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Review Article

Necroptosis in ischemia-reperfusion injury of lean and steatotic livers☆

Hua Sun [a, b], Tara McKeen [a], Hua Wang [b], Hong-Min Ni [a, *]

[a] Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS, USA
[b] Department of Oncology, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China

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ABSTRACT

Hepatic ischemia-reperfusion (IR) injury is a major complication during liver transplantation, liver resection, and other clinical situations. Increased hepatic IR injury in steatotic livers is a major reason for rejecting the use of steatotic livers for liver transplantation. Necroptosis is implicated in the pathogenesis of fatty liver diseases, including non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD). Necroptosis is one type of regulated cell death and is regulated by three key proteins: receptor-interacting protein 1 (RIP1), receptor-interacting protein 3 (RIP3), and mixed-lineage kinase domain-like protein (MLKL). In this review, we examine the necroptosis status in the steatotic liver diseases NAFLD and ALD as well as its role in hepatic IR injury of lean and steatotic livers.

1. Introduction

According to the Nomenclature Committee on Cell Death, necroptosis is a novel form of cell death known as “regulated necrosis” and is one type of regulated cell death.[1] Necroptosis is mediated by receptor-interacting protein 1 (RIP1), receptor-interacting protein 3 (RIP3), and its downstream molecule mixed-lineage kinase domain-like protein (MLKL).[2,3] As the definition of necroptosis infers, this phenomenon is morphologically similar to necrosis and is activated by a pathway also common to apoptosis, including death receptor activation.[4–6] Limited evidence is available regarding the linkage between necroptosis and the pathogenesis of inflammatory liver diseases including alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), and hepatic ischemia-reperfusion (IR) injury.[7–10]

IR injury occurs when the blood flow to a particular organ is disrupted, resulting in a lack of oxygen (ischemia) and disruption of energy and cellular metabolism. The blood flow is eventually restored to the organ (reperfusion); however, because of the prior ischemia, this can lead to tissue injury. Hepatic IR injury is a major complication in many clinical situations, including but not limited to liver surgery and trauma.[11–13] A better understanding of hepatic IR injury may lead to improved patient outcomes. In this review, we discuss the underlying mechanisms of necroptosis and hepatic IR injury and compare the role of necroptosis in hepatic IR injury of lean and steatotic livers.

1.1. Necroptosis: general concepts

Necroptosis is a form of regulated cell death initiated by disturbance of the extracellular or intracellular microenvironment as detected by specific death receptors, mainly Fas and tumor necrosis factor receptor 1 (TNFR1).[14–16] Necroptosis critically depends on the sequential activation of RIP1, RIP3, and MLKL at the molecular level.[2,3] The engagement of death receptors, particularly TNFR1, is the trigger for RIP1 and RIP3 activation and has been well characterized (Fig. 1). Tumor necrosis factor-alpha (TNF-α), a pleiotropic cytokine, regulates cell death, cell survival, and inflammation. TNF-α can induce either caspase-dependent apoptosis or RIP1/3-MLKL-mediated necroptosis depending on the cellular context. Two TNF receptors exist, and the various effects of TNF-α are mainly dependent on TNFR1. Once TNF-α binds to TNFR1, a multi-protein complex including TNF receptor-associated death domain (TRADD), TNF receptor-associated factor 2 (TRAF2), cellular inhibitor of apoptosis protein 1 (cIAP1), cIAP2, linear ubiquitin chain assembly complex (LUBAC), and RIP1 is formed, with the end result being ubiquitination of RIP1. Ubiquitination of

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* Corresponding author. Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS, USA.
E-mail address: hni@kumc.edu (H.-M. Ni).

