κ B), hypoxia-inducible factor-1 α (HIF-1 α), and tumor necrosis factor receptor (TNFR)-associated factor 3 (TRAF3). Nrf2 is an emerging cellular regulator that plays a critical role in maintaining redox homeostasis and inhibiting oxidative stress, for example, by regulating downstream target proteins in the HO-1 signaling pathway.⁴¹ Li et al.⁴² also pointed out that pretreatment of fisetin promoted cytoplasmic Nrf2 translocate into the nucleus and further elevated HO-1 expression to alleviate cell apoptosis and oxidative stress in IR liver model. Attentively, immune-responsive gene 1 (IRG1), one of the most sensitive genes in proinflammatory conditions, encodes for the enzyme producing itaconate which negatively regulates the inflammatory response. After I/R, IRG1^{-/-} caused downregulating expression of Nrf2 and its downstream target genes (NADPH quinone oxidoreductase 1 [NQO-1] and HO-1) as well as the upregulation of F4/80-positive macrophages and IL-6 mRNA expression compared with WT mice. However, restoring IRG could reverse oxidative damage by transfecting a plasmid carrying IRG1.⁴³ Another study exhibited pretreating veratric acid (VA) also relieved HIRI by Nrf2/HO-1/NQO-1 pathway.⁴⁴ Simultaneously, studies have pointed out that the recovery of NADPH oxidase (Nox) 4 activity can produce a similar effect to the activation of Nrf2 signaling pathway. In mice subjected to HIRI, the low doses of sitagliptin could keep the balance of nitrative and halogenative and reduce oxidative stress and inflammatory

response *via* increasing 3-bromotyrosine concentration and upregulating Nrf2 expression, reducing IL-6 and mid-kine expression and free 3-nitrotyrosine concentration, while partly restoring expression of Nox 4.⁴⁵ These oxidative stress reactions triggered by Nrf2 also interact with many inflammatory reactions or inflammatory transcription factors, thus forming a complex interactive network. Mao et al.⁴⁶ found that Nrf2 at T369 was directly phosphorylated due to the overexpression of p21-activated kinase 4 (PAK4, a serine/threonine kinases), causing nuclear export and proteasomal degradation to exacerbate oxidative stress in hepatocyte. Inversely, gastrodin (GSTD), a bioactive compound extracted from a traditional Chinese herb (*Gastrodia elata* Blume), contributes to the anti-inflammatory and antioxidant effects to protect cells against IR-stressed livers. The pre-administration of GSTD dose-dependently ameliorated surgery-caused HIRI and attenuated oxidative stress and inflammation by inducing HO-1 expression and inhibiting toll-like receptor 4 (TLR4), along with activating Nrf2 and p38 mitogen-activated protein kinases (P38/MAPK) signaling.⁴⁷ Notably, the MAPK pathway can trigger the phosphorylation of p38, c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinase (ERK), leading to the activation of the NF- κ B inflammatory pathway. Mice subjected to I/R injury by surgery showed the increased NF- κ B/p65 phosphorylation and release of TNF- α and IL-6. Nevertheless, kaempferol (KAE), an ingredient isolated and extracted from *Penthorum chinense* Pursh, reduced transaminase, MDA and raised SOD and GSH in a dose-dependent manner and inhibited inflammation *via* activated Nrf2/HO-1 signaling pathway and restraining NF- κ B phosphorylation.⁴⁸

In the field of tumor research, it has been well established that fibrocyte growth factor 18 (FGF18), which can regulate the epithelial-mesenchymal transition (EMT) of cell cancer, is involved in the regulation of HIRI to Nrf2. Specifically, injection of FGF18 has been shown to activate Nrf2 by downregulating ubiquitin carboxyl-terminal hydrolase 16 (USP16) expression, leading to increased ubiquitination of kelch-like ECH-associated protein 1 (KEAP1), which ultimately attenuates surgically induced HIRI.⁴⁹ Interestingly, vascular endothelial growth factor (VEGF) and Nrf2 are closely related under ROS stimulation, and Nrf2 has functioned as a transcription factor in macrophages, not only maintaining cancer cell growth under oxidative

stress but also regulating HIF-1 α /VEGF signaling pathways to promote the EMT of cancer cells;⁵⁰ however, in the field of HIRI, the regulation of Nrf2 on VEGF is still under study. It is reported that Nrf2 knockdown restricted the expression of VEGF by limiting expression of HIF-1 α , failing to palliate oxidative stress in colon tumor cells following hypoxia-mimicking CoCl₂ treatment.⁵¹ Whereas, surprisingly, overexpression of HIF-1 α could exert protective effects on hepatic IRI by upregulating activation of the A2B adenosine receptor (A2BAR) to prevent adenosine-mediated damage in HIRI with surgery.⁵² The finding suggests that HIF-1 α is critical to VEGF function played in hypoxia, but its role is currently controversial in HIRI progress. Notably, HIF-1 α is also a transcription factor that mediates the expression of

the transmembrane protein CEACAM1 (CC1). Recent mechanisms have found that HIF-1 α can inhibit hepatic injury in HIRI surgical mice and human donor liver grafts by binding polypyrimidine track-binding protein-1 (Ptbp1) to promote the increase of RNA splicing factor (CEACAM1-short). Specifically, CEACAM1-short exerted a cellular protective effect by inhibiting MAP3K and the p38 pathway (Fig. 2).⁵³

The mechanism of discovered abnormal cell death signaling during HIRI and corresponding treatment strategies

Cell death is the initially direct cell-damaging conse-

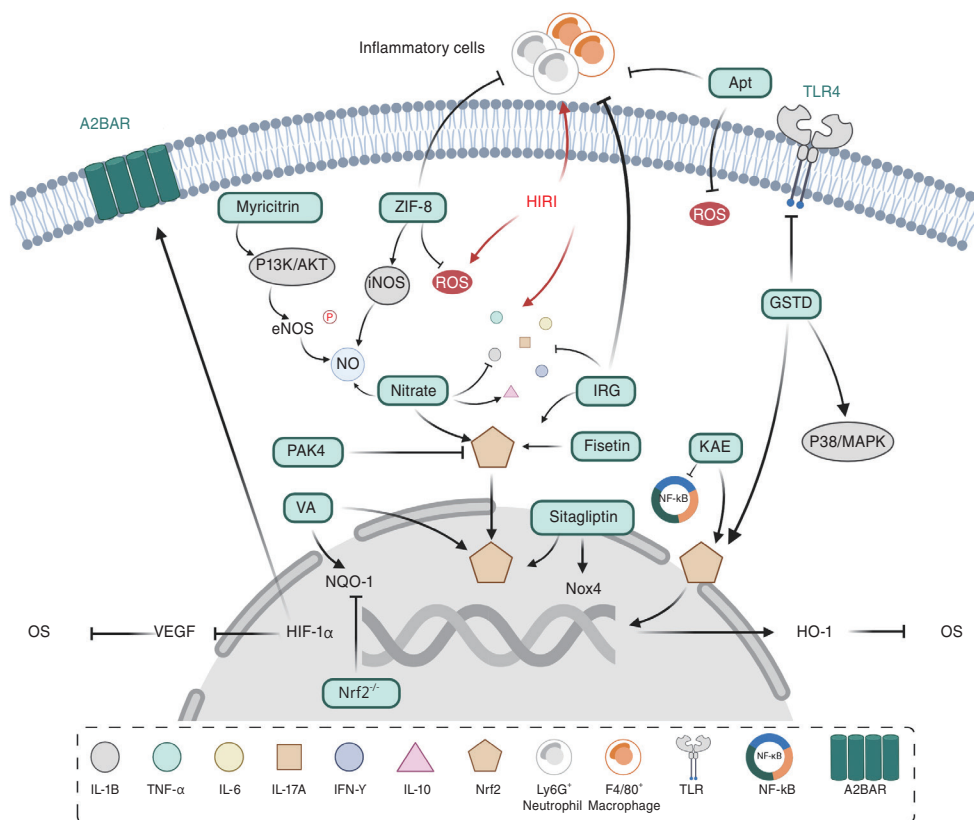


Figure 2. Signaling pathway and targeted therapeutic agents against oxidative stress during HIRI. Oxidative stress could influence HIRI through PI3K/ AKT, Nrf2/HO-1, P38/MAPK, HIF-1 α /VEGF, and NF- κ B signaling pathways, and regulatory factors including Nrf2, HO-1, NF- κ B, HIF-1 α and TRAF3 also participate in HIRI development. HIRI, hepatic ischemia-reperfusion injury; A2BAR, A2B adenosine receptor; TLR4, toll-like receptor 4; ROS, reactive oxygen species; ZIF-8, zeolitic imidazolate framework-8; PI3K, phosphatidylinositol 3-kinase; AKT, protein kinase B; NO, nitric oxide; iNOS, inducible nitric oxide synthase; eNOS, endothelial NOS; GSTD, gastrodin; IRG, immune-responsive gene; PAK4, p21-activated kinase 4; KAE, kaempferol; NF- κ B, nuclear factor kappa B; P38, p38 mitogen-activated protein kinases; MAPK, mitogen-activated protein kinases; VA, veratric acid; NQO-1, NADPH quinone oxidoreductase 1; Nox 4, NADPH oxidase; VEGF, vascular endothelial growth factor; HIF-1 α , hypoxia-inducible factor-1alpha; HO-1, heme oxygenase-1; IL-1 β , interleukin-1 β ; TNF- α , tumor necrosis factor-alpha; IL-6, interleukin-6; IL-17A, interleukin-17A; IFN- γ , interferon-gamma; IL-10, interleukin-10; Nrf2, nuclear factor erythroid 2-related factor 2.

quence of HIRI, including apoptosis, autophagy, pyroptosis and ferroptosis. Generally, there are two main pathways involved in apoptosis of HIRI process, namely exogenous pathway activated by death receptors and endogenous pathway. The intrinsic apoptosis pathway, also known as mitochondrial death pathway, is activated by different death signals, which eventually cause mitochondrial dysfunction and further induce apoptosis. Under the stimulation of ROS, apoptotic markers B-cell lymphoma-2 (Bcl-2) family and Bcl-2-associated X protein (Bax) can form oligomer complexes, which damage MMP and lead to the release of cytochrome c (Cyt C, proteins encoded by nuclear genes) as well as downstream cascade reaction of cell apoptosis.⁵⁴ In the rat model of HIRI following surgery, chlorogenic acid (CGA) pretreatment reduced the levels of MDA and ROS while increasing SOD and GSH levels to relieve oxidative stress. CGA also decreased hepatocyte injury by suppressing the mitochondria-mediated apoptotic pathway accompanied by reduced expression levels of Bax, Cyt-c, cleaved-caspase9, cleaved-caspase3, ENDOG, and apoptosis-inducing factor (AIF).⁵⁵ Saleh and El-Shorbagy⁵⁶ demonstrated the same therapeutic effect by chitosan administration on liver I/R injury. The intriguing discovery was that sevoflurane (SEV) preconditioning led to the downregulation of miR-124-3p and the increased expression of TRAF3, which not only decreased the expression of apoptosis-related factors but also promoted the ubiquitination and degradation of cAMP-responsive element-binding protein (CREB) to collectively alleviate HIRI both *in vivo* and *in vitro*.⁵⁷ It is well known that small mothers against decapentaplegic (SMAD), ERK, JNK, transforming growth factor-beta-activated kinase 1 (TAK1), p38 MAPK, NF- κ B or PI3K/AKT are all common participants through direct or indirect involvement in the process of apoptosis. More concretely, some studies have found that SMAD was also involved in the process of apoptosis, which may be related to miR-210.⁵⁸ Notably, in a murine hepatic IRI model by surgery, ghrelin administration delayed the transition from HIRI to liver fibrosis process *via* the inhibition of SMAD and ERK signaling pathways.⁵⁹ Pan et al.⁶⁰ also found that the level of miR-210 was increased after hepatic I/R injury but was accompanied by less inflammatory responses and hepatocytes apoptosis in miR-210 KO mice. Mechanistically, miR-210 could restrain SMAD4 expression, impair SMAD4-mediated anti-apoptosis and thus exacerbate cell death and

liver damage. Similarly, miRNA can also suppress gene expression through specific binding to genes responsible for regulating apoptosis. In a rat HIRI by surgery, administration of agomir-miR-34a-5p markedly decreased the expression of hepatocyte nuclear factor 4 α (HNF4 α), cleavage cas-3, Bax, JNK, and P38, thereby ameliorating HIRI.⁶¹

As a matter of fact, among these pathways involved, JNK was highly concerned first as a regulatory target to improve apoptosis in HIRI. Based on the above conclusions, the researchers pay close attention to regulating the processes of HIRI by targeting either the upstream or downstream targets of JNK. In mice and cell line exposed to HIRI, the overexpression of liver-specific Yes-associated protein (YAP) promoted autophagy to thus reduce apoptosis *via* upregulating the phosphorylation of JNK. However, it is worth noting that the interaction between YAP and transcriptional enhanced associate domain (TEAD) could reverse the activation effect of YAP on JNK, resulting in the upregulation of apoptosis levels.⁶² In mice model exposed to hepatic I/R injury by surgery, Guo et al.⁶³ found that TAK1 was another key upstream target of JNK/p38 proapoptosis signaling pathway, and six-transmembrane epithelial antigen of the prostate 3 (Steap3), an iron regulatory protein, could directly interact with TAK1 and promote TAK1-JNK/p38 signaling activation to accelerate hepatic cell apoptosis. As the study progressed further, tripartite motif-containing (TRIM) 27 was investigated as another key regulator and TAK1-related target to attenuate hepatic I/R injury through reducing inflammation and cell apoptosis. It has been reported that TRIM27 significantly diminished TAK1 activity *via* promoting the degradation of TAK1 binding protein 2/3 (TAB2/3) repressing K63-linked polyubiquitin, thus contributing to the inhibition of downstream JNK/p38 and NF- κ B signaling pathways.⁶⁴ Concurrently, Zhang et al.⁶⁵ in the same lab further discovered regulator of G-protein signaling 14 (RGS14), a complex scaffolding protein that integrates the G-protein and MAPK signaling pathways, not only inhibited the activation of TAK1 and downstream P38/JNK signaling but also reduced CD11b⁺ cell infiltration in the liver tissues to exert protective effects on HIRI in hepatocyte-specific RGS14 transgenic (RGS14-TG) mice. With deeper investigation, researchers searched out new regulatory targets that apoptosis-regulating kinase 1 (ASK1) could also regulate intracellular signaling pathways, such as JNK and p38MAPK, thereby further induc-

ing cell apoptosis. Researchers have found that the interaction between ring finger protein 5 (RNF5) and ubiquitinated phosphoglycerate mutase family member 5 (PGAM5) could repress ASK1-JNK-p38 signaling pathways *via* inhibiting ASK1 phosphorylation and increasing PGAM5 degradation to protect against HIRI.⁶⁶ Attentively, N-acetylgalactosaminyltransferase-4 (GALNT4) is the first step to catalyze protein O-glycosylation, which interacted with ASK1 and inhibited N-terminal dimerization and activation of ASK1, resulting in repression of downstream JNK/p38 and NF- κ B signaling pathways to alleviate HIRI induced by surgery.⁶⁷

In addition to the JNK pathway, PI3K/Akt signaling pathway also extensively regulates apoptosis and HIRI. In recent years, numerous studies have aimed to identify key targets involved in the regulation of PI3K/Akt on the one hand, and to seek more effective protective drugs against this pathway on the other hand. β -Arrestin-2 (*ARRB2*) belongs to a member of regulatory proteins involved in the G protein-coupled receptors (GPCRs) signaling pathway and also takes part in PI3K/Akt-mediated apoptosis; the knock-down of *ARRB2* resulted in high expression of caspase 3 and significant inhibition of PI3K/Akt signaling, leading to more severe apoptosis in mice after HIRI.⁶⁸ Lipoxin A4 receptor (ALXR), as another key target protein that can bind to GPCRs, is thought to participate in infiltration or apoptosis through AKT signaling. It has been reported that marresin 1 (MaR1) administration repressed inflammatory response, oxidative stress, and hepatocyte apoptosis *via* enhancing the ALXR/AKT signaling pathway, resulting in alleviation of I/R-mediated liver damage.⁶⁹ Moreover, Li et al.⁷⁰ found another protein that myristoylated alanine-rich C-kinase substrate (MARCKS), a regulatory molecule in actin dynamics, could not only activate p38/JNK MAPK but also suppress PI3K/AKT signal to exacerbate inflammation and cell apoptosis. Interestingly, MiR-142-3p overexpression could alleviate HIRI by decreasing MARCKS levels. At the same time, natural products have been found to improve apoptosis and HIRI by directly regulating PI3K/AKT activity. Methyl eugenol (ME), a natural enylbenzene, also attenuated inflammation and apoptosis by activating the PI3K/AKT pathway.⁷¹ Additionally, Ding et al.⁷² further explained the pro-survival effect of MAPK in endogenous apoptosis. In mice subjected to HIRI by surgery, cordycepin precondition inhibited MAPK phosphorylation and NF- κ B expression to reduce Beclin-1 and LC3-induced au-

tophagy, on the one hand, decreased BAX and caspase 9/3-induced apoptosis in a dose-dependent manner by inhibiting activation of the MAPK/NF- κ B signaling pathway.

