



Review Article

Distinct Types of Cell Death and Implications in Liver Diseases: An Overview of Mechanisms and Application



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Abstract

Cell death is associated with a variety of liver diseases, and hepatocyte death is a core factor in the occurrence and progression of liver diseases. In recent years, new cell death modes have been identified, and certain biomarkers have been detected in the circulation during various cell death modes that mediate liver injury. In this review, cell death modes associated with liver diseases are summarized, including some cell death modes that have emerged in recent years. We described the mechanisms associated with liver diseases and summarized recent applications of targeting cell death in liver diseases. It provides new ideas for the diagnosis and treatment of liver diseases. In addition, multiple cell death modes can contribute to the same liver disease. Different cell death modes are not isolated, and they interact with each other in liver diseases. Future studies may focus on exploring the regulation between various cell death response pathways in liver diseases.

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Introduction

As a fundamental process of life, cell death plays a crucial role in embryonic development. This process maintains the

body's homeostasis by continuously eliminating damaged cells.¹ According to the description listed in the Nomenclature Committee on Cell Death 2018, cell death manifests as macroscopic morphological changes. There are three classic cell death modes: apoptosis, autophagy, and necrosis.² With the further development of scientific research, a variety of new cell death modes have been discovered. These modes have different signaling pathways and morphological and molecular mechanisms.^{2,3} Different cell death modes can be regulated by various signaling pathways. When stimulated by internal or external factors, the activation or inhibition of various signaling pathways can cause cell death. There are many different types of liver disease. According to the etiology, liver diseases are divided into: viral hepatitis, alcoholic liver disease, nonalcoholic fatty liver disease, genetic causes, autoimmune causes and liver diseases caused by drugs or other factors, and so on. The final common pathway of any chronic damage to the liver is persistent wound healing, leading to hepatic parenchymal fibrosis.⁴ During different types of liver disease, such as liver fibrosis, alcoholic liver disease (ALD), steatohepatitis, or liver failure, the most prominent manifestation is hepatocyte death, which results in impaired liver function.^{5–7} This review mainly summarizes the mechanisms of various cell death modes that mediate liver disease, including some cell death modes that have emerged in recent years. This review provides new ideas for the diagnosis and treatment of liver diseases.

Modes of cell death associated with liver diseases

Apoptosis

Before introducing the mechanism of apoptosis, the B-cell lymphoma 2 (Bcl-2) family needs to be mentioned. Bcl-2 family proteins are divided into three groups according to their roles and domains: antiapoptotic Bcl-2-like proteins (including Bcl-2, Bcl-xL, Bcl-w, Mcl-1, and A1/Bfl-1), proapoptotic Bax-like proteins (including Bax, Bak, and Bok/Mtd) and only proapoptotic BH3 proteins (including Bid, Bim/Bod, Bad, Bmf, Bik/Nbk, Blk, Noxa, Puma/Bbc3, and Hrk/DP5).⁸ Apoptosis is activated by two pathways: the intrinsic (mitochondrial) pathway and the extrinsic (death receptor) pathway. Studies have shown that the intrinsic pathway is triggered by the Bcl-2 family, which is normally activated by mitochondrial dysfunction, endoplasmic reticulum (ER) stress, lysosomal permeabilization, and nuclear DNA damage.⁹ Then, toxic proteins such as cytochrome c are released from the intermembrane space through permeabilization of the outer mi-

Keywords: Cell death; Hepatocytes; Application; Liver diseases.

Abbreviations: ALD, alcoholic liver disease; ALT, alanine transaminase; APAP, acetaminophen; AST, aspartate aminotransferase; CypD, cyclophilin D; DAMP, damage-associated molecular modes; ER, endoplasmic reticulum; Fas-l, Fas ligand; Fer-1, Ferrostatin-1; GPX4, glutathione peroxidase 4; GSDMD, gasdermin D; GSDMD-NT, gasdermin D N-terminal fragment; GSH, glutathione; HBV, hepatitis B virus; HBe, HBV C protein; HBe, HBV E protein; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HSCs, hepatic stellate cells; HFD, high-fat diet; IL, interleukin; JNK, cJun-N-terminal Kinase; KC, kupffer cells; LPS, lipopolysaccharide; MCD, methionine–choline-deficient; MLKL, mixed lineage kinase domain protein; MPT, mitochondrial permeability transition; NASH, nonalcoholic steatohepatitis; NAFLD, nonalcoholic fatty liver disease; NETs, neutrophil extracellular traps; Nrf2, nuclear factor erythroid 2-related factor 2; PAMP, pathogen-associated molecular modes; RIPK, receptor interacting protein kinases; RIP1, receptor-interacting protein; ROS, reactive oxygen species; SLC27A5, solute carrier family 27 member 5; TAC, tricarboxylic acid cycle; TNF, tumor necrosis factor; TNF-R, TNF receptor; TRAIL, TNF-related apoptosis-inducing ligand.