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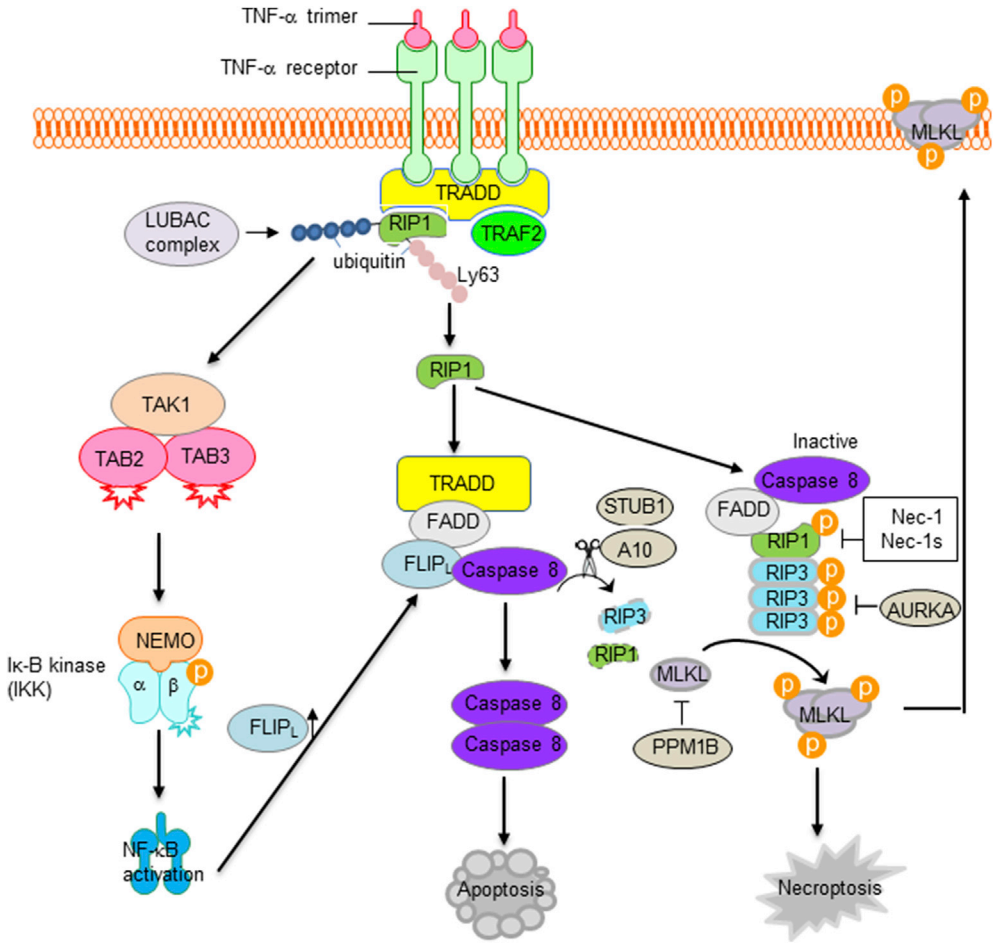

Fig. 1. Signal pathways in TNF-induced survival and cell death. TNF-α stimulation results in NF-κB activation, which prevents cell death. TNF receptor dissociation of RIP1 results in the formation of TRADD, FADD, and $FLIP_L$ complex to activate caspase 8 and induce apoptosis. When caspase 8 is inactivated, RIP1 and RIP3 interact with each other to form heterodimers resulting in assembly of the necrosome, which further recruits MLKL to form the necrosome and thus induce necroptosis. Abbreviations: AURKA, aurora kinase A; FADD, Fas-associated death domain; FLIP, FADD-like interleukin 1β-converting enzyme-inhibitory protein; IKK, inhibitor of NF-κB kinase; LUBAC, linear ubiquitin chain assembly complex; MLKL, mixed-lineage kinase domain-like protein; Nec-1, necrostatin-1; NEMO, NF-κB essential modulator; NF-κB, nuclear factor-kappa B; PPM1B, protein phosphatase, Mg^{2+}/Mn^{2+} dependent 1B; RIP, receptor-interacting protein; STUB1, STIP1 homology and U-box-containing protein 1; TAB, TAK1-binding protein; TAK1, transforming growth factor β-activated kinase 1; TNF-α, tumor necrosis factor-alpha; TNFR2, TNF receptor-associated factor 2; TRADD, TNF receptor-associated death domain.