Emerging evidence suggested that the changes in epigenetic modification states such as histone modification and small ubiquitin-like modifier (SUMOylation) can influence apoptosis in HIRI process. Histone deacetylase (HDAC) is mainly responsible for removing acetyl from histone, which is related to inhibition of gene transcription and expression. There are studies finding that carbon monoxide (CO) inhalation pretreatment prominently increased the mRNA expression of SIRT1 and activity of SIRT1 deacetylase by decreasing expression of miR-34a, causing the up-regulation of NF- κ B p65 and p53 deacetylation to inhibit NF- κ B p65/p53-related apoptotic response in mouse with HIRI surgery.⁷³ Moreover, LP342, as a candidate of the HDAC inhibitors (HDACi), was found to inhibit HDAC I and promote NF- κ B p65 acetylation, subsequent depressing of JNK activation and caspase 3 level to alleviate inflammation and apoptosis in male mice with HIRI surgery.⁷⁴ Observingly, SUMOylation is a novel form of protein post-translational modification that inhibits gene transcription of the protein. In mice with HIRI surgery, protein inhibitor of activated STAT1 (PIAS1), a small ubiquitin-like modifier (SUMO) E3 ligase, reduced hepatocyte inflammatory response and apoptosis by promoting nuclear factor of activated T cells 1 (NFATc1) SUMOylation.⁷⁵ Meanwhile, NFATc1 downregulation caused by SUMOylation decreased histone deacetylase 1 (HDAC1) and HDAC1-mediated interferon regulatory factor 1 (IRF1) and resulted in inactivating the p38 MAPK signaling, thus alleviating HIRI-related symptoms.⁷⁵

In addition to apoptosis, pyroptosis is another form of programmed cell death that is initiated by inflammasomes and plays a crucial role in eliminating pathogens and reducing infection. This process can be identified by monitoring levels of apoptosis-associated speck-like protein (ASC), gasdermin D (GSDMD), and caspase-1, as well as the presence of IL-1 β and IL-18.⁷⁶ Following HIRI surgery, the secretion of ikaros by infiltrating macrophages activated inflammasomes, suppressed SIRT1 expression, and accelerated hepatic pyroptosis and inflammatory response in an adenosine monophosphate-activated protein kinase (AMPK)-dependent manner in mice. Myeloid-specific disruption of SIRT1 activated caspase-1-GSDMD processing,

resulting in the increased release of IL-1 β and IL-18 to exacerbate HIRI.⁷⁷ As a matter of fact, the methods of inhibiting pyroptosis for improving HIRI are usually divided into two types: on the one hand, activating the relevant signaling pathway of a non-dependent pathway of caspase; on the other hand, directly inhibiting caspase function. Caspase 1 and Caspase 11 are proteins associated with cell death and inflammation, which activate inflammatory factors and detect sterile stressors (DAMPs).⁷⁸ Wu et al.⁷⁹ pointed out that dexmedetomidine (DEX), a highly selective α 2-adrenergic agonist, repressed ASC and GSDMD-N levels and inhibited the activation of the nucleotide-binding oligomerization domain (Nod)-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome and pyroptosis by increasing miRNAs-494 expression, inhibiting JUND (a

proinflammatory transcription factor) and activating the PI3K/AKT/Nrf2 pathway in hypoxia/reoxygenation (H/R) cells. Concurrently, NLRP3 silencing regulated pyroptosis by downregulating caspase 1 activation and IL-1 β and IL-18 contents.⁷⁹ Kolachala et al.⁸⁰ further confirmed that in mice fed a high-fat diet (HFD) and undergoing HIRI, there was a significant increase in the expression of GSDMD and caspase-1. However, the double gene knockout of Caspase 1/11 or the deletion of the Caspase 1 gene improved liver injury by inhibiting inflammation and pyroptosis. Meanwhile, it was verified that suppressing Caspase 1 could suppress pyroptosis and play a protective role in hepatocytes, but not Caspase 11.⁸⁰

Apart from apoptosis and pyroptosis, ferroptosis is a hot research topic but is relatively rare compared to the other

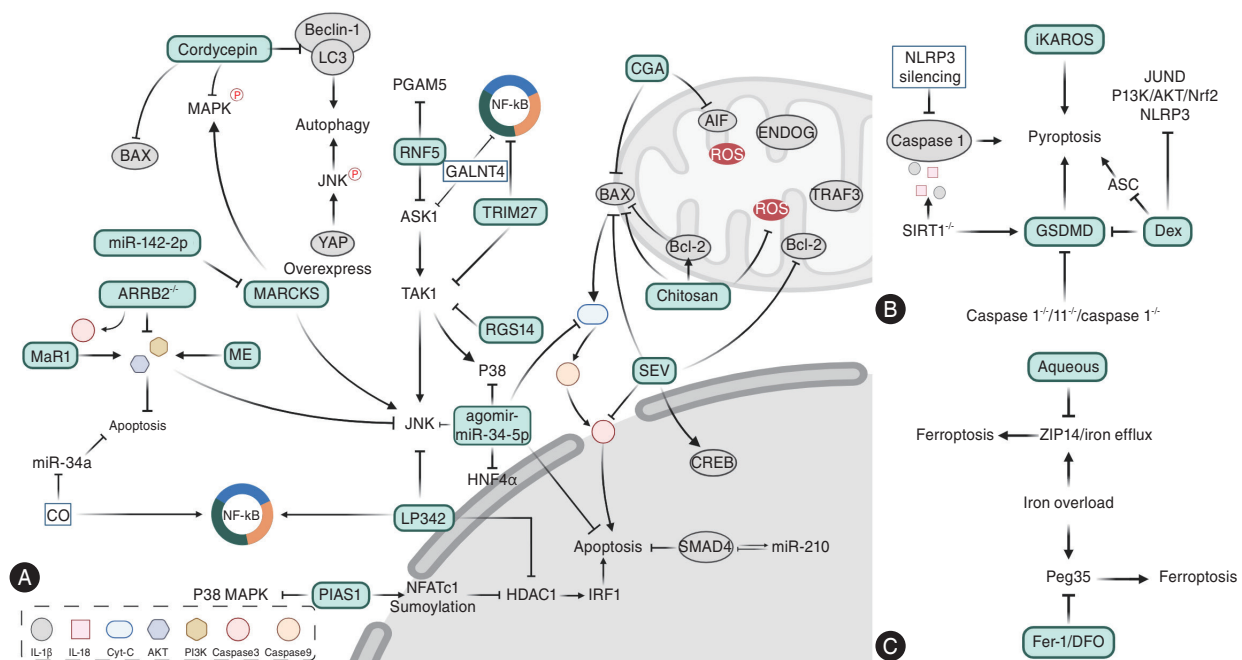


Figure 3. Potential strategies against cell death during HIRI. (A) SMAD, ERK, JNK, TAK1, p38 MAPK, NF- κ B, and PI3K/AKT influence HIRI by affecting apoptosis. (B) Several mechanisms influence HIRI progression by affecting pyroptosis. (C) Potential role of ferroptosis in HIRI. Bcl-2, B-cell lymphoma-2; Bax, Bcl-2-associated X protein; MAPK, mitogen-activated protein kinases; JNK, c-Jun N-terminal kinase; YAP, Yes-associated protein; *ARRB2*, β -Arrestin-2; MaR1, maresin 1; ME, methyl eugenol; CO, carbon monoxide; NF- κ B, nuclear factor kappa B; MARCKS, myristoylated alanine-rich C-kinase substrate; PGAM5, phosphoglycerate mutase family member 5; RNF5, ring finger protein 5; ASK1, apoptosis-regulating kinase 1; TAK1, transforming growth factor-beta-activated kinase 1; JNK, c-Jun N-terminal kinase; P38, p38 mitogen-activated protein kinases; MAPK, mitogen-activated protein kinases; PIAS1, protein inhibitor of activated STAT1; NFATc1, nuclear factor of activated T cells 1; GALNT4, N-acetylgalactosaminyltransferase-4; TRIM27, tripartite motif-containing 27; RGS14, regulator of G-protein signaling 14; HNF4 α , hepatocyte nuclear factor 4 α ; CGA, chlorogenic acid; AIF, apoptosis-inducing factor; TRAF3, tumor necrosis factor receptor (TNFR)-associated factor 3; SEV, sevoflurane; CREB, cAMP-responsive element-binding protein; HDAC1, SUMOylation decreased histone deacetylase 1; IRF1, interferon regulatory factor 1; IL-1 β , interleukin-1 β ; IL-18, interleukin-18; Cyt C, cytochrome c; AKT, PI3K, protein kinase B; phosphatidylinositol 3-kinase; NLRP3, nucleotide-binding oligomerization domain (Nod)-like receptor family pyrin domain-containing 3; SIRT1, sirtuin1; GSDMD, gasdermin-D; Nrf2, nuclear factor erythroid 2-related factor 2; ASC, apoptosis-associated speck-like protein; Dex, dexmedetomidine; ZIP14, zinc transporter14; PEG35, polyethylene glycol 35 kDa; Fer-1, ferrostatin-1; DFO, deferoxamine.

two in HIRI study. When induced by a high iron diet, ferroptosis leads to iron overload, lipid peroxidation, and increased expression of Ptgs2 (a marker of ferroptosis), exacerbating HIRI.⁸¹ However, treatment with ferroptosis-specific inhibitors like ferrostatin-1 (Fer-1), α -tocopherol, or iron chelators such as deferoxamine (DFO) can prevent cell death and reduce inflammatory damage in the liver.⁸¹ Proverbially, phospholipids are essential for oxidation in the process of ferroptosis and are regulated by long-chain acyl-coenzyme A synthase 4 (ACSL4). This study further suggested that GP78 upregulated the expression of Ptgs2 and ACSL4, promoting lipid remodeling to accelerate ferroptosis in gp78 overexpressed mice feeding MCD diets after surgery HIRI, which could be reversed by rosiglitazone, an inhibitor of ACSL.⁸² Most recently, we found that aqueous extract of *Radix Rehmanniae Praeparata* regulated intracellular iron metabolism by inhibiting zinc transporter14 (ZIP14)-mediated iron uptake and promoting hepcidin-mediated iron efflux processes to ameliorate HIRI,⁸³ suggesting a possibility of improving HIRI by inhibiting ferroptosis (Fig. 3).

The involvement of intracellular DAMPs in HIRI progression and related pharmacological therapy

DAMPs are intracellular molecules with immunoregulatory properties that are released during cell damage, death, or activation. These DAMPs, including HMGB1, IL-33, and ST2 growth stimulator expression factors, can trigger the release of inflammatory mediators, enhance the adhesion and infiltration of inflammatory cells, and modulate immune responses.⁸⁴ For instance, pretreatment with the natural product γ -oryzanol (ORY) could restrain the HMGB1/NLRP3/IL-1 β signaling pathways, thereby reducing inflammation caused by HIRI.⁸⁵ It is important to investigate whether DAMPs contribute to the increase in pro-inflammatory factors that worsen inflammation and tissue damage, or if these pro-inflammatory cytokines stimulate the release of more DAMPs, further amplifying the immune response.

Acknowledgedly, the main and most studied receptor for HMGB1 is TLR4. In a mouse model of surgical ischemia and a hydrogen peroxide-induced hepatocyte model, the pretreatment of pinocembrin, a monomer of flavonoid ex-

tracted from *Penthorum chinense Pursh*, scavenged ROS and attenuated tissue damage in liver accompanied by decreased levels of Bax and MDA and increased Bcl2, SOD and GSH levels *via* inhibiting the HMGB1/TLR4 signaling pathway.⁸⁶ Interestingly, after being fed with inulin, intestinal microbiota in mice can produce large amounts of propionic acid (PA),⁸⁷ of which administration also restrained HMGB1/TLR4 pathway and downregulated the production of inflammatory cytokines and chemokines in macrophages, which alleviated IR-induced liver injury.⁸⁸ However, this study only detected the protein expression of HMGB1 and TLR4 at this time, but there was a lack of discussion on the pathway relationship and direct evidence. Subsequently, Kawasoe et al.⁸⁹ further verified the effect of the domain 1 of recombinant thrombomodulin (rTMD1) on the HMGB1/TLR4 pathway on protein interaction to facilitate HIRI function. rTMD1 pretreatment diminished the activation of Ly-6G⁺ neutrophils and CD11b⁺ macrophages by blocking the binding of HMGB1 and TLR4 and subsequent reducing HMGB1/TLR4 pathways and the release of pro-inflammatory cytokines and chemokines after reperfusion. It is worth noting that rTMD1 can still improve the damage caused by HMGB1/TLR4 signal, even after the IR insult has started.⁸⁹ In addition to HMGB1/TLR4 regulation mode, the level of plasma thrombin, the main effector protease to activate thrombomodulin (TM), is mainly dependent on domain 1 (rTMD1) for its regulatory role.⁹⁰ was strikingly increased in clinical patients after hepatectomy. Noguchi et al.⁹¹ further found that as a highly selective inhibitor of thrombin, dabigatran pretreatment could inactivate and degrade the excessive expression of HMGB1 to decrease neutrophilic recruitment by increasing the expression of TM and downregulate the expression of inflammatory cytokines (TNF- α and IL-6) and VCAM-1 caused by HMGB1 infiltration in mice model of HIRI. Subsequently, the researchers found that HMGB1-induced damage in HIRI is also closely related to epigenetic modification. Sosa et al.⁹² indicated that human orthotopic liver transplant (OLT) recipients with IRI showed the significant upregulation of disulfide-HMGB1 (dis-HMGB1), histone acetyltransferase GTF3C4 and decrease of histone deacetylase HDAC5, which further elevated hyperacetylation of HMGB1 and translocated into cytoplasmic vesicles as a pro-inflammatory cytokine, ulteriorly activating pro-inflammatory macrophages and promoting TNF- α secretion from macrophages by combining TLR4 to induce cell death.

Once activated by whatever form in HIRI, HMGB1 will inevitably exacerbate inflammatory damage and immune responses by affecting multiple signaling pathways, such as the relatively most studied NF- κ B. Nogo-B (Reticulon 4B), a protein mainly localized to the endoplasmic reticulum, was largely expressed on macrophages and facilitated macrophage infiltration in mouse ischemic livers. Nogo-B^{KO} deficiency inhibited phosphorylation of NF- κ B p65 and expression of Nogo-B deletion-induced YAP, which further decreased M1 macrophage polarization in HMGB1-stimulated bone marrow-derived macrophages (BMMs), which could be eliminated with YAP siRNA intervention *in vivo* and *in vitro*.⁹³ This above study indicated that the regulation of immune response by HMGB1 is a worthy research direction in HIRI. With further deeper research, in mice subjected to IR procedure following Annexin A1 N-terminal peptide Acetyl 2-26 (Ac2-26) preconditioning, neutrophil infiltration was reduced, accompanied by fewer Ly6G⁺ cells and MPO

activity by suppressing the activation of HMGB1/TLR4/NF- κ B signaling pathway.⁹⁴ Furthermore, multiple studies have developed that a nanostructured lipid carrier of berberine isolated from traditional medicinal plants (NLC BBR) therapy⁹⁵ or lncRNA potassium voltage-gated channel subfamily Q member 1 opposite strand/antisense transcript 1 (KC-NQ1OT1) knockdown⁹⁶ could palliate HIRI *via* restraining the HMGB1/TLR4/NF- κ B inflammatory signaling. In addition to TLR4, our recent study demonstrated that HMGB1 may also influence TLR3 to aggravate HIRI. Likewise, acteoside (ACT) could prevent HMGB1 binding with TLR3/4 on LSECs and the subsequent activation of IRF1 nuclear translocation, as well as restrain neutrophils chemotaxis, which eventually exerts protective effect from an immune perspective in mice with HIRI surgery.⁹⁷ Besides TLR4/3, TLR9 is another pivotal innate immune receptor. Allyson *et al.* elaborated that dis-HMGB1 promoted mass generation of monocyte cytokines as well as stimulated the polar-