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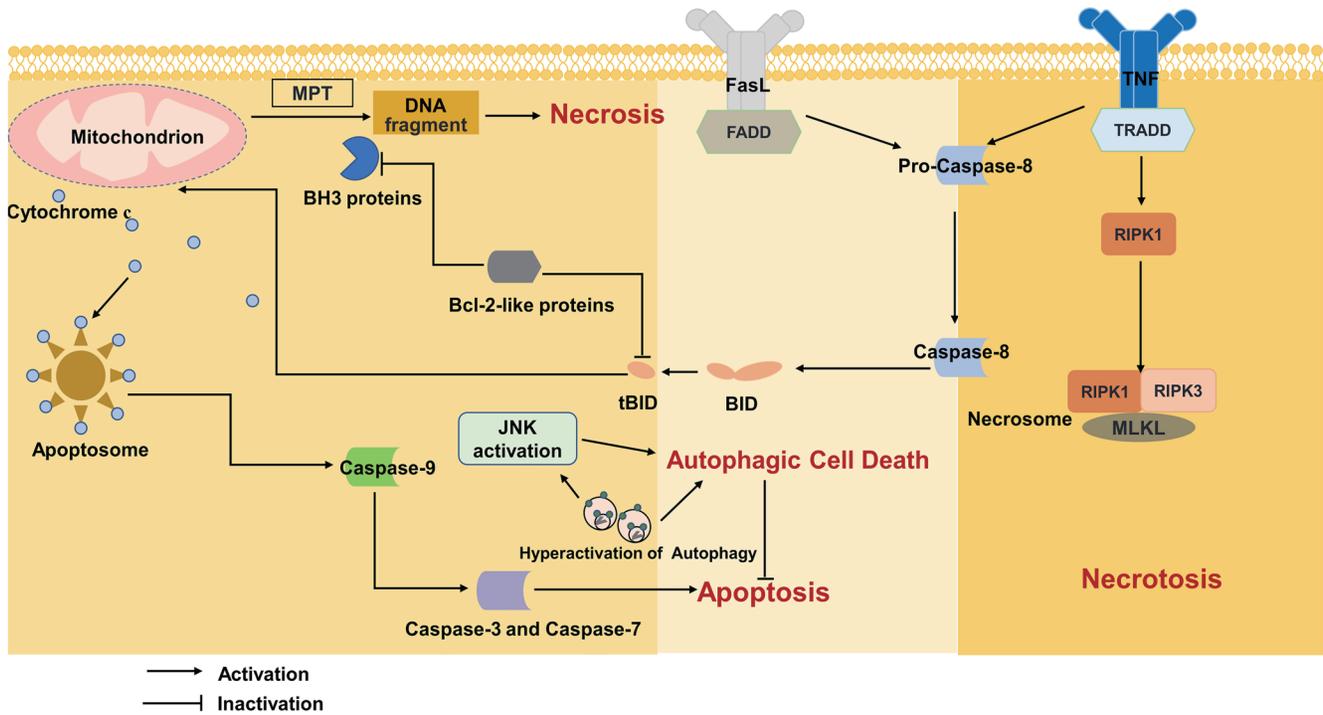


Fig. 1. Mechanism of apoptosis, necrosis, necroptosis and autophagy. FADD, fas-associated protein; Fas-L, fas ligand; JNK, cJun-N-terminal Kinase; MLKL, mixed lineage kinase domain protein; MPT, mitochondrial permeability transition; RIPK1, receptor interacting protein kinases 1; RIPK3, receptor interacting protein kinases 3; TNF, tumor necrosis factor; TRADD, TNFR1-associated death domain protein.

tochondrial membrane. Ultimately, caspase is activated and promotes apoptosis.^{5,8} Studies have shown that caspases related to apoptosis include promoters including caspase-8 and -9 and effectors including caspase-3, -6, and -7.¹⁰ In addition to being death receptor ligands, tumor necrosis factor (TNF), TNF-related apoptosis-inducing ligand (TRAIL) and Fas ligand (Fas-L) trigger extrinsic apoptosis pathways by binding to death receptors.¹¹ Several studies have shown that apoptosis mediated by death receptors is a key feature of various liver diseases.¹² The progression of liver diseases is influenced by the balance between apoptosis and antiapoptotic capacity.¹³ During liver injury, as hepatocyte apoptosis and its pathophysiology vary among different liver diseases, this review will address them separately (Fig. 1).