RIP1 by cIAPs and LUBAC recruits transforming growth factor β-activated kinase 1 (TAK1)-binding protein (TAB) 2/3 to promote the activation of TAK1, which in turn activates the inhibitor of nuclear factor-kappa B (NF-κB) kinase (IKK) complex and the further NF-κB, thereby increasing the expression of genes associated with inflammation and cell survival. Two potential outcomes are possible when RIP1 is deubiquitinated: TNF-α triggers either apoptosis or necroptosis. Deubiquitinated RIP1 forms a complex with TRADD, Fas-associated death domain (FADD), FADD-like interleukin (IL)-1β-converting enzyme-inhibitory protein (FLIP), and caspase 8 to activate caspase 8 and subsequently trigger apoptosis. Necroptosis is generally blocked by apoptosis through caspase 8-mediated cleavage and inactivation of RIP1 and RIP3. In the absence of functional caspase 8, RIP1 and RIP3 interact with each other via their RIP homotypic interaction motif domains to form heterodimers resulting in the assembly of the necrosome, which is a critical step that leads to the phosphorylation and activation of MLKL; this activation further recruits MLKL to form the necrosome and thus induce necroptosis.[2,17] Active RIP3 phosphorylates MLKL, resulting in the formation of MLKL oligomers (most likely trimers or tetramers). Phosphorylated MLKL oligomers translocate to the plasma membrane, where they activate cell-surface proteases of the ADAM family. These proteases can promote the shedding of plasma membrane-associated proteins or form Mg^{2+}-permeant channels to trigger plasma membrane permeabilization, leading to the lethal step of necroptosis.[18–21] The necrosome also increases the expression of inflammatory factors, including IL-1β and IL-6, which reportedly play important roles in ALD, NAFLD, and hepatic IR injury.[22]

RIP1, RIP3, and MLKL are three key molecules in necroptosis. Pharmacological inhibitors of RIP1, including necrostatin-1 (Nec-1) and its derivatives (Nec-1s), robustly inhibit TNFR1-driven necroptosis both *in vitro* and *in vivo*.[23,24] Furthermore, the essential contribution of MLKL to necroptosis has been confirmed by genetic studies as well as by pharmacological inhibition of MLKL with necrosulfonamide.[25,26] Several major negative regulators of the necrosome have been reported, including (i) STIP1 homology and U-box-containing protein 1 (STUB1), which promotes RIP1 and RIP3 ubiquitination followed by lysosomal degradation and in turn inhibits necroptosis;[27,28] (ii) A20, a deubiquitinating enzyme that deubiquitinates RIP3 and inhibits necrosome assembly and necroptosis;[28,29] (iii) protein phosphatase, Mg^{2+}/Mn^{2+}-dependent 1B (PPM1B), a protein phosphatase that dephosphorylates RIP3 and prevents MLKL recruitment to the necrosome;[30] and (iv) aurora

kinase A (AURKA), which directly interacts with RIP1 and RIP3 to inhibit necrosome activation.[31]

1.2. Hepatic IR injury: general concepts

Hepatic IR injury is often the result of liver injury from liver transplantation and liver resection.[32] The most effective treatment for end-stage liver disease is liver transplantation; however, limited numbers of organs are available. Therefore, an understanding of the mechanisms of how hepatic IR injury occurs secondary to liver transplantation is a relevant issue to preserve and maintain donated organs.[33] Hepatic IR injury occurs by depletion of oxygen and adenosine triphosphate (ATP), excessive inflammatory responses, and generation of reactive oxygen species (ROS) after reperfusion (Fig. 2).[11–13]