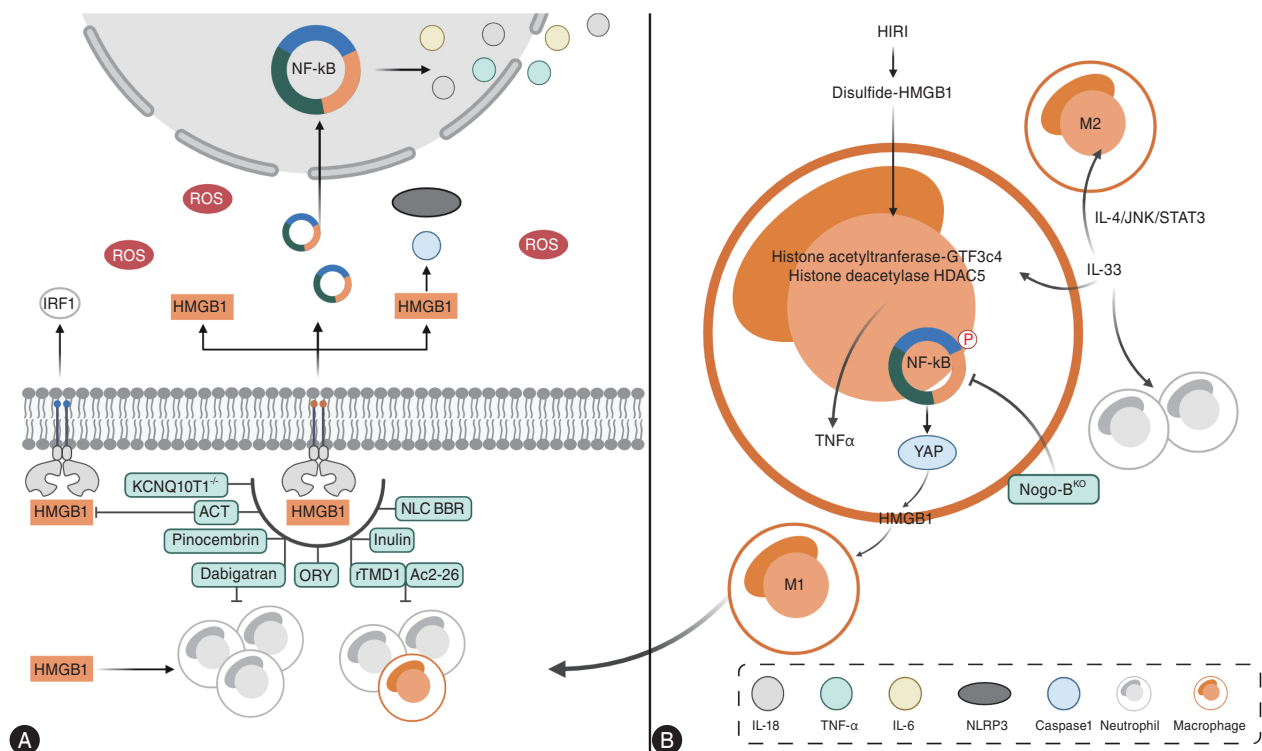


Figure 4. The mechanism of DAMPs involved in HIRI, especially HMGB1 and IL-33. (A) The mechanism of HMGB1 included in HIRI. (B) IL-33 is a double-edged sword during HIRI progression. NF- κ B, nuclear factor kappa B; ROS, reactive oxygen species; NLRP3, nucleotide-binding oligomerization domain (Nod)-like receptor family pyrin domain-containing 3; HMGB1, high mobility group box-1 protein; IRF1, interferon regulatory factor 1; KCNQ10T1, potassium voltage-gated channel subfamily Q member 1 opposite strand/antisense transcript 1; ACT, acteoside; ORY, γ -oryzanol; NLC BBR, nanostructured lipid carrier of berberine isolated from traditional medicinal plants; rTMD1, recombinant thrombomodulin; Ac2-26, acetyl 2-26; HDAC5, Histone deacetylase 5; TNF- α , tumor necrosis factor-alpha; IL-6, interleukin-6; IL-4, interleukin-4; IL-1 β , interleukin-1 β ; IL-33, interleukin-33; JNK, c-Jun N-terminal kinase; YAP, Yes-associated protein.

ization of monocyte-derived macrophage (MDM) from OLT patients by activating TLR4 and TLR9, which further contributed to an enhanced ability of MDM to present antigens and activate T cells and aggravated the degree of liver injury in OLT murine model of IRI.⁹⁸ Gratefully, restrained diSHMGB1 with the therapy of HMGB1 neutralizing antibodies or TLR4/9 inhibitors could reverse the above situation.

IL-33 plays a crucial role in DAMPs and can regulate immune responses and contribute to the progression of HIRI. Following HIRI in mice, there is a rapid release of IL-33, and studies have shown that IL-33-deficient mice exhibit reduced I/R injury, as evidenced by decreased neutrophil recruitment, lower levels of ALT, and reduced tissue damage.⁹⁹ Furthermore, IL-33 may serve as a potential biomarker for early hepatic I/R injury in human liver transplantation, as its serum levels increase immediately after reperfusion of the liver graft.⁹⁹ However, it is worth noting that subsequent studies have pointed out that IL-33 may also play a beneficial protective role, which may be related to the positive regulation of M2 macrophage in HIRI from some perspectives. Notably, after I/R injury, a low dose but long-course administration of IL-33 reduced hepatocyte edema and hepatic vessel congestion and were associated with the increasing polarization of CD45⁺CD11b⁺F4/80^{high} macrophages to the M2 type.¹⁰⁰ Mechanically, innate lymphoid cells (ILC2s) proliferation stimulated by exogenous IL-33 could promote M2 macrophage polarization *via* regulating the IL-4/JNK/Stat3 pathway.¹⁰⁰ This suggests that IL-33 is a double-edged sword and its intrinsic mechanism in HIRI needs to be further explored (Fig. 4).

Effect of intracellular inflammatory response on HIRI development and providing a promising solution

Although inflammatory targets have been covered in previous chapters, once HIRI happened, apart from the released DAMPs and immune cells mentioned above, hepatic inflammatory signaling is associated with many kinds of intracellular damage and have more complex effects on liver damage and immunologic process than we thought.^{101,102} As above research demonstrated, chemokines and chemokine receptors are also elements that trigger or respond to inflammation involved in HIRI. It has been widely regarded as a truism that TLR4 has a powerful recognition func-

tion like the eyes of the immune response, and is involved in inflammation and cell damage by TLR4-dependent or independent myeloid differentiation factor 88 (MyD88) pathway. At the same time, this process also involves many transcription factors that participate in TLR4 regulation. Notably, NF- κ B is involved in the TLR4-dependent MyD88 pathway or NLRP3 signal pathway, which can cause severe inflammation and cell damage. There is evidence that NF- κ B, a key regulator of intranuclear immunity, also has potential to become a downstream regulatory target of receptor tyrosine kinase (AXL) that has significant anti-inflammatory and antiapoptotic effects. Activated AXL decreased hepatic inflammation by promoting the expression level of suppressor of cytokine signaling-1 (SOCS-1) to inhibit TLR4 and its downstream signaling pathways (MyD88/NF- κ B) and inflammatory cytokines (TNF- α , IL-1 β , and IL-6) expression on one side, as well as alleviated hepatocyte apoptosis and improved HIRI *via* reducing the proapoptotic proteins BAX and cleaved caspase-3 levels, and increasing the antiapoptotic protein Bcl-2 levels on the other side.¹⁰³ In addition, telmisartan, a partial agonist of PPAR γ , also can directly decline the expression of pro-inflammatory cytokines and attenuate the downstream inflammatory response by inhibiting TLR4/NF- κ B signal pathway to protect liver IR.¹⁰⁴ Moreover, TRAF3, as an adaptor molecule, regulates NF- κ B pathways causing inflammatory responses. The ubiquitinated TRAF3 at Lys138 aggravated oxidative stress and activated the NF- κ B inflammation pathway induced by H/R in BRL-3A cells, while HOPE therapy effectively relieved IR.¹⁰⁵ On the other hand, morin (MRN), a natural flavanol isolated from plants of the Moraceae family, also counteracted the inflammation by repressing TLR4, NF- κ B and NLRP3 expression following the hepatic impairment.¹⁰⁶ It is important to recognize that both ASC and caspase-1 play crucial roles in the NLRP3 inflammasome and collectively impact inflammatory responses. Furthermore, pre-treatment with salidroside, an active compound derived from *Rhodiola* plants, has been shown to reduce the activation of NF- κ B and the NLRP3/ASC/caspase-1 inflammasome, as well as inhibit the TLR4-dependent MyD88 pathway, leading to a dose-dependent decrease in proinflammatory cytokine levels by blocking TLR4 activation. Noteworthy, the hepatoprotective effects of salidroside can be negated by the administration of a TLR4 agonist, LPS.¹⁰⁷

Proverbially, inflammation in the intestinal tract can po-

tentially alter the composition of intestinal microbes and their metabolites. Simultaneously, hepatic inflammation can be triggered by the affected intestinal tract and the intestinal microbes, leading to an intensification of inflammation. *Clostridium butyricum* (*C. butyricum*), a probiotic in human gut, restored liver function *via* decreasing the Firmicutes/Bacteroidetes ratio and increasing the relative abundance of probiotics such as *Lactobacillus* and *Bifidobacterium*. This was associated with a decline in the levels of short-chain fatty acids (SCFAs) and butyric acid levels in a rat model of HIRI. Meanwhile, *C. butyricum* supplement attenuated inflammation accompanied by reduced LPS, TNF- α and IL-6 levels and downregulated TLR4 and NF- κ B p65 expressions.¹⁰⁸ Other evidence further explained the mechanism about how intestinal flora regulates inflammation induced by HIRI. The pretreatment of prophylactic antibiotics (Abx) could attenuate HIRI associated with the reduction of CCR2⁺ monocytes recruitment by reshaping the gut microbiota including decreasing the relative abundance of Firmicutes and increasing the relative abundance of *Clostridium*, increasing the levels of hepatic unconjugated bile acid species (unconjugated BAs, especially UDCA), and activating liver farnesoid X receptor (FXR).¹⁰⁹ Further, the activation of FXR decreased the expression of TLR4 and suppressed its downstream MAPK/NF- κ B signaling pathways and the CCL2-CCR2 axis both *in vivo* and *in vitro*, along with the decrease of TLR4, p-ERK1/2, p-P38 and p-JNK1/2 expression.¹⁰⁹

Considering that ischemia and reperfusion usually lead to the impairment of whole hepatic function, hepatocyte regeneration and functional recovery after liver injury have also been a hot research direction. Schmitz et al.¹¹⁰ found the inhalation of noble gas argon upregulated the pro-inflammatory response by increasing expression of cytokines encompassing IL-1 β and IL-6 and reduced hepatic regeneration capacity by declining the mitotic index and the cell cycle marker Ki-67 levels to aggravate liver damage after the mice with HIRI surgery.¹¹⁰ Observingly, when hepatocytes are damaged, autophagy can restore the volume and function of the liver by eliminating abnormal hepatocytes and enhancing hepatocyte regeneration. Pituitary adenylate cyclase-activating polypeptide was reported to exert hepatoprotective effects by amplifying hepatocellular regeneration in autophagy-dependent manner, which was relied on the improvement of CREB activation and Kruppel-

like factor 4 (KLF4) expression.¹¹¹ On the other hand, in cases of liver dysfunction following I/R injury, autophagy activation may also play a negative regulatory effect under certain circumstances. For instance, autophagy may also decline the proliferation of hepatocytes by enhancing the activation of non-parenchymal hepatocytes. As research pointed out, IRF8, a pivotal upstream intracellular regulatory factor, caused cytokine secretion and autophagy formation. Shi et al.¹¹² elucidated that IRF8 knockout inhibited inflammatory injury and improved HIRI, whereas, unlike IRF8 knockout mice, the IRF8 overexpression of mice exerted a higher exacerbation of liver injury. Further research showed that IRF8-mediated chemokine release (CXCL1, CXCL9 and CCL2) significantly aggravated the liver structure damage *via* accelerating autophagy formation and the NF- κ B pathway in Adeno-Associated Virus 8-IRF8-I/R (AAV8-IRF8-I/R) mice, which could be remedied by hydroxychloroquine (HCQ) or secukinumab pretreatment.¹¹² Notoriously, the chemokine receptor CXCR4 stands out for its pleiotropic roles, for example, as potential substrates and receptors of dipeptidyl peptidase-4 (DPP-4) and also as a receptor for CXCL12. Sitagliptin acted as DPP-4 inhibitors, of which pretreatment downregulated the expression of chemokine stromal-derived factor 1 (SDF1) and CXCR4 to protect liver in HIRI animals (Fig. 5).¹¹³

Regulation of immune cell on HIRI development and establishing protective approaches

The immunity events contributing to hepatocyte damage are fairly complicated and involve the interaction between hepatocytes, neutrophils, macrophages, KC and other immune cells in the pathophysiological process of HIRI. Specifically, there is no consentaneous conclusion on this question about how do immune cells attack hepatocytes to exacerbate injury or ameliorate inflammation during HIRI. As is known to all, neutrophils have chemotactic, phagocytotic and bactericidal properties, and they are attracted to the site of injury by chemotactic substances when HIRI occurs. Chronic plus binge alcohol (Gao-binge)-fed mice underwent hepatic IRI surgery and showed neutrophil infiltration and the increase in mixed lineage kinase-like protein (MLKL) plasma membrane translocation.¹¹⁴ However, the deletion of *Mkl1* had non-significant protective effects against IR liver injury in ethanol-fed mouse livers.¹¹⁴ Fortu-

nately, dermcidin (DCD) is a peptide derived from sweat glands and its analog is contained on a Cys (C)→Ser (S) substitution at residue 34 (DCD-C34S), all observably preventing IR damage at the beginning of reperfusion. Further, DCD attenuated Gr-1-positive neutrophils infiltration and I/R-elicited inflammation by suppressing the phosphorylation of epidermal growth factor receptor (EGFR) and AKT as well as reducing the expression of a neutrophilic CXC chemokine, CXCL2 and NO production.¹¹⁵ Besides CXCL2, the adhesion and infiltration of neutrophils are also related to other adhesion molecules. Other evidence demonstrates that endomucin (EMCN) is a negative regulator of leukocyte adhesion. And the overexpression of EMCN inhibited leukocyte adhesion and the infiltration of CD11b⁺Ly6G⁺ neutrophils caused by HIRI *via* preventing integrin subunit beta 2 (ITGB2, LFA-1) on neutrophils binding to intercellular adhesion molecule-1 (ICAM-1) on liver sinusoidal endothe-

lial cells (LSECs) to abrogate liver injury.⁶⁰ Similarly, CD321 also is an essential junctional adhesion molecule (JAM) to advance neutrophil migration into inflammatory tissues.¹¹⁶ CD321, the essential molecules for trans-endothelial migration of leukocytes, could interact with lymphocyte function-associated antigen 1 (LFA-1) on CD45⁺ neutrophils to dramatically increase infiltrated neutrophil in a murine HIRI model, while anti-CD321 monoclonal antibody (90G4 mAb) treatment could significantly block neutrophil and leukocytes infiltration in the early phase of HIRI.¹¹⁶ Not only CD321, but carabin, as the negative regulator of calcineurin, also can affect neutrophil migration by interacting with calcineurin and Ras.¹¹⁷ Carabin-deficient mice (LysMCr: Carabinfl/fl) showed more severe damage and inflammatory symptoms associated with CD11b⁺Ly6G⁺ neutrophils recruitment *via* the activation of Ras-ERK and calcineurin-NFAT (nuclear factor of activated T cells) pathways, which

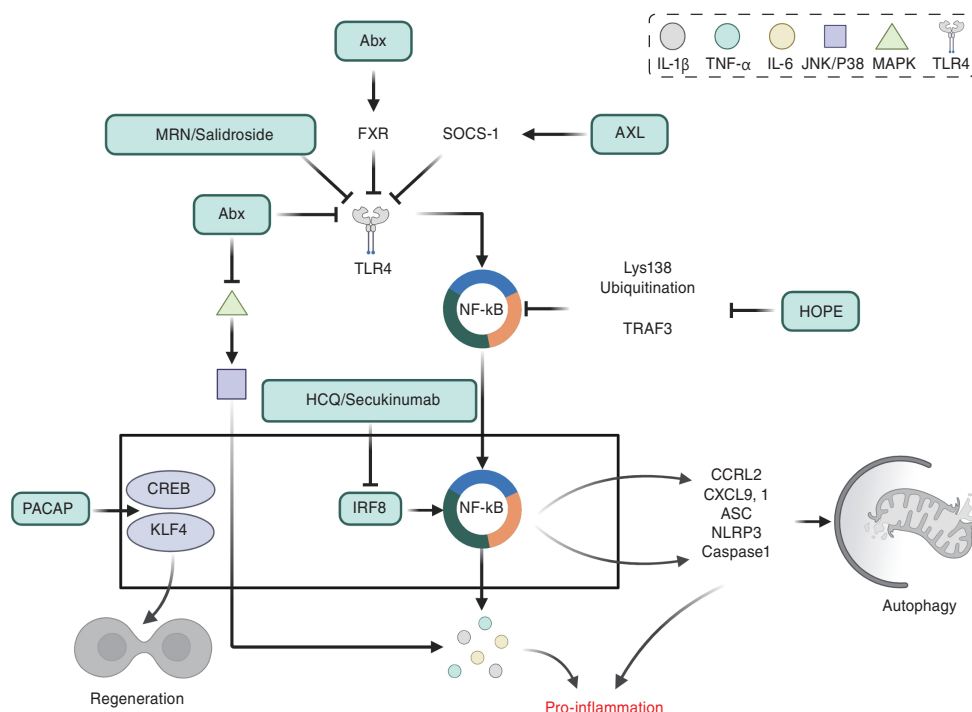


Figure 5. Pathophysiological effects and consequences of intracellular inflammation in HIRI. Inflammation could influence HIRI through TLR4, JNK/p38, NF-κB and other kinds of signal pathways. Abx, antibiotics; MRN, morin; FXR, farnesoid X receptor; SOCS-1, suppressor of cytokine signaling-1; AXL, receptor tyrosine kinase; TLR4, toll-like receptor 4; NF-κB, nuclear factor kappa B; HOPE, hypothermic oxygenated perfusion; TRAF3, tumor necrosis factor receptor (TNFR)-associated factor 3; HCQ, hydroxychloroquine; PACP, pituitary adenylate cyclase-activating polypeptide; CREB, cAMP-responsive element-binding protein; KLF4, kruppel-like factor 4; IRF8, interferon regulatory factor 8; CCRL2, C-C motif chemokine receptor-like 2; CXCL1, C-X-C Motif Chemokine Ligand 1; CXCL9, C-X-C Motif Chemokine Ligand 9; ASC, apoptosis-associated speck-like protein; NLRP3, nucleotide-binding oligomerization domain (Nod)-like receptor family pyrin domain-containing 3; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; IL-4, interleukin-4; IL-1β, interleukin-1β; TLR4, toll-like receptor 4; JNK, c-Jun N-terminal kinase; P38, p38 mitogen-activated protein kinases; MAPK, mitogen-activated protein kinases.

could be reversed by treatment with anti-P-selectin, E-selectin and $\alpha v\beta 3$ integrin.¹¹⁷

Furthermore, neutrophils can aggravate HIRI by inducing chemokines to the injured site, but also intensify HIRI through the formation of neutrophil extracellular traps (NETs).¹¹⁸ The knockdown of histidine-rich glycoprotein (HRG) led to the increased expression of inflammatory factors (IL-6 and TNF- α), MPO-DNA, nucleosome and Ly6G⁺ neutrophil accumulation, as well as up-regulation of NETs formation, accompanied by the downregulation of FXR and hypoxia inducible factor 2 alpha subunit (HIF2A) in HIRI mice, and gave rise to the susceptibility of the methionine-choline-deficient diet (MCD) mice to HIRI.¹¹⁹ Another study pointed out that resveratrol, as a natural polyphenol found in kinds of plants, its preintervention repressed endothelin 1 secreted by neutrophils in an autocrine manner to ameliorate oxidative stress and the inflammatory response of neutrophils accompanied by decreased NETs generation via suppressing the ERK/c-Fos signaling pathway.¹²⁰ In addition to neutrophils themselves affecting the immune microenvironment and aggravating HIRI, what is noteworthy is that NETs could activate LSECs by promoting the expression of integrins on LSEC to augment infiltration of neutrophil pro-inflammatory immune cells in a murine model of liver IRI. Interestingly, exercise training (ExT) preconditioning as a non-pharmacological therapy before the operation attenuated neutrophils infiltration by intercepting NET formation.¹²¹

Among these immune cells, macrophages are divided into BMDM and liver-derived macrophages called KCs. Notably, Kupffer cells, colonized in the liver and distributed along the LSECs, have caught the researchers' attention in HIRI process. Under TNF- α stimulation, KCs can secrete neutrophil chemotactic agents and pro-inflammatory factors, conclusively inducing neutrophil immune response.¹²² HIRI mice also showed the recruitment of KCs, as well as release of proinflammatory cytokines and ROS derived from the activation of KCs and neutrophils. But a hydrophilic carbohydrate-derived nanoparticle (C-NP) was synthesized as an effective nanoantioxidant against HIRI and suppressed the inflammatory effect of the above immune cells by removing excessive ROS.¹²³ Proverbially, Rao et al.¹²⁴ ultimately indicated that the expression of Nrf2 in KCs was significantly increased in HIRI mice. Myeloid-specific Nrf2-KO mice (Nrf2^{M-KO}) with IR stress-induced liver injury

showed more hepatic ROS, inflammation, and hepatocellular apoptosis/necrosis that were associated with the activation of the Ras homolog family member A (RhoA)/Rho-associated coiled-coil containing protein kinase (ROCK) pathway by repressing the target gene tissue inhibitor of metalloproteinase 3 (Timp3). Sulforaphane (SFN) promoted Nrf2 translocation in KCs to enhance Nrf2 interacting with the antioxidant response element (ARE), and declined hepatic injury in ARE-*luc* mice after IRI.¹²⁵ Moreover, researchers have indicated that except for Nrf2, immune regulatory factors IL-18 and a stimulator of interferon genes (STING) also actively participate in macrophages regulation, respectively effecting the activation of macrophages¹²⁶ and the release of apoptosis signals by macrophages.¹²⁷ Aging mice exhibited more serious HIRI associated with excessive proinflammation in macrophages by intensifying intrahepatic NLRP3 activation in STING-dependent manner.¹²⁸ Further research showed that hepatic STING significantly upregulated the expression of caspase 1-GSDMD and inflammatory cytokines IL-1 β /IL-18 to cause inflammation and damage but which were prominently restrained by the treatment of knockdown STING in macrophages, VX-765 (caspase 1 inhibitor) or liposome-encapsulated clodronate (the macrophage inhibitor) during HIRI. *In vitro*, STING strengthened caspase 1-GSDMD processing and inflammatory response by increasing intracellular Ca²⁺ to initiate Kupffer cell pyroptosis after H/R, which could be prominently mitigated by BAPTA-AM (a calcium chelator) administration.¹²⁹

Moreover, VEGF contains a variety of receptor subtypes that may play their own unique roles in HIRI. Therapy of pro-lymphangiogenic factors like VEGF-D can improve liver repair in mice model of HIRI and attenuate hepatic surgery I/R injury by fortifying the number of hepatic restorative macrophages and the expression of mannose receptor (MR), Fizz1 and IL-10.¹³⁰ In contrast, chemokines and chemokine receptors can be secreted by pro-inflammatory KCs and regulate cell migration further to aggravate injury during HIRI. Wang et al.¹³¹ also reported that after reperfusion, proinflammatory phenotype of the C1QC_KC cluster specifically expressed CXCL12 and responded to the CXCR4 receptor on the T/B cell clusters to upregulate the migration of cells, as well as highly expressed TNFAIP3 interacting protein 3 (TNIP3). Interestingly, TNIP3 secreted by inflammatory cells could ameliorate HIRI by decreasing

inflammation via inhibiting NF- κ B pathway.¹³¹ In addition, a new subpopulation of KCs that is colony-stimulating factor 3 (CSF3) positive KCs, namely inflammation-associated Kupffer cell subtype (iKC), has been found to exacerbate HIRI injury through the IL-17 signaling pathway, but also activated T cells in adaptive immunity to participate in the immune response of HIRI. At the same time, CSF3⁺KCs can facilitate the antigen presentation of dendritic cells (DCs) through JAG1/Notch2 and CD47/signal regulatory protein gamma (SIRPG) axes, indirectly promote the recruitment of CCR7⁺ CD8⁺ T cells in transplanted steatotic liver microenvironment, and ultimately aggravate inflammatory damage caused by I/R injury.¹³²

Interestingly, the researchers indicated that KCs and BMDM may co-regulate HIRI processes by regulating similar signaling pathways. Principally, CD47 and signal regulatory protein alpha (SIRP α) are immune test sites, but play a positive dominant role in the immune response of HIRI process. In mice model of IR-induced sterile inflammatory liver injury, adoptive transfer of mesenchymal stem cell (MSC) showed that CD47 interacted with macrophage SIRP α . This interaction inhibited proinflammatory mediators by enhancing downstream SMO activity and increased nuclear expression of Gli1 and Notch1 intracellular domain (NICD) of macrophage, which caused Dvl2 upregulation. Dvl2 upregulation acts as a negative regulator of NLRP3-driven inflammatory response, thereby inhibiting NEK7/NLRP3 activity.¹³³ Notably, as a Nod-like receptor, NLRP3 is related to the regulation of Notch signal of immune cells mediated by Jagged1 (JAG1).¹³⁴ Jin et al.¹³⁵ found that in Notch1-proficient (Notch1^{FL/FL}) model of liver I/R injury, JAG1-mediated myeloid Notch1 signaling pathway not only reduced macrophage infiltration under IR stress by promoting heat shock transcription factor 1 (HSF1)/Snail activation and inhibiting TXNIP/NLRP3 activation, but also reduced apoptosis of hepatocytes under IR stress. Interestingly, Snail suppressed pro-inflammatory cytokines such as TNF- α , IL-1 β and CXCL-1 and ameliorated the inflammatory response and apoptosis through the downregulation of NLRP3 and caspase-1 in the Notch1^{FL/FL} model. Moreover, adoptive transfer of lentivirus expressing HSF1 transgenic macrophages could improve inflammatory response and hepatocellular apoptosis by enhancing Snail expression and inhibiting NLRP3, thioredoxin-interacting protein (TXNIP), and ASC expression in Notch1 knockout model (Notch1^{M-KO}).¹³⁵

Along with the deepening of study, the researchers pointed out that the polarization of liver macrophages and its related immune response are closely related to HIRI. HIRI mice exhibited KCs M1 polarization and the increase of TNF- α , IL-1 β and IL-6, which was decreased by liraglutide treatment.¹³⁶ Another study further demonstrated the intracellular mechanism affecting the direction of hepatic macrophages polarization. Alpha B-crystallin (CRYAB) promoted the differentiation of liver macrophages into M2 phenotype through activating AKT1/mammalian target of rapamycin (mTOR), thereby alleviating the liver IRI in RAW 264.7 cell hypoxia/reoxygenation (H/R) model. It was also found that miR-450b-5p can directly target CRYAB and inhibit the Akt/mTOR phosphorylation and CRYAB level in liver IRI, but miR-450b-5p inhibitors can improve the above situation.¹³⁷ Besides, Zhou et al.¹³⁸ similarly proved that macrophage migrated to injury location and polarized to M1 state *in vitro* by activating cathepsin E (Cat E)/AKT/mTOR signaling pathway, which could be inhibited by plasma membrane-bound G protein-coupled bile acid receptor (TGR5) in myeloid cells. While ursodeoxycholic acid administration could relieve HIRI by activating TGR5 and facilitating macrophage M2 polarization. Attentively, Zito et al.¹³⁹ pointed out that peripheral blood mononuclear cells (PBMC)-derived M1-like macrophages were a befitting model to research HIRI *in vitro*. It elaborated that human amnion-derived mesenchymal stromal/stem cells (hAMSCs) pre-conditioning by the IFN- γ pre-treatment could also restrain inflammation induced by M1-differentiated macrophages via reducing IL-1 β , IL-12 and phosphotransferase system gene (PTSG) 2 levels, activating hepatocyte growth factor (HGF)-met signaling (Fig. 6).¹³⁹

DISCUSSION

During surgery, the ischemic process of HIRI is generally divided into hot ischemia and cold ischemia stages. In hot ischemia, due to the lack of oxygen and various metabolic substrates and relatively higher levels of liver metabolism, hepatic ischemic damage occurs faster and is more severe.¹⁰² In contrast, cold ischemia can increase organ tolerance to ischemia, but is often used in transplant surgery. Contrary to the general perception, the high incidence of HIRI is not limited to lobectomy, liver transplantation and

other operations but also occurs in the process of restoring blood supply in patients with ischemic organs and tissues. Therefore, we emphatically focused on the mechanism of action and treatment of hepatic hot ischemia-reperfusion injury. Recently, researchers have explored corresponding targeted drugs based on pathogenesis to block the damage process, but due to the complexity of HIRI pathogenesis, further exploration of the underlying mechanism is still necessary. Although researchers have attempted to comprehensively investigate the pathological mechanism, they

have focused mainly on the damage process caused by oxidative stress, followed by the apoptosis process and therapeutic targets. Hence, to further understand the current status of HIRI treatment, we searched for articles related to HIRI clinical practice. However, we found that most human sample studies were limited to exploring the target of abnormal expression after HIRI, and mechanistic studies or pharmacodynamic identification were subsequently performed in animal or cell models.^{91,109,140-142} However, as expected, Zhang et al.^{143,144} found that from incision

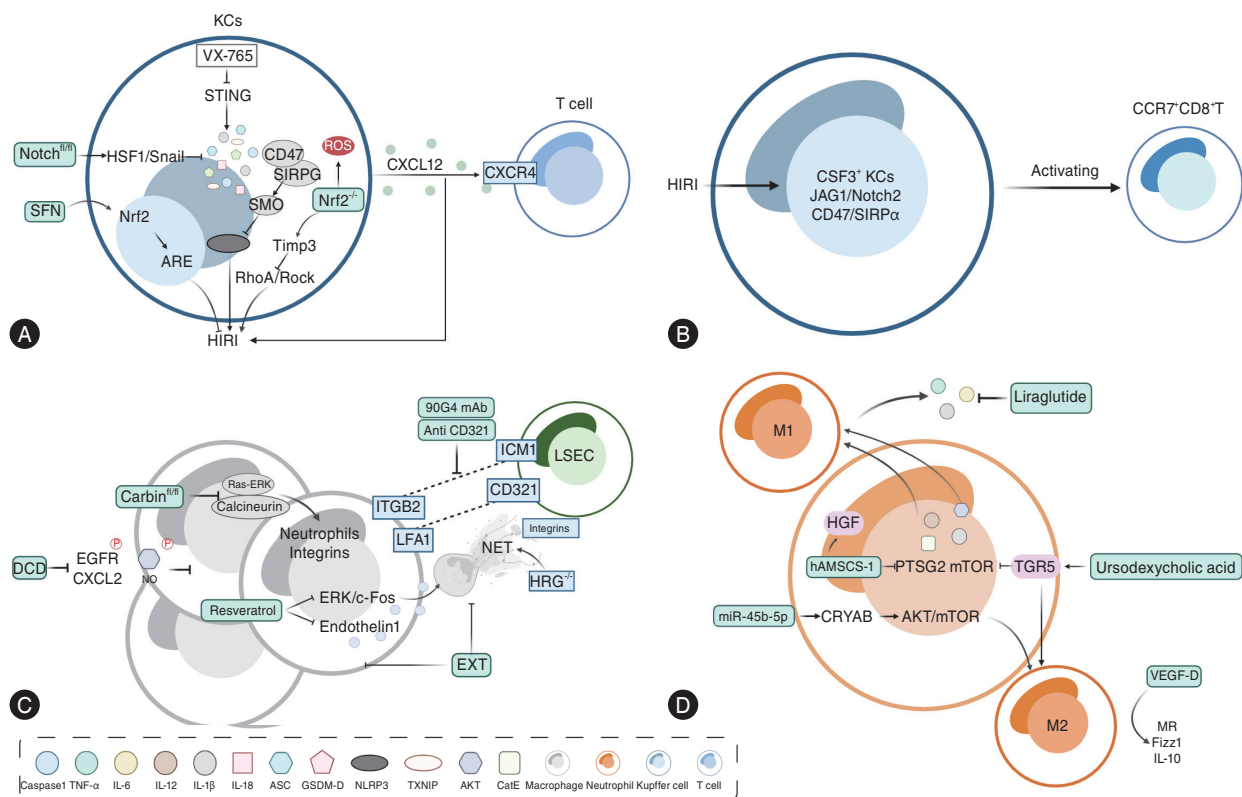


Figure 6. The mechanisms by which immune cells affect HIRI. (A) Immune response happened in KCs during HIRI. (B) CSF3⁺KCs promote the recruitment of CCR7⁺ CD8⁺ T cells through JAG1/Notch2 and CD47/SIRPG axes. (C) The neutrophils infiltration and NET formation are closely related to HIRI. (D) The polarization of liver macrophages and its related immune response play an important role in HIRI. HIRI, hepatic ischemia-reperfusion injury; TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6; IL-12, interleukin-12; IL-1 β , interleukin-1 β ; IL-18, interleukin-18; ASC, apoptosis-associated speck-like protein; GSDMD, gasdermin-D; NLRP3, nucleotide-binding oligomerization domain (Nod)-like receptor family pyrin domain-containing 3; TXNIP, thioredoxin-interacting protein; AKT, protein kinase B; Cat E, cathepsin E; KCs, Kupffer cells; T cell, T lymphocyte; LSEC, liver sinusoidal endothelial cell; STING, stimulator of interferon genes; SFN, sulforaphane; HSF1, heat shock transcription factor 1; SIRPG, signal regulatory protein gamma; ARE, antioxidant response element; Nrf2, nuclear factor erythroid 2-related factor 2; Timp3, target gene tissue inhibitor of metalloproteinase 3; RhoA, Ras homolog family member A; ROCK, Rho-associated coiled-coil containing protein kinase; CXCL12, C-X-C Motif Chemokine Ligand 12; CXCR4, chemokine (C-X-C motif) receptor 4; JAG1, jagged1; SIRP α , signal regulatory protein alpha; DCD, dermcidin; EGFR, epidermal growth factor receptor; CXCL2, C-X-C Motif Chemokine Ligand 2; NO, nitric oxide; ERK, extracellular signal-related kinase; ITGB2, LFA-1, integrin subunit beta 2; ICAM-1, intercellular adhesion molecule-1; NET, neutrophil extracellular trap; HRG, histidine-rich glycoprotein; Ext, exercise training; HGF, hepatocyte growth factor; hAMSCs, human amnion-derived mesenchymal stromal/stem cells; PTSG2 phosphotransferase system gene 2; mTOR, mammalian target of rapamycin; TGR5, plasma membrane-bound G protein-coupled bile acid receptor; CRYAB, Alpha B-crystallin; AKT, protein kinase B; VEGF, vascular endothelial growth factor; MR, mannose receptor; IL-10, interleukin-10.

to the end of surgery, injecting DEX in pediatric patients undergoing liver transplantation could reduce postoperative liver enzyme levels and HIRI severity. This is relevant with another animal study we pointed out earlier. Concretely, DEX could improve HIRI through the miR-494/JUND/PI3K/AKT/Nrf2 axis,⁷⁹ suggesting the materiality of the above targets in the field of HIRI therapy.