Two distinct phases of liver injury occur after IR.[34–36] The early phase of liver injury occurs within the first 2 h of reperfusion. The lack of oxygen in hepatocytes during ischemia causes ATP depletion, increased anaerobic metabolism, and acidosis. This leads to swelling of sinusoidal endothelial cells (SECs) and Kupffer cells (KCs), which in turn contributes to microcirculatory dysfunction and further hepatic neutrophil accumulation. In the meantime, KCs are activated and release ROS and proinflammatory cytokines, including TNF-α and IL-1β.[35] Studies have shown that several circulating small proteins, also known as the complement cascade, are involved in the induction of KC-induced oxidant stress, the priming of KCs and neutrophils for enhanced ROS generation, and the continuous accumulation of neutrophils in the liver to initiate the inflammatory response.[37] Depletion of serum complement significantly attenuates the KC-induced oxidant stress and prevents the accumulation of neutrophils in the liver during the initial reperfusion. Deletion of complement component 3 and inhibition of complement with complement receptor 2-complement receptor 1-related protein y reduces the inflammatory response and hepatic IR injury.[38] In addition to complement stimulation after IR, $CD4^+$ T lymphocytes are rapidly recruited to the post-ischemic liver after IR.[39] Liver-recruited $CD4^+$ T lymphocytes are activated by cytokines TNF-α and IL-1β from activated KCs.[40] Depletion of $CD4^+$ T lymphocytes in mice with antibody reportedly results in a reduction of subacute-phase injury and inflammation as well as neutrophil infiltration.[39] $CD4^+$ T-lymphocyte-deficient mice also show decreased neutrophil recruitment and inflammatory responses.[41]

Mitochondria have a distinguished role in the cascade of organ damage due to IR. Mitochondria are a primary target in the very early injury phase. Similar to ROS production by KCs, ROS can be generated from mitochondria in activated SECs and hepatocytes following IR.[42] Ischemia results in mitochondrial dysfunction, which may impair the electron flow and increase superoxide formation during IR. A prolonged duration of ischemia triggers mitochondrial oxidant stress in the liver.[42,43] ROS increases membrane

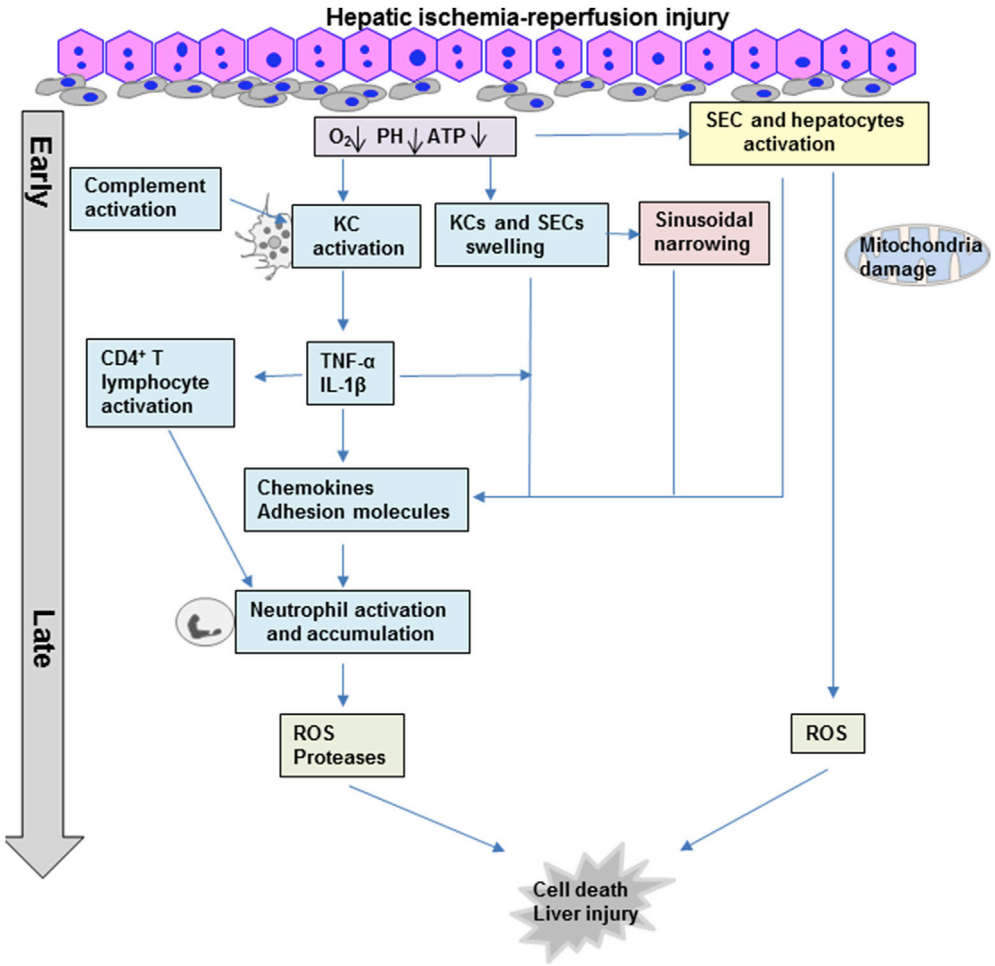

Fig. 2. Schematic process of hepatic IR injury. Hepatic IR injury occurs due to depletion of oxygen and ATP, excessive inflammatory responses, mitochondrial uncoupling, and ROS generation after reperfusion. Abbreviations: ATP, adenosine triphosphate; IL-1β, interleukin-1β; KCs, Kupffer cells; ROS, reactive oxygen species; O_2, oxygen; SECs, sinusoidal endothelial cells; TNF-α, tumor necrosis factor-alpha.

permeability, thereby inducing mitochondria permeability transition (MPT); this further induces oxidative stress in the liver.[44]

The late phase of IR injury occurs 2 h post-reperfusion and is characterized by the recruitment of neutrophils to the post-ischemic liver, resulting in damage to hepatocytes through ROS and proteases. Proinflammatory cytokines released from KCs activate multiple types of cells to produce and release chemokines and induce adhesion molecules (intercellular cell adhesion molecule and vascular cell adhesion molecule) from SECs and neutrophils.[13,45] These proinflammatory mediators facilitate the recruitment of neutrophils and promote neutrophil activation and accumulation, thereby contributing to the progression of liver injury by releasing ROS and proteases.[13,45] In addition, the MPT caused by ROS further induces mitochondrial oxidative stress, which contributes to IR injury.[44]

In summary, the three main mechanisms contributing to hepatic IR injury are excessive inflammatory responses, mitochondrial uncoupling, and oxidative stress. Targeting these factors may help to develop strategies to prevent hepatic IR injury.

2. Necroptosis in fatty liver disease

Fatty liver disease, including ALD and NAFLD, is a major health issue worldwide. NAFLD is associated with diabetes, obesity, and other metabolic diseases.[46] The estimated prevalence of NAFLD is about 20% worldwide and continues to increase.[47] The pathogenesis of fatty liver disease is characterized by hepatic steatosis, inflammation, and fibrosis and can progress to cirrhosis and hepatocellular carcinoma.[48]

Studies of necroptosis in fatty liver diseases have mainly focused on NAFLD (Table 1). The first study of necroptosis in NAFLD was conducted by Gautheron *et al.*,[8] who found that RIP3 was upregulated in both human non-alcoholic steatohepatitis (NASH) and in a methionine- and choline-deficient (MCD) diet-induced NASH mouse model. RIP3 also mediates liver injury, inflammation, and liver fibrosis through necroptosis. These findings were later confirmed by Afonso *et al.*,[49] who found that necroptosis increased in patients with NAFLD and in a high-fat choline-deficient (HFCD) or MCD diet-induced NASH mouse model. Pharmacological use of RIP1 inhibitor Nec-1 or RIP3 gene silencing protected necroptosis in primary murine hepatocytes. Genetic deletion of RIP3 protected against MCD-induced liver injury, steatosis, inflammation, fibrosis, and oxidative stress. However, Roychowdhury *et al.*[7] showed that deletion of RIP3 exacerbated high-fat diet (HFD) induced liver injury, which was associated with increased inflammation, hepatocyte death, and fibrotic responses.

In contrast, studies of necroptosis in ALD are limited. Our group[9] used a chronic-plus-binge alcohol feeding mouse model to examine this issue. We found that RIP3 protein was increased by chronic alcohol exposure through impaired hepatic proteasomal functions. Deletion of RIP3 ameliorated alcohol-induced liver injury and steatosis. Pharmacological inhibition of RIP1 by Nec-1s decreased inflammation in this chronic-plus-binge alcohol feeding mouse model.