Of concern, some studies have pointed out that mitochondria are the first affected organelles in the two processes of ischemia and reperfusion, and in the occurrence and development of HIRI, mitochondrial dysfunction and mitochondrial autophagy have a critical regulating effect in liver injury, death and regeneration. Interestingly, in addition to the commonly used mouse model, Feng et al.¹⁴⁵ demonstrated that in a canine HIRI model, the inhibition of BMAL1, a transcription factor that regulates circadian rhythms, improved hepatocyte injury by decreasing sympathetic nerve activity in the celiac ganglion (CG) and the liver, reducing IL-1 β , TNF- α , and MDA levels, and elevating GSH levels. However, the regulatory role of BMAL1 in the mouse HIRI model has not been mentioned in studies, but some studies have pointed out that melatonin enhanced autophagy through the SIRT1-BMAL1 pathway to restrain cerebral ischemia-reperfusion injury in diabetic mice, which also provides research ideas for therapy HIRI.¹⁴⁶ In future, researchers can consider directly applying animal models with specific knock-out of mitochondrial autophagy associated genes (PINK1 or Parkin) to investigate the role of mitochondria in surgically induced HIRI, as well as explore the protective effect of drugs on HIRI caused by mitochondrial dysfunction and its possible mechanism. Meanwhile, mitochondria are the enforcers of apoptosis, of which structure and function changes occur earlier than chromosomes. Probably because various targets and pathways (including but not limited to Bcl-family, caspases, JNK pathway or PI3K/Akt pathway) are activated and eventually enter mitochondria, causing apoptosis of mitochondrial pathway. The researchers focused on the role of common apoptosis-related pathways in the regulation of different HIRI animal models or HIRI therapeutics. For example, Guo et al.⁶³ proved that TAK1/JNK/p38 signaling can intensify the occurrence of apoptosis in HIRI process by using global Steap3-KO mice in the study. Zhang et al.⁶⁵ used RGS14-KO mice as an animal model of ischemia-reperfusion surgery to explore the negative regulation of TAK/JNK

signaling pathway, and finally demonstrated that RGS14 could reverse inflammation and apoptosis. The above research direction has become the mainstream trend, but it is noteworthy that scholars often overlook apoptosis caused by exogenous factors (such as death receptor activation), lysosomal penetration, and endoplasmic reticulum stress pathway in HIRI. In addition, calcium overload is also one of the pivotal factors causing ischemia-reperfusion injury, which has been widely reported in cardiac ischemia-reperfusion or cerebral ischemia-reperfusion studies, but it has rarely been reported in HIRI studies in the past ten years. It is worth discussing whether it is a neglected key research direction that calcium overload can aggravate mitochondrial damage. Meanwhile, there is evidence that the deficiency of metabolic substrates, especially glucose, is an important factor affecting energy supply and aggravating injury. Yu et al.¹⁴⁷ pointed out that methyltransferase 3 (METTL3)-mediated RNA N6-methyladenosine (m⁶A) modification can promote the expression of phosphoenolpyruvate carboxykinase (PCK1) to eventually facilitate gluconeogenesis and supplement glycogen, effectively relieving HIRI caused by surgery. This suggests that maintaining glucose homeostasis may be an emerging direction for the prevention and treatment of HIRI.¹⁴⁷ It is believed that these are also crucially conquered research directions that can further explore the pathogenesis of HIRI.

As mentioned above, complex signaling molecular networks and pathways are involved in mediating hepatocyte damage. However, complex regulatory mechanisms also affect the communication between hepatocytes and other hepatic resident cells, resulting in cellular crosstalk events during the occurrence of HIRI. Interactions between hepatocytes and other organelles occur *via* molecules such as TLR4, NF- κ B, Nrf2 or interleukin to transmit signals. Moreover, LSECs influence vascular homeostasis, cell adhesion, intrahepatic inflammation and hepatocyte death. During HIRI, dabigatran pretreatment inhibited inflammation to improve HIRI by regulating the number of LSECs and reducing the expression of TNF- α , IL-6 and VCAM-1.⁹¹ Inflammatory factors such as IL-1 β , IL-6, and TNF- α , as well as immune cells such as neutrophils and macrophages, are pivotal indicators for evaluating the level of inflammation in HIRI. Therefore, whether LSECs are also involved in the intracellular communication with immune cells such as macrophages and neutrophils may be further verified. In

Table 1. List of experimental animal studies and pharmacological strategies to protect livers against HIRI

Number	Animal strain	Animal gender/age/weight	Gene knock-out animal	Ischemic time	Reperfusion time	Drug pretreatment or natural product-based agent (drug) treatment (+)	Target	Reference
1	C57BL/6J mice	Male	-	30 min	40 min	-	CAST, ATG5	29
2	C57BL/6J mice	Male/8–10 weeks	-	60 min	45 min	-	Neutrophils	116
3	C57BL/6J mice	-	MCJ knockout (KO)	30/90 min	1 h	-	MCJ, TNF, IL-6/EGF	15
4	C57BL/6 mice	Male/6–8 weeks	-	60 min	1 h	Platinum nano-antioxidant pretreatment	Reactive oxygen species, nitric oxide, macrophage and neutrophil	39
5	SD rats	Male/8–10 weeks/250–300 g	-	30 min	1 h	-	HOPE, HECTD3/ TRAF3	105
6	Wistar rats	Male/250–300g	-	45 min	1 h	COS pretreatment	Bcl-2/Bax, TNF- α and TGF- β	56
7	C57BL/6J mice	Male/8–10 weeks/25 g	Trim27 knockout (Trim27-KO) mice and hepatocyte-specific Trim27 transgene (Trim27-HTG) mice	60 min	1, 3, 6, and 12 h	-	TAK1, JNK/p38	64
8	C57BL/6 mice	Male/8–10 weeks	RNF5 knockdown	60 min	1, 3, 6, and 24 h	-	ASK1, JNK/p38	66
9	C57BL/6J mice	Male/8–12 weeks/25–28g	-	60 min	1, 6 h	Maresin 1 pretreatment	ALXR/Akt	69
10	C57BL/6J mice	8–12 weeks	Mettl3flox/flox, Mettl3 cKO	60 min	1, 6 h	PCK1 inhibitor pretreatment	METTL3/m6A-PCK1	147
11	C57BL/6 mice	Male	Nrf2 KO	60 min	1, 6 h	-	USP16/KEAP1/Nrf2	49
12	C57BL/6 mice	Male/6–8 weeks	ARRB2 knockout	90 min	1, 6, and 12 h	-	PI3K/Akt	68
13	C57BL/6J mice	Male/8 weeks/20–26 g	-	60 min	1, 6, 12, and 24 h	-	miR-142a-3p/HMGB1	96
14	SD rats	-	-	-	1, 24 h	Salidroside pretreatment (+)	TLR-4/NF- κ B/NLRP3	107
15	Wistar rats	Male/72 weeks	-	60 min	2 h	-	VEGF, LSEC	36
16	Wistar rats	Male/200–250g	-	60 min	2 h	Obeticholic acid pretreatment	MMPs	150

Table 1. Continued

Number	Animal strain	Animal gender/age/weight	Gene knock-out animal	Ischemic time	Reperfusion time	Drug pretreatment or natural product-based agent (drug) treatment (+)	Target	Reference
17	Wistar rats	Male/220–250 g	-	30 min	2 h	-	PPAR γ /TLR4/NF- κ B	104
18	C57BL/6J mice	6 weeks	HRG knockdown	60 min	2, 4, or 6 h	-	FXR, HIF-2 α , neutrophil, NET	119
19	C57BL/6J mice	Male/12 weeks	Caspase 11-/- and Caspase1-/-/11-/-	40 min	2, 4, 6, and 24 h	-	GSDMD, caspase 1, IL-1 β	80
20	C57BL/6J mice	Male/6–8 weeks	GLP-1R-/- mice	60 min	2, 6, or 24 h	Liraglutide pretreatment	GLP-1R, Kupffer cells	136
21	BALB/c mice	Male/20–25 g	-	60 min	3 h	CoQ10 pretreatment	Virus-mimicking liposomal system based on dendritic lipopeptides	19
22	C57BL/6J mice	Male/6–8 weeks/18–22 g	Gfer+/+ mice and heterozygous ALR-knockout (ALR-KO) Gfer+/- mice	60 min	3 h	-	ALR, autophagy	30
23	C57BL/6J mice	Male/6–8 weeks	-	60 min	3 h	Deferoxamine pretreatment	Pigs2	81
24	C57BL/6J mice	Male/6 weeks	Heterozygous knockout of the ALR gene (ALR+/-)	90 min	3 h	-	Drp1 SUMOylation, ALR	26
25	-	Male	RGS14 knockout (RGS14-KO) and hepatocyte-specific RGS14 transgenic (RGS14-TG) mice	60 min	3, 6 h	-	TAK1, RGS14, JNK and p38	65
26	C57BL/6 mice	Male/6–8 weeks	-	60 min	3, 6, and 12 h	-	Drp1, FTO	25
27	C57BL/6J mice	6 weeks	-	60 min	3, 6, 9, 12, and 24 h	Recombinant mouse Gas6 protein pretreatment	AXL, SOCS-1	103
28	SD rats	Male/12–14 weeks	-	45 min	3, 36, or 96 h	-	Ki-67, IL-1 β and IL-6	110

Table 1. Continued

Number	Animal strain	Animal gender/age/weight	Gene knock-out animal	Ischemic time	Reperfusion time	Drug pretreatment or natural product-based agent (drug) treatment (+)	Target	Reference
29	Brown Norway rats	Male/250–320 g	-	30 min (And cold ischemia for 4 h)	4 h	-	HOPE, FMN, NADH	23
30	C57BL/6 mice	Male/8–9 weeks/21–25 g	-	60 min	4 h	Dabigatran pretreatment	HMGB-1, TM	91
31	SD rats	Male/160–200 g	-	60 min	4 h	Chlorogenic acid pretreatment	HMGB1/TLR-4/NF- κ B	55
32	Wistar rats	Male/9 weeks/250–300 g	-	15 min	6 h	Metformin pretreatment	ROS, TNF- α	32
33	Wistar rats	-	-	30 min	6 h	Clostridium butyricum pretreatment	TNF- α , IL-6, TLR4, NF- κ B	108
34	Wistar rats	Male/250–300 g	-	30 min	6 h	HIF-1 α agonist pretreatment	HIF-1 α , A2BAR	52
35	C57BL/6 mice	8–10 weeks/18–22 g	-	60 min	6 h	Kaempferol (KAE) pretreatment (+)	Nrf2/HO-1	48
36	C57BL/6 mice	Male/8–12 weeks	-	60 min	6 h	Exercise Training (12m/min-1hour)/4 weeks	Macrophage, neutrophil	121
37	C57BL/6 mice	Male	Myeloid-specific SIRT1-deficient (mSirt1-KO)	60 min	6 h	-	SIRT1	77
38	C57BL/6 mice	-	-	60 min	6 h	Fisetin pretreatment	Nrf2/HO-1	42
39	C57BL/6 mice	Male/22–26 g	HO-1 heterozygous mutant (HO-1 ^{+/-}) mice	60 min	6 h	Gastrodin(GSTD) pretreatment (+)	TLR4, p38MAPK/ Nrf2/HO-1	47
40	C57BL/6 mice	Male/18–22 g	-	60 min	6 h	Pinocembrin (PIN) pre-administration (+)	HMGB1/TLR4	86
41	C57BL/6 mice	Male/6–8 weeks/18–22 g	-	60 min	6 h	Gavaged before the experiment	Nrf2, HO-1 and NQO-1	44
42	C57BL/6 mice	Male/8 weeks	-	60 min	6 h	Resveratrol pretreatment (+)	ERK, neutrophils	120
43	C57BL/6 mice	Male/8 weeks/20–30 g	-	60 min	6 h	Sodium nitrate pretreatment	Nrf2	151

Table 1. Continued

Number	Animal strain	Animal gender/age/weight	Gene knock-out animal	Ischemic time	Reperfusion time	Drug pretreatment or natural product-based agent (drug) treatment (+)	Target	Reference
44	C57BL/6 mice	Male/8–10 weeks/20–25 g	-	60 min	6 h	The lectin-like domain of thrombomodulin pretreatment	HMGB-1, TNF- α , IL-6, IL-1 β , and CXCL-2	89
45	C57BL/6 mice	Male/8–10 weeks	-	60 min	6 h	γ -Oryzanol (ORY) pretreatment (+)	HMGB1, NLRP3, caspase-1 (p20), and IL-1 β	85
46	C57BL/6 mice	Male/8–10 weeks/20–25 g	-	60 min	6 h	Inulin pretreatment	HMGB1, TLR-4	88
47	C57BL/6 mice	Male/8–10 weeks/24–27 g	miR-210 KO mice	60 min	6 h	-	miR-210, SMAD4	60
48	C57BL/6J mice	Male/8–14 weeks	Galnt4-KO	60 min	6 h	-	ASK1-JNK/p38	67
49	C57BL/6J mice	Male/6–8 weeks	-	60 min	6 h	Methyl eugenol (ME) treatment (+)	PI3K/Akt	71
50	C57BL/6J mice	Male/8 weeks	-	60 min	6 h	Pretreated with myricitrin before subjected to I/R	PI3K/Akt, NO	40
51	C57BL/6J mice	Male/6–8 weeks/20 g	-	60 min	6 h	-	STAT1/STAT3, macrophage	17
52	C57BL/6J mice	Male/9–10 weeks/23–27 g	-	60 min	6 h	Acteoside (ACT) pretreatment (+)	HMGB1, TLR3/4, IRF1	97
53	SD rats	Male/8–12 weeks	-	60 min	6 h	-	JNK/p38	61
54	C57BL mice	Male/5–6 weeks/9–21 g	-	60 min	6 h	Panax notoginseng mixture (PNM) pretreatment (+)	NR3C2, SRC and GAPDH	152
55	Lewis rats	Male	-	60 min	6 h	-	VEGF-sdf1, LSECs	153
56	Beagles dogs	Adult male/20–28 weeks/8–12 kg	Bmal1 knockdown group (KO-Bmal1)	60 min	6 h	-	Bmal1/TNF- α /IL-1 β	145
57	C57BL/6 mice	Male/8–10 weeks	gp78 HKO	90 min	6 h	Rosiglitazone and Ferrostatin-1 pretreatment	gp78/ACSL4	82
58	C57BL/6 mice	6–8 weeks	Myeloid-specific SMO knockout (SMOM-KO) mice	90 min	6 h	Mesenchymal stem cells pretreatment	Hedgehog/SMO/Gli1 pathway	133
59	C57BL/6 mice	Male/8–10 weeks/23–27 g	DJ-1 KO (Dj-1 $^{-/-}$) mice	90 min	6 h	-	DJ-1, mitophagy, PARKIN	31