In summary, necroptosis contributes to both NAFLD and ALD. However, the mechanism of necroptosis is controversial, possibly because of the different models used in each study.

3. Hepatic IR injury in lean and steatotic livers

The current standard of care for end-stage liver disease is liver transplantation; however, not enough donated healthy livers are available for everyone on the transplant list.[33] The shortage of donor organs has led to more aggressive acceptance and use of liver grafts from extended-criteria donors, including the use of steatotic livers.[22] Moderate to severe donor liver steatosis (>30%–60% graft macrosteatosis) increases the risk of IR injury as demonstrated by the evidence that patients with fatty liver have reduced tolerance to IR injury and increased mortality after transplantation.[22,50] However, the mechanisms by which fatty liver increases IR injury are not fully understood. This limits the safe use of fatty livers as potential grafts and further limits the therapeutic modalities to improve postoperative care for an extensive fatty liver graft.

Animal studies have consistently demonstrated that hepatic steatosis exacerbates hepatic IR injury (Table 2).[51–69] Four potential mechanisms by which NAFLD can exacerbate IR injury have been proposed. The first is an increased microcirculatory disturbance, which may cause prolonged ischemia.[51,52,57] Microcirculatory dysfunction is the main underlying mechanism of steatotic IR injury. The second is extensive mitochondrial dysfunction, in which ATP synthesis is lower in steatotic than non-steatotic livers during IR and mitochondrial uncoupling protein 2 (UCP2) is upregulated.[56,70] Deletion of mitochondrial UCP2 ameliorates IR injury in steatotic livers. The third is increased inflammation and ROS overproduction.[56,60,70] Greater accumulation of inflammatory cells, including macrophages and neutrophils, occurs in steatotic livers with IR injury than in lean livers.[60] Complement C3 deficiency protects against hepatic IR injury in steatotic liver through inhibition of inflammation.[56] Induction of heme oxygenase-1 protects against steatotic IR injury through its anti-inflammatory and antioxidant effects.[70] The fourth mechanism is an increase in peroxisome proliferator-activated receptor-gamma (PPAR-γ).[71] Increased PPAR-γ levels in steatotic livers subjected to IR increase the liver's susceptibility to injury, which is associated with increased levels of adiponectin, oxidative stress, and IL-1. However, most studies have utilized either genetic models of obesity such as *ob/ob* mice, or severe but less physiologically relevant diets such as the MCD diet. Several recent studies have demonstrated that necroptosis could be another mechanism in steatotic IR injury.[63,64] This will be discussed further later.

The main mechanisms involved in hepatic IR injury in ALD models are increased inflammation with increased production of TNF-α and some other cytokines and excessive ROS production.[65–67] Induction of heme oxygenase-1 also protects against IR injury in ALD.

Table 1
Necroptosis studies in fatty liver diseases.

References	Species	Diet	Inhibitor	Effect on liver injury
Roychowdhury *et al.*[7]	RIP3 KO	HFD		More injury
Wang *et al.*[9]	RIP3 KO	Gao Binge		Protection
Afonso *et al.*[49]	WT	HFCD or MCD	Nec-1 or RIP3 silencing	Protection
Afonso *et al.*[49]	RIP3 KO	HFCD or MCD		Protection
Gautheron *et al.*[8]	RIP3 KO	MCD		Protection

Abbreviations: HFCD, high-fat choline-deficient; HFD, high-fat diet; MCD, methionine- and choline-deficient; Nec-1, necrostatin-1; RIP3, receptor-interacting protein 3; WT, wild-type.

Table 2
List of experimental models of hepatic IR injury of fatty liver.