Table 1. Continued

Number	Animal strain	Animal gender/age/weight	Gene knock-out animal	Ischemic time	Reperfusion time	Drug pretreatment or natural product-based agent (drug) treatment (+)	Target	Reference
60	C57BL/6 mice	Male/8–10 weeks		90 min	6 h	-	caspase 1, GSDMD, STING	129
61	C57BL/6 mice	Male/8–12 weeks	-	90 min	6 h	Pituitary adenylate cyclase-activating polypeptide pretreatment	PACAP, mitophagy	111
62	C57BL/6 mice	Male/ 8 weeks and 100 weeks	-	90 min	6 h	-	STING-NLRP3 axis	128
63	C57BL/6 mice	Male/6–8 weeks	Nogo-B knockout (Nogo-BKO), FloxP-Nogo-B (Nogo-BFL/FL)	90 min	6 h	-	Hippo/YAP pathway	93
64	C57BL/6J mice	Male/6–8 weeks	Liver-specific FXR knockdown	90 min	6 h	-	FXR, MAPK, NF- κ B, monocytes	109
65	C57BL/6J mice	Male/6–8 weeks	Liver-specific YAP knockdown (YAP-LKD) mice	90 min	6 h	-	YAP/JNK	62
66	C57BL/6J mice	8 weeks	TGR5 knockout (TGR5-/-)	90 min	6 h	-	TGR5, macrophage	138
67	-	6–8 weeks	Floxed Notch1 (Notch1FL/FL) mice, myeloid-specific Notch1 knockout (Notch1M-KO) mice	90 min	6 h	-	Jagged1/Notch1/HSF1/Snail	135
68	C57BL/6 mice	Male/8 weeks	EC-specific Notch activation (NICeCA)	60 min	6, 12 h	-	LSECs, Notch	154
69	C57BL/6 mice	-	Carabin flox/flox (Carabinfl/fl)	60 min	6, 12, or 24 h	-	Neutrophils	117
70	C57BL/6 mice	Male/8–10 weeks/21–25 g	-	60 min	6, 12, or 24 h	-	IL-33, Kupffer cells	100
71	C57BL/6N mice	Male/8–12 weeks	Mkl1-/- mice	45 min	6, 24 h	-	MLKL, neutrophil	114
72	C57BL/6NJ mice	8–12 weeks	IRG1 knockout (KO); IRG1-/- and Nrf2 KO (Nrf2-/-)	60 min	6, 24 h	-	IRG1/itaconate	43

Table 1. Continued

Number	Animal strain	Animal gender/age/weight	Gene knock-out animal	Ischemic time	Reperfusion time	Drug pretreatment or natural product-based agent (drug) treatment (+)	Target	Reference
73	BALB/c mice	Male/6–8 weeks/21–25 g	-	45 min	6, 24 h	Cordycepin pretreatment (+)	MAPK/NF-κB	72
74	C57BL/6 mice	8–10 weeks/20–30 g	Transgenic ARE-luc mice	90 min	6, 24, and 72 h	Sulforaphane treatment (+)	Nrf2, ARE	125
75	C57BL/6 mice	Male/8 weeks	-	45 min	6, 24, 48, 72, 96, and 120 h	-	VEGF-C, VEGF-D, VEGFR3 and reparative macrophages	130
76	C57BL/6J mice	Male/12 weeks	-	45 min	6 h, 24 h, 15 d, and 8 w	Ghrelin pretreatment	Smad and ERK	59
77	C57BL/6 mice	Male/8 weeks	Hepatocyte- and myeloid-specific Pak4 knockout	45 min	12 h	p21-activated kinase 4 inhibition pretreatment	Nrf2	46
78	BALB/c mice	-	-	60 min	12 h	Intravenously injected before surgery	ROS, C5a	33
79	C57BL/6 mice	6–8 weeks	IRF8 knockout	60 min	12 h	-	IRF8, NF-κB, neutrophil	112
80	C57BL/6 mice	Male/8–12 weeks/20–26 g	-	60 min	12 h	-	NFATc1/HDAC1/IRF-1/p38 MAPK signaling axis	75
81	C57BL/6 mice	-	-	60 min	12, 60 h	Carbohydrate-derived nanoparticle pretreatment	ROS	123
82	Wistar rats	Male/180–240 g	-	30 min	24 h	Berberine (BBR) pretreatment (+)	HMGB1/TLR4/ NF-κB	95
83	SD rats	Male/200–250 g	-	30 min	24 h	Morin (MRN) pretreatment (+)	TLR4/ NF-κB, Nrf2/ HO-1	106
84	C57BL/6 mice	Male/8 weeks/22–25 g	-	45 min	24 h	Annexin A1 N-terminal peptide Acetyl 2-26 pretreatment	HMGB1/TLR4/ NF-κB axis and neutrophil	94
85	Wistar rats	Male/8–12 weeks	-	60 min	24 h	Sitagliptin pretreatment	SDF-1α/CXCR4	113
86	C57BL/6 mice	male/8–9 weeks/20–25g	-	60 min	24 h	-	Mip-2, AKT, NO	115

Table 1. Continued

Number	Animal strain	Animal gender/age/weight	Gene knock-out animal	Ischemic time	Reperfusion time	Drug pretreatment or natural product-based agent (drug) treatment (+)	Target	Reference
87	Wistar rats	8–12 weeks	-	60 min	24 h	Sitagliptin pretreatment	Nox4	45
88	SD rats	9–10 weeks/190–210 g	-	60 min	24 h	Dexametomidine pretreatment	miR-494/JUND/ PI3K/AKT/Nrf2 axis	79
89	SD rats	8 weeks/400 g	-	-	24 h	-	CSF3 Kupffer cells	132
90	C57BL/6 mice	Male/8–10 weeks/18–21 g	-	90 min	24 h	-	miR-124-3p/ TRAF3/CREB axis	57
91	C57BL/6 mice	6–8 weeks	Myeloid-specific Nrf2-knockout (Nrf2M-KO)	90 min	-	Recombinant Timp3 pretreatment	Nrf2, Timp3, RhoA/ROCK pathway	124

(+) indicates that the article uses natural product-based agent (drug) treatment.

general, under normal circumstances, immune cells can flow through the blood and accumulate in the liver to maintain normal physiological activities. However, the hepatic microcirculation dysfunction causes the abnormal accumulation of immune cells, which is one of the pivotal factors regulating the development of HIRI. Actually, neutrophils in the blood are on standby in general, and once stimulated by I/R, macrophages are responsible for releasing signals that tell neutrophils to start working. They are then recruited together at the site of inflammation to exert a proinflammatory effect. Without the help of macrophages to recruit neutrophils to the lesion location attack site, neutrophils simply wander aimlessly in the blood. Remarkably, although KCs and macrophages derived from monocytes are both macrophages that can polarize into M1 or M2 states and further influence liver microenvironment and even HIRI development, here, we found that their mechanisms of action or sites of action in HIRI are different. However, researchers seem to easily overlook this detail, which emphasizes both types of macrophages, and even debate whether macrophages or KCs are the standard model for *in vitro* studies of HIRI. This suggests that the establishment of standard *in vivo* or *in vitro* models that mimic the internal environment for studying HIRI may be important.

As mentioned above, the inconsistency of experimental models could cause serious controversy and lead to paradoxes in the research results. In Table 1, we counted the conditions of HIRI modeling animals for 91 articles, including animal strain, gender, age, weight, gene knock-out animal (yes or no), ischemic time, reperfusion time, drug pretreatment (yes or no), natural product-based agent (drug) (yes or no) and target. And concretely, we found that in these animal models, ischemia time was 15, 30, 40, 45, 60, and 90 minutes, respectively accounting for 1.1%, 10%, 1.1%, 7.8%, 59.5%, and 16.8%; reperfusion operation time of 6 h accounted for 51%, while other reperfusion operation time made up the remaining 49%; in the experiments, more than 90% of the articles explicitly labeled male mice or rats; older animals accounted for 1%, young mice (8 weeks) accounted for 62.5%; the use of natural product-based agent (drug) as a means of HIRI treatment accounted for 14.8%. Interestingly, even the same target may have different results in animals of different ages. As expected, we found that NO plays a negative regulatory role in elderly mice treated with ischemia for 3 hours³⁶ and a protective

role in young mice treated with ischemia for 60 minutes.³⁷ This implies that the disunity of the model may be one of the important hindrances to the exploration of HIRI mechanism and efficacy verification of medicine. In addition, consistent with our statistical results, Dong et al.¹⁴⁸ also showed that males were more prone to HIRI than females by the up-regulated expression of male-specific gene SRY. This seems to indicate a tendency that researchers should also pay more attention to whether different animal modeling methods can be applied to explore the pathogenesis of HIRI in different clinical diseases and whether they can better propose the direction of potential drug development.

In addition, an increasing number of researchers have begun to seek new therapeutic methods or drugs to interfere with the HIRI process, such as intermittent clamping or ischemic preconditioning to interfere with the degree of injury or preconditioning with exercise training to alleviate HIRI. However, these methods may have certain limitations, such as a high price, high risk coefficient and nonsignificant therapeutic effect. In particular, since we also observed that many studies used animal models of pretreatment, we should consider the feasibility of pre-administration in the clinic and whether the models used have value in practical applications. Notably, this pretreatment operation is particularly suitable for the application of natural product-based agents (drugs) because it aligns with the preventive nature of natural product-based agents (drugs), which aim to prevent the occurrence and progression of diseases in advance. In the future, more studies should focus on the intervention effects and potential mechanisms of Chinese medicine in the prevention and improvement of HIRI after surgery. Besides, we also pay attention to two new research targets, namely HOPE and alternative splicing (AS). Firstly, HOPE, as an emerging mechanical perfusion preservation technology, has the advantages of high safety and flexibility, while having received limited coverage in the HIRI space. In HIRI, oxygen entering the ischemic liver accelerates ROS formation, but HOPE not only inhibits the rate of ROS synthesis but also gives time for mitochondrial function (oxygen reserve function) to recover, ultimately easing oxidative stress levels.¹⁴⁹ On the other hand, AS refers to the selection of different splicing site combinations to produce different mRNA splicing isomers, resulting in the final protein product showing different functions. Other content such as ¹⁵⁰⁻¹⁵⁴ references refer to the

table. Recently, Kenneth's study showed that ischemia induced AS of CEACAM1, which was divided into CEACam1-long and CEACam1-short and further interacted with HIF-1 α to improve HIRI.^{53,155} However, in the field of HIRI, the modification of CEACAM1 by AS technology to reduce the injury degree of HIRI and the related research after liver transplantation are still few, which is one of the potential research directions. Thus, larger, randomized, double-blind and multicenter clinical studies are needed to further demonstrate the above targets involved in the above strategies and the role of TCM in hepatic IRI.

CONCLUSION

In summary, our objective is to identify overlooked targets, mechanisms and research directions in drug development by analyzing previous studies. Nevertheless, only English articles are included in this article; thus, there may be some omissions of articles in other languages. Collectively, effective prevention or suppression of HIRI has profound significance to enhance the success rate of surgery, improve the quality of life for the patient with severe liver disease, and also reduce the burden on the medical system. Therefore, we strongly encourage researchers to investigate the effectiveness of natural product-based agent (drug) in the occurrence and progression of HIRI, which will serve as a valuable reference for clinical and scientific research on the prevention or intervention of HIRI.

Authors' contributions

Xiaojiaoyang Li: conceptualization, investigation, supervision, writing-review & editing. Jia Liu: investigation, validation, writing-original draft, writing-review & editing. Ranyi Luo: methodology, writing-original draft. Yin hao Zhang: methodology.

Acknowledgements

This work was supported by: National Natural Science Foundation of China (Grant NO. 82274186 to XL); National Key Research and Development Program on Modernization of Traditional Chinese Medicine (Grant NO. 2022YFC3502100 to XL); Fundamental Research Funds for the Central Universities (Grant NO. 2023-JYB-JBZD-046 to XL); National High-Level Talents Special Support Program to XL.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

REFERENCES

- Ye L, He S, Mao X, Zhang Y, Cai Y, Li S. Effect of hepatic macrophage polarization and apoptosis on liver ischemia and reperfusion injury during liver transplantation. *Front Immunol* 2020;11:1193.
- Devarbhavi H, Asrani SK, Arab JP, Nartey YA, Pose E, Kamath PS. Global burden of liver disease: 2023 update. *J Hepatol* 2023;79:516-537.
- George J, Lu Y, Tsuchishima M, Tsutsumi M. Cellular and molecular mechanisms of hepatic ischemia-reperfusion injury: The role of oxidative stress and therapeutic approaches. *Redox Biol* 2024;75:103258.
- Yuan D, Collage RD, Huang H, Zhang X, Kautza BC, Lewis AJ, et al. Blue light reduces organ injury from ischemia and reperfusion. *Proc Natl Acad Sci U S A* 2016;113:5239-5244.
- Dar WA, Sullivan E, Bynon JS, Eltzschig H, Ju C. Ischaemia reperfusion injury in liver transplantation: Cellular and molecular mechanisms. *Liver Int* 2019;39:788-801.
- Bavarsad K, Riahi MM, Saadat S, Barreto G, Atkin SL, Sahabkar A. Protective effects of curcumin against ischemia-reperfusion injury in the liver. *Pharmacol Res* 2019;141:53-62.
- Pantazi E, Bejaoui M, Folch-Puy E, Adam R, Roselló-Catafau J. Advances in treatment strategies for ischemia reperfusion injury. *Expert Opin Pharmacother* 2016;17:169-179.
- Jaeschke H, Lemasters JJ. Apoptosis versus oncotic necrosis in hepatic ischemia/reperfusion injury. *Gastroenterology* 2003;125:1246-1257.
- Peralta C, Jiménez-Castro MB, Gracia-Sancho J. Hepatic ischemia and reperfusion injury: effects on the liver sinusoidal milieu. *J Hepatol* 2013;59:1094-1106.
- Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X, et al. Ferroptosis: process and function. *Cell Death Differ* 2016;23:369-379.
- Tang SP, Mao XL, Chen YH, Yan LL, Ye LP, Li SW. Reactive oxygen species induce fatty liver and ischemia-reperfusion injury by promoting inflammation and cell death. *Front Immunol* 2022;13:870239.
- Liu H, Man K. New insights in mechanisms and therapeutics for short- and long-term impacts of hepatic ischemia reperfusion injury post liver transplantation. *Int J Mol Sci* 2021;22:8210.
- Vartak R, Porras CA, Bai Y. Respiratory supercomplexes: structure, function and assembly. *Protein Cell* 2013;4:582-590.
- Schusdziarra C, Blamowska M, Azem A, Hell K. Methylation-controlled J-protein MCJ acts in the import of proteins into human mitochondria. *Hum Mol Genet* 2013;22:1348-1357.
- Goikoetxea-Usandizaga N, Serrano-Maciá M, Delgado TC, Simón J, Fernández Ramos D, Barriales D, et al. Mitochondrial bioenergetics boost macrophage activation, promoting liver regeneration in metabolically compromised animals. *Hepatology* 2022;75:550-566.
- Teixeira da Silva R, Machado IF, Teodoro JS, Panisello-Roselló A, Roselló-Catafau J, Rolo AP, et al. PEG35 as a preconditioning agent against hypoxia/reoxygenation injury. *Int J Mol Sci* 2022;23:1156.
- Shang L, Ren H, Wang S, Liu H, Hu A, Gou P, et al. SS-31 Protects liver from ischemia-reperfusion injury via modulating macrophage polarization. *Oxid Med Cell Longev* 2021;2021:6662156.
- Zeng H, Saari JT, Johnson WT. Copper deficiency decreases complex IV but not complex I, II, III, or V in the mitochondrial respiratory chain in rat heart. *J Nutr* 2007;137:14-18.
- Zhuang R, Chen M, Zhou Y, Cheng W, Zhang T, Ni Y, et al. Virus-mimicking liposomal system based on dendritic lipopeptides for efficient prevention ischemia/reperfusion injury in the mouse liver. *ACS Macro Lett* 2021;10:215-222.
- Frye RA. Characterization of five human cDNAs with homology to the yeast SIR2 gene: Sir2-like proteins (sirtuins) metabolize NAD and may have protein ADP-ribosyltransferase activity. *Biochem Biophys Res Commun* 1999;260:273-279.
- He S, Gao Q, Wu X, Shi J, Zhang Y, Yang J, et al. NAD⁺ ameliorates endotoxin-induced acute kidney injury in a sirtuin1-dependent manner via GSK-3 β /Nrf2 signalling pathway. *J Cell Mol Med* 2022;26:1979-1993.
- Teodoro JS, Varela AT, Duarte FV, Gomes AP, Palmeira CM, Rolo AP. Indirubin and NAD⁺ prevent mitochondrial ischaemia/reperfusion damage in fatty livers. *Eur J Clin Invest* 2018;48:e12932.
- Schlegel A, Muller X, Mueller M, Stepanova A, Kron P, de Rougemont O, et al. Hypothermic oxygenated perfusion protects from mitochondrial injury before liver transplantation. *EBioMedicine* 2020;60:103014.
- Lin J, Duan J, Wang Q, Xu S, Zhou S, Yao K. Mitochondrial dynamics and mitophagy in cardiometabolic disease. *Front*