References	Species	Diet	Ischemia (min)	Reperfusion (hr)	Effect on liver injury
El-Badry *et al.*[51]	*ob/ob* mouse	Chow	45	3	More injury *vs.* lean control
Hasegawa *et al.*[52]	*ob/ob* mouse	Chow	60	1, 6, 12 and 24	More injury *vs.* lean control
Evans *et al.*[53]	*ob/ob* or *ob/ob* UCP2 KO mouse	Chow	15	1 and 24	More injury *vs.* lean control UCP2 KO worse *vs. ob/ob*
Massip-Salcedo *et al.*[54]	*ob/ob* rat		60	24	More injury *vs.* lean control
Serviddio *et al.*[55]	Rat	MCD	60	24	More injury *vs.* lean control
He *et al.*[56]	WT mouse	HFD	45	24	More injury *vs.* lean control
Teoh *et al.*[57]	WT *foz/foz* mouse	HFD or chow	60	2–24	More injury *vs.* lean control
Tevar *et al.*[58]	WT mouse	MCD	90	1, 4 and 8	More injury *vs.* lean control
Gupta *et al.*[59]	WT mouse	HFD	20	24	More injury *vs.* lean control
Fujii *et al.*[60]	WT mouse	HFD	60	6 and 24	More injury *vs.* lean control
Luo *et al.*[61]	WT mouse	MCDHF	15	3	More injury *vs.* lean control
Marsman *et al.*[62]	Rat	MCD	40	24	More injury *vs.* lean control
Liss *et al.*[63]	WT mouse	HTF-C, WD	60	4 and 24	More injury *vs.* lean control Increased necroptosis proteins
Ni *et al.*[64]	WT mouse	WD	45	1, 6 and 24	More injury *vs.* lean control
Yamada *et al.*[65]	Rat	Ethanol diet 6–8 wks	30	1, 4 and 24	More injury *vs.* control diet
Kim *et al.*[66,67] Cho *et al.*[68]	Rat	Ethanol diet 5 wks	60	5	More injury *vs.* control diet

Abbreviations: HFD, high-fat diet; HTF-C, high trans-fat, fructose, and cholesterol; MCD, methionine- and choline-deficient; MCDHF, methionine- and choline-deficient plus high fat; Nec-1, necrostatin-1; RIP3, receptor-interacting protein 3; UCP2, uncoupling protein 2; WD, Western diet; WT, wild-type.

In summary, liver steatosis exacerbates IR injury, and the mechanisms involved in hepatic IR injury may differ depending on the method used to induce experimental steatosis.

4. Necroptosis in hepatic IR injury in lean and steatotic livers

During the past 20 years, significant controversy has arisen about the type of cell death caused by hepatic IR: apoptotic cell death or necrotic cell death.[72–76] In most studies, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL), presumably as an apoptosis marker, has been labeled for cell death. However, our group and others have found that TUNEL can be detected not only in apoptosis but also in necrotic cell death because necrosis also causes deoxyribonucleic acid (DNA) degradation.[72,77] Moreover, Yang *et al.*[73] found that plasma biomarkers of necrotic cell death, such as micro-RNA 122, full-length cytokeratin 18, and high-mobility group box 1 protein, increased dramatically and were well correlated with the histological characteristics of necrosis. In contrast, there is a lack of morphological evidence of apoptotic cell death and caspase 3 activity in the post-ischemic liver. Moreover, in plasma, there was an absence of caspase 3 activity and only a minor increase caspase-cleaved fragment of cytokeratin 18, which is an apoptosis marker. A quantitative comparison of the release of full-length cytokeratin 18 (necrosis) and cytokeratin 18 (apoptosis) indicated that most cell death occurred by necrosis during IR and only a minor proportion occurred by apoptosis. However, MPT, mitochondrial uncoupling, Bax translocation, and mitochondrial cytochrome *c* release have been observed during IR injury and various types of drug-induced necrosis, which also play important roles in apoptosis (especially type 2 apoptosis).[72,78,79] Part of the confusion concerning the roles of apoptosis and necrosis in IR arises from the assumption that apoptotic and necrotic mechanisms are distinct and separate; in actuality, however, the pathways leading to necrosis and apoptosis can be shared by necroptosis.[14–16]

Limited evidence indicates that necroptosis may play a pivotal role in the pathogenesis of inflammatory liver diseases including ALD, NAFLD, and hepatic IR injury (Tables 1 and 3).[7–10] Intensive ongoing research is focusing on the identification of new preconditioning strategies for diminishing the adverse effects of allograft steatosis after liver transplantation. Most new therapies tested in murine models have demonstrated that hepatocyte damage and inflammation are major contributors that exacerbate hepatic IR injury in steatotic liver grafts.[50,80,81] Nec-1, a pharmacological inhibitor of RIP1, reportedly protects against IR injury in the intestine, liver, kidney, and heart,[10,82–84] suggesting that targeting RIP1-RIP3-MLKL-mediated necroptosis may be beneficial for IR injury. However, Nec-1 has also been demonstrated to inhibit indoleamine 2,3-dioxygenase and is not a specific inhibitor of RIP1.[85] Therefore, the exact role of RIP1-RIP3-MLKL-mediated necroptosis in hepatic IR injury is still unclear.

The role of necroptosis in hepatic IR injury is still controversial. Rosentreter *et al.*[86] used a warm IR mouse model and treated the mice with the RIP1 inhibitor Nec-1, and the authors concluded that Nec-1 did not protect the mice from IR injury. However, Hong *et al.*[10] used a similar model and found that Nec-1 did in fact protect the mice from hepatic IR injury by inducing autophagy. Besides using pharmacological inhibitors to examine the effect of

Table 3
Necroptosis in hepatic IR injury in lean and steatotic livers.

References	Species	Diet	Ischemia (min)	Reperfusion (hr)	Inhibitor	Effect on liver injury
Rosentrete *et al.*[86]	WT mouse	Chow	90	4	Nec-1	No protection
Hong *et al.*[10]	WT mouse	Chow	60	3	Nec-1	Protection
Saeed *et al.*[87]	RIP3 KO mouse	Chow	60	4		No protection
Ni *et al.*[64]	MLKL KO, RIP3 KO, and RIP3 kinase-dead knock-in mouse	Chow or WD	45	1, 6 and 24		MLKL KO mice were protected from IR injury; RIP3 KO or RIP3 kinase-dead knock-in mice were protected against IR injury at late time point

Abbreviations: IR, ischemia-reperfusion; MLKL, mixed-lineage kinase domain-like protein; Nec-1, necrostatin-1; RIP3, receptor-interacting protein 3; WD, Western diet; WT, wild-type.

necroptosis in hepatic IR models, genetically modified animal models have also been used in multiple studies. A recent study showed that deletion of RIP3 did not protect against IR injury in mice.[87] However, that study was limited by the fact that it only examined IR injury 4 h post-reperfusion. Our group found that a Western diet increased several key molecules in necroptosis (RIP1, RIP3, and MLKL) through inhibition of proteasome activities. Hepatic IR injury further increased in mice fed a Western diet compared with their lean littermates. Deletion of MLKL protected against hepatic IR injury in both lean and steatotic livers at 6 and 24 h post-reperfusion, while RIP3 deletion or a RIP3 kinase-dead knock-in status protected against IR injury at late time points for both lean and steatotic mice.[64]

Few studies have focused on hepatic IR injury in alcoholic fatty liver.[66–68,88] Most of these studies focused on inflammation in this model. Moreover, there is no evidence indicating that necroptosis plays a role in hepatic IR injury of alcoholic fatty liver. Studying the role of necroptosis of hepatic IR injury in alcoholic fatty liver is our ongoing project.

Activation of the RIP1-RIP3-MLKL necroptosis pathway, which promotes the neutrophil extracellular traps (NETs) in cultured neutrophils and bone marrow-derived cells, has been described in several recent studies.[89–91] Increased NETs have also been shown to promote IR injury in the liver and heart.[92,93] However, whether RIP1-RIP3-MLKL-mediated NETs may promote hepatic IR injury requires further investigation.

In summary, necroptosis plays a key role in hepatic IR injury in NAFLD; however, its role in ALD needs to be further investigated.

5. Conclusions

Necroptosis plays an important role in fatty liver diseases, including ALD and NAFLD, as well as in hepatic IR injury in lean and steatotic livers with NAFLD. However, its role in hepatic IR injury in ALD remains unclear. A better understanding of the mechanism of necroptosis during hepatic IR injury in fatty liver will further assist in designing and developing necroptosis-specific therapeutics in the near future.

Authors' contributions

All the authors contributed to writing this manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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