- Cardiovasc Med 2022;9:917135.
25. Du YD, Guo WY, Han CH, Wang Y, Chen XS, Li DW, et al. N6-methyladenosine demethylase FTO impairs hepatic ischemia-reperfusion injury via inhibiting Drp1-mediated mitochondrial fragmentation. *Cell Death Dis* 2021;12:442.
 26. Huang J, Xie P, Dong Y, An W. Inhibition of Drp1 SUMOylation by ALR protects the liver from ischemia-reperfusion injury. *Cell Death Differ* 2021;28:1174-1192.
 27. Sun X, Yang Y, Zhong XZ, Cao Q, Zhu XH, Zhu X, et al. A negative feedback regulation of MTORC1 activity by the lysosomal Ca²⁺ channel MCOLN1 (mucolipin 1) using a CALM (calmodulin)-dependent mechanism. *Autophagy* 2018;14:38-52.
 28. Hanna RA, Campbell RL, Davies PL. Calcium-bound structure of calpain and its mechanism of inhibition by calpastatin. *Nature* 2008;456:409-412.
 29. Flores-Toro J, Chun SK, Shin JK, Campbell J, Lichtenberger M, Chapman W, et al. Critical roles of calpastatin in ischemia/reperfusion injury in aged livers. *Cells* 2021;10:1863.
 30. Kong WN, Li W, Bai C, Dong Y, Wu Y, An W. Augmenter of liver regeneration-mediated mitophagy protects against hepatic ischemia/reperfusion injury. *Am J Transplant* 2022;22:130-143.
 31. Xu M, Hang H, Huang M, Li J, Xu D, Jiao J, et al. DJ-1 deficiency in hepatocytes improves liver ischemia-reperfusion injury by enhancing mitophagy. *Cell Mol Gastroenterol Hepatol* 2021;12:567-584.
 32. An W, Kang JS. Effect of metformin on myocardial injury induced by hepatic ischemia-reperfusion in rats. *Front Pharmacol* 2022;13:822743.
 33. Zhang X, Hu J, Becker KV, Engle JW, Ni D, Cai W, et al. Antioxidant and C5a-blocking strategy for hepatic ischemia-reperfusion injury repair. *J Nanobiotechnology* 2021;19:107.
 34. Zhang YP, Liu XR, Yang MW, Yang SL, Hong FF. New progress in understanding roles of nitric oxide during hepatic ischemia-reperfusion injury. *World J Hepatol* 2022;14:504-515.
 35. Ferreira-Silva M, Faria-Silva C, Carneiro MC, Simões S, Marinho HS, Marcelino P, et al. Quercetin liposomal nanoformulation for ischemia and reperfusion injury treatment. *Pharmaceutics* 2022;14:104.
 36. Hide D, Warren A, Fernández-Iglesias A, Maeso-Díaz R, Peralta C, Le Couteur DG, et al. Ischemia/reperfusion injury in the aged liver: The importance of the sinusoidal endothelium in developing therapeutic strategies for the elderly. *J Gerontol A Biol Sci Med Sci* 2020;75:268-277.
 37. Wu J, Xue X, Fan G, Gu Y, Zhou F, Zheng Q, et al. Ferulic acid ameliorates hepatic inflammation and fibrotic liver injury by inhibiting PTP1B activity and subsequent promoting AMPK phosphorylation. *Front Pharmacol* 2021;12:754976.
 38. Anavi S, Tirosh O. iNOS as a metabolic enzyme under stress conditions. *Free Radic Biol Med* 2020;146:16-35.
 39. Mu J, Li C, Shi Y, Liu G, Zou J, Zhang DY, et al. Protective effect of platinum nano-antioxidant and nitric oxide against hepatic ischemia-reperfusion injury. *Nat Commun* 2022;13:2513.
 40. Shen Y, Shen X, Cheng Y, Liu Y. Myricitrin pretreatment ameliorates mouse liver ischemia reperfusion injury. *Int Immunopharmacol* 2020;89:107005.
 41. Ma Q. Role of nrf2 in oxidative stress and toxicity. *Annu Rev Pharmacol Toxicol* 2013;53:401-426.
 42. Li Z, Wang Y, Zhang Y, Wang X, Gao B, Li Y, et al. Protective effects of fisetin on hepatic ischemia-reperfusion injury through alleviation of apoptosis and oxidative stress. *Arch Med Res* 2021;52:163-173.
 43. Yi Z, Deng M, Scott MJ, Fu G, Loughran PA, Lei Z, et al. Immune-responsive gene 1/itaconate activates nuclear factor erythroid 2-related factor 2 in hepatocytes to protect against liver ischemia-reperfusion injury. *Hepatology* 2020;72:1394-1411.
 44. Yu Q, Chen S, Tang H, Zhang X, Tao R, Yan Z, et al. Verrucic acid alleviates liver ischemia/reperfusion injury by activating the Nrf2 signaling pathway. *Int Immunopharmacol* 2021;101:108294.
 45. Trocha M, Fleszar MG, Fortuna P, Lewandowski Ł, Gostomska-Pampuch K, Sozański T, et al. Sitagliptin modulates oxidative, nitrative and halogenative stress and inflammatory response in rat model of hepatic ischemia-reperfusion. *Antioxidants (Basel)* 2021;10:1168.
 46. Mao Y, Han CY, Hao L, Lee Y, Son JB, Choi H, et al. p21-activated kinase 4 inhibition protects against liver ischemia/reperfusion injury: Role of nuclear factor erythroid 2-related factor 2 phosphorylation. *Hepatology* 2022;76:345-356.
 47. Yuan B, Huang H, Qu S, Zhang H, Lin J, Jin L, et al. Gastrodin pretreatment protects liver against ischemia-reperfusion injury via activation of the Nrf2/HO-1 pathway. *Am J Chin Med* 2020;48:1159-1178.
 48. Chen Y, Li T, Tan P, Shi H, Cheng Y, Cai T, et al. Kaempferol from *Penthorum chinense* pursh attenuates hepatic ischemia/reperfusion injury by suppressing oxidative stress and inflammation through activation of the Nrf2/HO-1 signaling pathway.

- Front Pharmacol 2022;13:857015.
49. Tong G, Chen Y, Chen X, Fan J, Zhu K, Hu Z, et al. FGF18 alleviates hepatic ischemia-reperfusion injury via the USP16-mediated KEAP1/Nrf2 signaling pathway in male mice. *Nat Commun* 2023;14:6107.
50. Feng R, Morine Y, Ikemoto T, Imura S, Iwahashi S, Saito Y, et al. Nrf2 activation drive macrophages polarization and cancer cell epithelial-mesenchymal transition during interaction. *Cell Commun Signal* 2018;16:54.
51. Kim TH, Hur EG, Kang SJ, Kim JA, Thapa D, Lee YM, et al. NRF2 blockade suppresses colon tumor angiogenesis by inhibiting hypoxia-induced activation of HIF-1 α . *Cancer Res* 2011;71:2260-2275.
52. Zhang X, Du P, Luo K, Li Y, Liu Z, Wang W, et al. Hypoxia-inducible factor-1 α protects the liver against ischemia-reperfusion injury by regulating the A2B adenosine receptor. *Bioengineered* 2021;12:3737-3752.
53. Dery KJ, Kojima H, Kageyama S, Kadono K, Hirao H, Cheng B, et al. Alternative splicing of CEACAM1 by hypoxia-inducible factor-1 α enhances tolerance to hepatic ischemia in mice and humans. *Sci Transl Med* 2023;15:eadf2059.
54. Wang Q, Zhou H, Bu Q, Wei S, Li L, Zhou J, et al. Role of XBP1 in regulating the progression of non-alcoholic steatohepatitis. *J Hepatol* 2022;77:312-325.
55. Li K, Feng Z, Wang L, Ma X, Wang L, Liu K, et al. Chlorogenic acid alleviates hepatic ischemia-reperfusion injury by inhibiting oxidative stress, inflammation, and mitochondria-mediated apoptosis in vivo and in vitro. *Inflammation* 2023;46:1061-1076.
56. Saleh H, El-Shorbagy HM. Chitosan protects liver against ischemia-reperfusion injury via regulating Bcl-2/Bax, TNF- α and TGF- β expression. *Int J Biol Macromol* 2020;164:1565-1574.
57. Wang YL, Zhang Y, Cai DS. Hepatoprotective effects of sevoflurane against hepatic ischemia-reperfusion injury by regulating microRNA-124-3p-mediated TRAF3/CREB axis. *Cell Death Discov* 2022;8:105.
58. Hu HH, Chen DQ, Wang YN, Feng YL, Cao G, Vaziri ND, et al. New insights into TGF- β /Smad signaling in tissue fibrosis. *Chem Biol Interact* 2018;292:76-83.
59. Yang Y, Liu R, Qu Y, Zhao J, Tong L, Ye S, et al. Ghrelin ameliorates transformation of hepatic ischemia-reperfusion injury to liver fibrosis by blocking Smad and ERK signalling pathways, and promoting anti-inflammation and anti-oxidation effects. *Transpl Immunol* 2022;73:101597.
60. Pan WM, Wang H, Zhang XF, Xu P, Wang GL, Li YJ, et al. miR-210 Participates in hepatic ischemia reperfusion injury by forming a negative feedback loop with SMAD4. *Hepatology* 2020;72:2134-2148.
61. Zheng X, Wang G, Yuan J, Li N, Yan B, Yan J, et al. hsa-miR-34a-5p Ameliorates hepatic ischemia/reperfusion injury via targeting HNF4 α . *Turk J Gastroenterol* 2022;33:596-605.
62. Zhu S, Wang X, Chen H, Zhu W, Li X, Cui R, et al. Hippo (YAP)-autophagy axis protects against hepatic ischemia-reperfusion injury through JNK signaling. *Chin Med J (Engl)* 2024;137:657-668.
63. Guo WZ, Fang HB, Cao SL, Chen SY, Li J, Shi JH, et al. Six-transmembrane epithelial antigen of the prostate 3 deficiency in hepatocytes protects the liver against ischemia-reperfusion injury by suppressing transforming growth factor- β -activated kinase 1. *Hepatology* 2020;71:1037-1054.
64. Chen SY, Zhang HP, Li J, Shi JH, Tang HW, Zhang Y, et al. Tripartite motif-containing 27 attenuates liver ischemia/reperfusion injury by suppressing transforming growth factor β -activated kinase 1 (TAK1) by TAK1 binding protein 2/3 degradation. *Hepatology* 2021;73:738-758.
65. Zhang JK, Ding MJ, Liu H, Shi JH, Wang ZH, Wen PH, et al. Regulator of G-protein signaling 14 protects the liver from ischemia-reperfusion injury by suppressing TGF- β -activated kinase 1 activation. *Hepatology* 2022;75:338-352.
66. Ding MJ, Fang HR, Zhang JK, Shi JH, Yu X, Wen PH, et al. E3 ubiquitin ligase ring finger protein 5 protects against hepatic ischemia reperfusion injury by mediating phosphoglycerate mutase family member 5 ubiquitination. *Hepatology* 2022;76:94-111.
67. Zhou J, Guo L, Ma T, Qiu T, Wang S, Tian S, et al. N-acetylgalactosaminyltransferase-4 protects against hepatic ischemia/reperfusion injury by blocking apoptosis signal-regulating kinase 1 N-terminal dimerization. *Hepatology* 2022;75:1446-1460.
68. Chen X, Zhang J, Xia L, Wang L, Li H, Liu H, et al. β -Arrestin-2 attenuates hepatic ischemia-reperfusion injury by activating PI3K/Akt signaling. *Aging (Albany NY)* 2020;13:2251-2263.
69. Tang D, Fu G, Li W, Sun P, Loughran PA, Deng M, et al. Maresin 1 protects the liver against ischemia/reperfusion injury via the ALXR/Akt signaling pathway. *Mol Med* 2021;27:18.
70. Li Y, Gao M, Xu LN, Yin LH, Qi Y, Peng JY. MicroRNA-142-3p attenuates hepatic ischemia/reperfusion injury via targeting of myristoylated alanine-rich C-kinase substrate. *Pharmacol*

- Res 2020;156:104783.
71. Wang M, Zhang J, Zhang J, Sun K, Li Q, Kuang B, et al. Methyl eugenol attenuates liver ischemia reperfusion injury via activating PI3K/Akt signaling. *Int Immunopharmacol* 2021;99:108023.
 72. Ding J, Yang W, Jiang Y, Ji J, Zhang J, Wu L, et al. Cordycepin Protects against Hepatic Ischemia/Reperfusion Injury via Inhibiting MAPK/NF- κ B Pathway. *Mediators Inflamm* 2022;2022:5676256.
 73. Kim HJ, Joe Y, Yu JK, Chen Y, Jeong SO, Mani N, et al. Carbon monoxide protects against hepatic ischemia/reperfusion injury by modulating the miR-34a/SIRT1 pathway. *Biochim Biophys Acta* 2015;1852:1550-1559.
 74. Samuvel DJ, Krishnasamy Y, Li L, Lemasters JJ, Chou CJ, Zhong Z. LP342, a novel histone deacetylase inhibitor, decreases nitro-oxidative stress, mitochondrial dysfunction and hepatic ischaemia/reperfusion injury in mice. *RPS Pharm Pharmacol Rep* 2023;2:rqa013.
 75. Luo J, Li J, Li T, Zhang Z, Chen G, Li Q, et al. PIAS1 Alleviates hepatic ischemia-reperfusion injury in mice through a mechanism involving NFATc1 SUMOylation. *Dis Markers* 2022;2022:4988539.
 76. Burdette BE, Esparza AN, Zhu H, Wang S. Gasdermin D in pyroptosis. *Acta Pharm Sin B* 2021;11:2768-2782.
 77. Kadono K, Kageyama S, Nakamura K, Hirao H, Ito T, Kojima H, et al. Myeloid Ikaros-SIRT1 signaling axis regulates hepatic inflammation and pyroptosis in ischemia-stressed mouse and human liver. *J Hepatol* 2022;76:896-909.
 78. Szabo G, Petrasek J. Inflammasome activation and function in liver disease. *Nat Rev Gastroenterol Hepatol* 2015;12:387-400.
 79. Wu Y, Qiu G, Zhang H, Zhu L, Cheng G, Wang Y, et al. Dexmedetomidine alleviates hepatic ischaemia-reperfusion injury via the PI3K/AKT/Nrf2-NLRP3 pathway. *J Cell Mol Med* 2021;25:9983-9994.
 80. Kolachala VL, Lopez C, Shen M, Shayakhmetov D, Gupta NA. Ischemia reperfusion injury induces pyroptosis and mediates injury in steatotic liver thorough Caspase 1 activation. *Apoptosis* 2021;26:361-370.
 81. Yamada N, Karasawa T, Wakiya T, Sadatomo A, Ito H, Kamata R, et al. Iron overload as a risk factor for hepatic ischemia-reperfusion injury in liver transplantation: Potential role of ferroptosis. *Am J Transplant* 2020;20:1606-1618.
 82. Li C, Wu Y, Chen K, Chen R, Xu S, Yang B, et al. Gp78 deficiency in hepatocytes alleviates hepatic ischemia-reperfusion injury via suppressing ACSL4-mediated ferroptosis. *Cell Death Dis* 2023;14:810.
 83. Zhang Y, Jia K, Li Y, Ma Z, Fan G, Luo R, et al. Radix Rehmanniae Praeparata aqueous extract improves hepatic ischemia/reperfusion injury by restoring intracellular iron homeostasis. *Chin J Nat Med* 2023;21:1-17.
 84. Roh JS, Sohn DH. Damage-associated molecular patterns in inflammatory diseases. *Immune Netw* 2018;18:e27.
 85. Du Y, Zhong F, Cheng H, Li T, Chen Y, Tan P, et al. The dietary supplement γ -oryzanol attenuates hepatic ischemia reperfusion injury via inhibiting endoplasmic reticulum stress and HMGB1/NLRP3 inflammasome. *Oxid Med Cell Longev* 2021;2021:4628050.
 86. Ma T, Zhang H, Li T, Bai J, Wu Z, Cai T, et al. Protective effect of pinocembrin from *Penthorum chinense* Pursh on hepatic ischemia reperfusion injury via regulating HMGB1/TLR4 signal pathway. *Phytother Res* 2023;37:181-194.
 87. Le Bastard Q, Chapelet G, Javaudin F, Lepelletier D, Batard E, Montassier E. The effects of inulin on gut microbial composition: a systematic review of evidence from human studies. *Eur J Clin Microbiol Infect Dis* 2020;39:403-413.
 88. Kawasoe J, Uchida Y, Kawamoto H, Miyauchi T, Watanabe T, Saga K, et al. Propionic acid, induced in gut by an inulin diet, suppresses inflammation and ameliorates liver ischemia and reperfusion injury in mice. *Front Immunol* 2022;13:862503.
 89. Kawasoe J, Uchida Y, Miyauchi T, Kadono K, Hirao H, Saga K, et al. The lectin-like domain of thrombomodulin is a drug candidate for both prophylaxis and treatment of liver ischemia and reperfusion injury in mice. *Am J Transplant* 2021;21:540-551.
 90. Coughlin SR. Thrombin signalling and protease-activated receptors. *Nature* 2000;407:258-264.
 91. Noguchi D, Kuriyama N, Hibi T, Maeda K, Shinkai T, Gyoten K, et al. The impact of dabigatran treatment on sinusoidal protection against hepatic ischemia/reperfusion injury in mice. *Liver Transpl* 2021;27:363-384.
 92. Sosa RA, Terry AQ, Kaldas FM, Jin YP, Rossetti M, Ito T, et al. Disulfide high-mobility group box 1 drives ischemia-reperfusion injury in human liver transplantation. *Hepatology* 2021;73:1158-1175.
 93. Rao J, Cheng F, Zhou H, Yang W, Qiu J, Yang C, et al. Nogo-B is a key mediator of hepatic ischemia and reperfusion injury. *Redox Biol* 2020;37:101745.
 94. Bai C, Jiang Z, Jiang H, Yu S, Li M, Chu F, et al. Ac2-26 alleviates hepatic ischemia-reperfusion injury based on inhibiting

- the positive feedback loop of HMGB1/TLR4/NF- κ B/neutrophils. *Exp Ther Med* 2022;24:673.
95. Gendy AM, Elnagar MR, Allam MM, Mousa MR, Khodir AE, El-Haddad AE, et al. Berberine-loaded nanostructured lipid carriers mitigate warm hepatic ischemia/reperfusion-induced lesion through modulation of HMGB1/TLR4/NF- κ B signaling and autophagy. *Biomed Pharmacother* 2022;145:112122.
 96. Liang C, Peng Y, Sun H, Wang L, Jiang L, Zou S. Silencing lncRNA KCNQ1OT1 reduced hepatic ischemia reperfusion injury-induced pyroptosis by regulating miR-142a-3p/HMGB1 axis. *Mol Cell Biochem* 2023;478:1293-1305.
 97. Jia K, Zhang Y, Luo R, Liu R, Li Y, Wu J, et al. Acteoside ameliorates hepatic ischemia-reperfusion injury via reversing the senescent fate of liver sinusoidal endothelial cells and restoring compromised sinusoidal networks. *Int J Biol Sci* 2023;19:4967-4988.
 98. Terry AQ, Kojima H, Sosa RA, Kaldas FM, Chin JL, Zheng Y, et al. Disulfide-HMGB1 signals through TLR4 and TLR9 to induce inflammatory macrophages capable of innate-adaptive crosstalk in human liver transplantation. *Am J Transplant* 2023;23:1858-1871.
 99. Barbier L, Robin A, Sindayigaya R, Ducousso H, Dujardin F, Thierry A, et al. Endogenous interleukin-33 acts as an alarmin in liver ischemia-reperfusion and is associated with injury after human liver transplantation. *Front Immunol* 2021;12:744927.
 100. Zhang HM, Chen XJ, Li SP, Zhang JM, Sun J, Zhou LX, et al. ILC2s expanded by exogenous IL-33 regulate CD45+CD11b+F4/80high macrophage polarization to alleviate hepatic ischemia-reperfusion injury. *Front Immunol* 2022;13:869365.
 101. Li S, Fan G, Li X, Cai Y, Liu R. Modulation of type I interferon signaling by natural products in the treatment of immune-related diseases. *Chin J Nat Med* 2023;21:3-18.
 102. Li Y, Ma Z, Ding M, Jia K, Xu B, Zhou F, et al. Chuanxiong Rhizoma extracts prevent cholestatic liver injury by targeting H3K9ac-mediated and cholangiocyte-derived secretory protein PAI-1 and FN. *Chin J Nat Med* 2023;21:694-709.
 103. Wang Z, Liu D, Yan Q, Liu F, Zhan M, Qi S, et al. Activated AXL protects against hepatic ischemia-reperfusion injury by upregulating SOCS-1 expression. *Transplantation* 2022;106:1351-1364.
 104. Morsy MA, Abdel-Gaber SA, Rifaai RA, Mohammed MM, Nair AB, Abdelzaher WY. Protective mechanisms of telmisartan against hepatic ischemia/reperfusion injury in rats may involve PPAR γ -induced TLR4/NF- κ B suppression. *Biomed Pharmacother* 2022;145:112374.
 105. Zhou W, Zhong Z, Lin D, Liu Z, Zhang Q, Xia H, et al. Hypothermic oxygenated perfusion inhibits HECTD3-mediated TRAF3 polyubiquitination to alleviate DCD liver ischemia-reperfusion injury. *Cell Death Dis* 2021;12:211.
 106. Gendy A, Elnagar MR, Soubh A, Al-Mokaddem A, El-Haddad A, El-Sayed MK. Morin alleviates hepatic ischemia/reperfusion-induced mischief: In vivo and in silico contribution of Nrf2, TLR4, and NLRP3. *Biomed Pharmacother* 2021;138:111539.
 107. Liu Y, Lei Z, Chai H, Kang Q, Qin X. Salidroside alleviates hepatic ischemia-reperfusion injury during liver transplant in rat through regulating TLR-4/NF- κ B/NLRP3 inflammatory pathway. *Sci Rep* 2022;12:13973.
 108. Yang X, Yu H, Wei J, Wei Q, Huang H, Chen J, et al. The protective effects of dietary Clostridium butyricum supplementation on hepatic ischemia reperfusion injury in rats. *Acta Cir Bras* 2022;37:e370904.
 109. Liu H, Wang J, Ding Y, Shi X, Ren H. Antibiotic pretreatment attenuates liver ischemia-reperfusion injury by Farnesoid X receptor activation. *Cell Death Dis* 2022;13:484.
 110. Schmitz SM, Dohmeier H, Stoppe C, Alizai PH, Schipper S, Neumann UP, et al. Inhaled argon impedes hepatic regeneration after ischemia/reperfusion injury in rats. *Int J Mol Sci* 2020;21:5457.
 111. Xue Z, Zhang Y, Liu Y, Zhang C, Shen XD, Gao F, et al. PACAP neuropeptide promotes hepatocellular protection via CREB-KLF4 dependent autophagy in mouse liver ischemia reperfusion injury. *Theranostics* 2020;10:4453-4465.
 112. Shi G, Zhang Z, Ma S, Li Y, Du S, Chu Y, et al. Hepatic interferon regulatory factor 8 expression mediates liver ischemia/reperfusion injury in mice. *Biochem Pharmacol* 2021;192:114728.
 113. Krzystek-Korpacka M, Fleszar MG, Fortuna P, Gostomska-Pampuch K, Lewandowski Ł, Piasecki T, et al. Modulation of prostanooids profile and counter-regulation of SDF-1 α /CXCR4 and VIP/VIPAC2 expression by sitagliptin in non-diabetic rat model of hepatic ischemia-reperfusion injury. *Int J Mol Sci* 2021;22:13155.
 114. Chen H, McKeen T, Chao X, Chen A, Deng F, Jaeschke H, et al. The role of MLKL in hepatic ischemia-reperfusion injury of alcoholic steatotic livers. *Int J Biol Sci* 2022;18:1096-1106.
 115. Qiang X, Li J, Zhu S, He M, Chen W, Al-Abed Y, et al. Human dermcidin protects mice against hepatic ischemia-reperfu-

- sion-induced local and remote inflammatory injury. *Front Immunol* 2022;12:821154.
116. Yin E, Fukuhara T, Takeda K, Kojima Y, Fukuhara K, Ikejima K, et al. Anti-CD321 antibody immunotherapy protects liver against ischemia and reperfusion-induced injury. *Sci Rep* 2021;11:6312.
117. Ni X, Wu X, Zhu XX, Li JH, Yin XY, Lu L. Carabin deficiency aggravates hepatic ischemia-reperfusion injury through promoting neutrophil trafficking via ras and calcineurin signaling. *Front Immunol* 2022;13:773291.
118. Stephan A, Fabri M. The NET, the trap and the pathogen: neutrophil extracellular traps in cutaneous immunity. *Exp Dermatol* 2015;24:161-166.
119. Guo J, Akahoshi T, Mizuta Y, Murata M, Narahara S, Kawano T, et al. Histidine-rich glycoprotein alleviates liver ischemia/reperfusion injury in mice with nonalcoholic steatohepatitis. *Liver Transpl* 2021;27:840-853.
120. Wang Y, Sun X, Han X, Sun J, Li L, Zhang D, et al. Resveratrol improves hepatic ischemia-reperfusion injury by inhibiting neutrophils via the ERK signaling pathway. *Biomed Pharmacother* 2023;160:114358.
121. Yazdani HO, Kaltenmeier C, Morder K, Moon J, Traczek M, Loughran P, et al. Exercise training decreases hepatic injury and metastases through changes in immune response to liver ischemia/reperfusion in mice. *Hepatology* 2021;73:2494-2509.
122. Wang H, Xi Z, Deng L, Pan Y, He K, Xia Q. Macrophage polarization and liver ischemia-reperfusion injury. *Int J Med Sci* 2021;18:1104-1113.
123. Long Y, Wei H, Li J, Li M, Wang Y, Zhang Z, et al. Prevention of hepatic ischemia-reperfusion injury by carbohydrate-derived nanoantioxidants. *Nano Lett* 2020;20:6510-6519.
124. Rao J, Qiu J, Ni M, Wang H, Wang P, Zhang L, et al. Macrophage nuclear factor erythroid 2-related factor 2 deficiency promotes innate immune activation by tissue inhibitor of metalloproteinase 3-mediated RhoA/ROCK pathway in the ischemic liver. *Hepatology* 2022;75:1429-1445.
125. Liang W, Greven J, Qin K, Fragoulis A, Horst K, Bläsius F, et al. Sulfuraphane exerts beneficial immunomodulatory effects on liver tissue via a Nrf2 pathway-related mechanism in a murine model of hemorrhagic shock and resuscitation. *Front Immunol* 2022;13:822895.
126. Wang YY, Diao BZ, Zhong LH, Lu BL, Cheng Y, Yu L, et al. Maslinic acid protects against lipopolysaccharide/d-galactosamine-induced acute liver injury in mice. *Microb Pathog* 2018;119:49-53.
127. Ishikawa H, Barber GN. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. *Nature* 2008;455:674-678.
128. Zhong W, Rao Z, Rao J, Han G, Wang P, Jiang T, et al. Aging aggravated liver ischemia and reperfusion injury by promoting STING-mediated NLRP3 activation in macrophages. *Aging Cell* 2020;19:e13186.
129. Wu XY, Chen YJ, Liu CA, Gong JH, Xu XS. STING Induces liver ischemia-reperfusion injury by promoting calcium-dependent caspase 1-GSDMD processing in macrophages. *Oxid Med Cell Longev* 2022;2022:8123157.
130. Nakamoto S, Ito Y, Nishizawa N, Goto T, Kojo K, Kumamoto Y, et al. Lymphangiogenesis and accumulation of reparative macrophages contribute to liver repair after hepatic ischemia-reperfusion injury. *Angiogenesis* 2020;23:395-410.
131. Wang L, Li J, He S, Liu Y, Chen H, He S, et al. Resolving the graft ischemia-reperfusion injury during liver transplantation at the single cell resolution. *Cell Death Dis* 2021;12:589.
132. Yang X, Lu D, Wang R, Lian Z, Lin Z, Zhuo J, et al. Single-cell profiling reveals distinct immune phenotypes that contribute to ischaemia-reperfusion injury after steatotic liver transplantation. *Cell Prolif* 2021;54:e13116.
133. Sheng M, Lin Y, Xu D, Tian Y, Zhan Y, Li C, et al. CD47-mediated Hedgehog/SMO/GLI1 signaling promotes mesenchymal stem cell immunomodulation in mouse liver inflammation. *Hepatology* 2021;74:1560-1577.
134. Yu J, Zhu C, Wang X, Kim K, Bartolome A, Dongiovanni P, et al. Hepatocyte TLR4 triggers inter-hepatocyte Jagged1/Notch signaling to determine NASH-induced fibrosis. *Sci Transl Med* 2021;13:eabe1692.
135. Jin Y, Li C, Xu D, Zhu J, Wei S, Zhong A, et al. Jagged1-mediated myeloid Notch1 signaling activates HSF1/Snail and controls NLRP3 inflammasome activation in liver inflammatory injury. *Cell Mol Immunol* 2020;17:1245-1256.
136. Li SL, Wang ZM, Xu C, Che FH, Hu XF, Cao R, et al. Li-raglutide attenuates hepatic ischemia-reperfusion injury by modulating macrophage polarization. *Front Immunol* 2022;13:869050.
137. Huang Z, Mou T, Luo Y, Pu X, Pu J, Wan L, et al. Inhibition of miR-450b-5p ameliorates hepatic ischemia/reperfusion injury via targeting CRYAB. *Cell Death Dis* 2020;11:455.
138. Zhou H, Zhou S, Shi Y, Wang Q, Wei S, Wang P, et al. TGR5/Cathepsin E signaling regulates macrophage innate immune activation in liver ischemia and reperfusion injury. *Am J*

- Transplant 2021;21:1453-1464.
139. Zito G, Miceli V, Carcione C, Busà R, Bulati M, Gallo A, et al. Human amnion-derived mesenchymal stromal/stem cells preconditioning inhibits inflammation and apoptosis of immune and parenchymal cells in an in vitro model of liver ischemia/reperfusion. *Cells* 2022;11:709.
 140. Zhou J, Hu M, He M, Wang X, Sun D, Huang Y, et al. TNFAIP3 Interacting protein 3 is an activator of Hippo-YAP signaling protecting against hepatic ischemia/reperfusion injury. *Hepatology* 2021;74:2133-2153.
 141. Kojima H, Kadono K, Hirao H, Dery KJ, Torgerson T, Yao S, et al. T Cell CEACAM1-TIM-3 crosstalk alleviates liver transplant injury in mice and humans. *Gastroenterology* 2023;165:1233-1248.e9.
 142. Jin G, Guo N, Liu Y, Zhang L, Chen L, Dong T, et al. 5-aminolevulinate and CHIL3/CHI3L1 treatment amid ischemia aids liver metabolism and reduces ischemia-reperfusion injury. *Theranostics* 2023;13:4802-4820.
 143. Zhang L, Cui LL, Yang WH, Xue FS, Zhu ZJ. Effect of intraoperative dexmedetomidine on hepatic ischemia-reperfusion injury in pediatric living-related liver transplantation: A propensity score matching analysis. *Front Surg* 2022;9:939223.
 144. Zhang L, Li N, Cui LL, Xue FS, Zhu ZJ. Intraoperative low-dose dexmedetomidine administration associated with reduced hepatic ischemia-reperfusion injury in pediatric deceased liver transplantation: A retrospective cohort study. *Ann Transplant* 2021;26:e933354.
 145. Feng J, Zhang L, Xue E, Qiu Z, Hu N, Wang K, et al. Downregulation of Bmal1 expression in celiac ganglia protects against hepatic ischemia-reperfusion injury. *Biomolecules* 2023;13:713.
 146. Liu L, Cao Q, Gao W, Li BY, Zeng C, Xia Z, et al. Melatonin ameliorates cerebral ischemia-reperfusion injury in diabetic mice by enhancing autophagy via the SIRT1-BMAL1 pathway. *FASEB J* 2021;35:e22040.
 147. Yu S, Liu X, Xu Y, Pan L, Zhang Y, Li Y, et al. m6A-mediated gluconeogenic enzyme PCK1 upregulation protects against hepatic ischemia-reperfusion injury. *Hepatology* 2023 Dec 12. doi: 10.1097/HEP.0000000000000716.
 148. Dong J, Ke MY, Wu XN, Ding HF, Zhang LN, Ma F, et al. SRY is a key mediator of sexual dimorphism in hepatic ischemia/reperfusion injury. *Ann Surg* 2022;276:345-356.
 149. Czigany Z, Lurje I, Schmelzle M, Schöning W, Öllinger R, Raschzok N, et al. Ischemia-reperfusion injury in marginal liver grafts and the role of hypothermic machine perfusion: molecular mechanisms and clinical implications. *J Clin Med* 2020;9:846.
 150. Palladini G, Cagna M, Di Pasqua LG, Adorini L, Croce AC, Perlini S, et al. Obeticholic acid reduces kidney matrix metalloproteinase activation following partial hepatic ischemia/reperfusion injury in rats. *Pharmaceuticals (Basel)* 2022;15:524.
 151. Li S, Jin H, Sun G, Zhang C, Wang J, Xu H, et al. Dietary inorganic nitrate protects hepatic ischemia-reperfusion injury through NRF2-mediated antioxidative stress. *Front Pharmacol* 2021;12:634115.
 152. Hou W, Wei B, Liu HS. The protective effect of panax notoginseng mixture on hepatic ischemia/reperfusion injury in mice via regulating NR3C2, SRC, and GAPDH. *Front Pharmacol* 2021;12:756259.
 153. Wang X, Walkey CJ, Maretti-Mira AC, Wang L, Johnson DL, DeLeve LD. Susceptibility of rat steatotic liver to ischemia-reperfusion is treatable with liver-selective matrix metalloproteinase inhibition. *Hepatology* 2020;72:1771-1785.
 154. Zhang P, Yue K, Liu X, Yan X, Yang Z, Duan J, et al. Endothelial Notch activation promotes neutrophil transmigration via downregulating endomucin to aggravate hepatic ischemia/reperfusion injury. *Sci China Life Sci* 2020;63:375-387.
 155. Hirao H, Kojima H, Dery KJ, Nakamura K, Kadono K, Zhai Y, et al. Neutrophil CEACAM1 determines susceptibility to NETosis by regulating the S1PR2/S1PR3 axis in liver transplantation. *J Clin Invest* 2023;133:e162940.