Viral hepatitis

Hepatitis B virus (HBV) or hepatitis C virus (HCV) infection is the main cause of chronic viral hepatitis. The damage to hepatocytes caused by hepatitis virus is mainly mediated by the immune response. When hepatitis virus is copied in large quantities, cytotoxic T lymphocytes (CTLs) identify and kill hepatocytes that express hepatitis virus antigens and cause hepatocyte damage. Compared with acute viral hepatitis, chronic viral hepatitis is characterized by a large amount of apoptosis during continuous viral infection. Studies have shown that HBV and HCV promote the occurrence of apoptosis via cJun-N-terminal kinase (JNK) and CHOP through the intrinsic pathway.^{14,15} The extrinsic pathway is typically not sufficient to induce apoptosis, and it needs to be enhanced through the intrinsic pathway. When liver cells are infected by HCV, TNF α /TNF receptor (TNF-R), Fas/Fas-L, and TRAIL/TRAIL receptor-1 (also called death receptor 4, DR4) or TRAIL receptor-2 (also called death receptor 5, DR5) bind to CTLs and undergo apoptosis.¹⁶ Death receptors bind with

death ligands to form a complex that activates caspase-8,¹⁷ which triggers two signaling pathways. In the first pathway, caspase-8 activation directly leads to the activation of effector caspases (caspase-3, -6, -7), which results in massive amplification and promotes apoptosis.^{18,19} The second pathway is related to the mitochondria, recruited by the intrinsic pathway, and involves the cleavage of Bid. Mitochondria-dependent apoptosis is mediated by Bcl-2 family proteins converged at the mitochondrial permeability transition (MPT) pore, which regulates the release of apoptotic regulatory proteins like cytochrome C and caspase-9 is ultimately activated.²⁰ The hepatocyte is a so-called type II cell in which the activation of caspase-3, caspase -7 is inhibited, so the extrinsic pathway mainly acts through the second pathway.² HCV also induces apoptosis by generating reactive oxygen species (ROS) and reducing mitochondrial cross-membrane potential.²¹ During chronic HBV infection, liver expression of TNF α , TRAIL, and Fas is also related to hepatocyte apoptosis.^{22,23} The expression of Bax in liver cells is positively related to the number of apoptotic cells. HBV X protein can adjust TRAIL receptor expression during apoptosis.^{23,24}

ALD

Various death modes are involved in ALD, and apoptosis plays an important role in ALD.^{25,26} Caspase-3-positive hepatocytes were significantly increased in liver biopsies taken from patients with alcoholic hepatitis.²⁷ *In vivo* and *in vitro* experiments have shown that caspase inhibitors significantly attenuate alcohol-induced hepatocyte apoptosis.^{28,29} At present, the mechanisms of apoptosis in ALD can be summarized as follows: acetaldehyde metabolism drives the production of ROS, which leads to mitochondrial dysfunction and the release of the proapoptotic factor cytochrome c, which ultimately promotes caspase activation.³⁰ Alcohol

and its metabolites trigger ER stress and promote apoptosis via CHOP.^{14,25,26} Fas-I and TNF play important roles in the pathogenesis of ALD.^{31,32} Studies have shown that TNF and TNF-R levels were increased in ALD patients, and anti-TNF antibody treatment prevented alcoholic liver injury in animal models.^{26,33} However, anti-TNF antibodies are currently not clinically recommended for the treatment of ALD owing to increased infection and mortality rates.^{26,34} In addition, studies have also shown that there is crosstalk between the gut microbiome and the liver. Alcohol increases systemic bacterial production by altering intestinal permeability, which in turn stimulates Kupffer cells (KCs) and promotes TNF production and ultimately leads to apoptosis.³⁵ When cytotoxic T cell sensitivity is increased, Fas receptor expression is significantly increased in ALD through lymphocyte-mediated apoptosis.^{27,36} Through ER stress, alcohol promotes the phosphorylation of interferon regulatory factor 3, which leads to hepatocyte apoptosis via Bax.^{26,37}

Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH)

Studies have shown that free fatty acids (FFAs) stimulate TNF- α expression through the lysosomal pathway, which promotes hepatic fat metabolism and apoptosis.³⁸ The release of lysosomal enzymes into the cytosol is dependent on the specific lysosomal enzyme cathepsin B. This enzyme promotes TNF- α -mediated hepatocyte apoptosis by promoting mitochondrial depolarization, cytochrome c release and increased ROS production, thereby exacerbating liver damage.^{39,40} It has been reported that Fas protein expression is increased in NASH patients.⁴¹ In addition, elevated serum TNF- α levels were observed in patients with NASH.⁴² Studies have shown that Fas/Fas-I,^{43,44} TNF- α /TNF-R and TRAIL/TRAIL-R2 form a death-inducing signaling complex in NASH.⁴⁵⁻⁴⁷ This complex induces mitochondrial dysfunction through caspase-8-dependent cleavage of Bid. Mitochondrial dysfunction results in the release of cytochrome c, which activates caspase-3 and caspase-7,^{44,47} and then caspase-6 is activated. Recent studies have shown that activated caspase-6 cleaves Bid, and mitochondrial cytochrome c is released, which in turn activates caspase-3 and caspase-7, resulting in continuous apoptosis in hepatocytes.⁴⁸ In addition, ER stress plays an important role in the development of NAFLD.⁴⁹ Hepatocyte ballooning and apoptosis were detected in wild-type mice after the injection of ER stress inducers. Studies have shown that ER stress leads to apoptotic cell death through JNK-mediated Bim activation.^{26,50} Thus, apoptosis is the predominant mode of cell death in NAFLD. Both the intrinsic and extrinsic pathways play important roles.

Hepatic fibrosis and cirrhosis

Viral hepatitis, ALD, NAFLD, autoimmune hepatitis, and drug-induced liver injury can contribute to liver fibrosis.⁵¹ Apoptotic bodies that form during apoptosis can be engulfed by phagocytes in the liver, such as KCs.⁵² Studies have shown that apoptosis is not only a mode of death but is also associated with inflammatory responses. When the level of hepatocyte apoptosis exceeds the clearance capacity of phagocytes, apoptotic bodies accumulate and release their proinflammatory factors. The phagocytosis of apoptotic bodies by KCs enhances the expression of the death ligands Fas and TNF- α and promotes hepatocyte apoptosis. It also promotes the production of transforming growth factor β (TGF- β), which leads to liver fibrosis.⁵³ Studies have shown that the phagocytosis of apoptotic bodies by hepatic stellate cells (HSCs) directly stimulates fibrogenesis.⁵⁴ In addition, the destruction of the antiapoptotic factor Bcl-xL in hepatocytes leads to continuous apopto-

sis in hepatocytes, which increases the production of hepatic TGF- β and leads to liver fibrosis.⁵⁵ In conclusion, chronic liver diseases cause continuous apoptosis in hepatocytes, induce hepatocyte fibrosis, and gradually cause liver cirrhosis.

Hepatocellular carcinoma (HCC)

HCC is closely related to chronic inflammation and fibrosis, and is a common end-stage of chronic liver disease. Apoptosis is a prominent defense mechanism against hepatocarcinogenesis. An imbalance of proapoptotic and antiapoptotic members (e.g., activation of the antiapoptotic protein BCL-xL and downregulation of the proapoptotic protein Bax) has been observed in human HCC.⁵⁶ Most HCCs show insensitivity to TNF-related apoptosis-inducing ligands or Fas-mediated cell death.⁵⁷ As a strong inducer of apoptosis, sorafenib treatment of different HCC cell lines increased the levels of pro-apoptotic mRNA and proteins and decreased the levels of anti-apoptotic proteins.⁵⁸

Necrosis

Necrosis is generally considered to be an unprogrammed form of cell death. Necrosis is characterized by cell swelling and plasma membrane rupture.⁵⁹ Under oxidative stress or toxin stimulation, MPT occurs.⁶⁰ MPT leads to the opening of mitochondrial pores through interactions with the mitochondrial protein cyclophilin D (CypD).⁶¹ The subsequent disturbance in the ion gradient across the inner mitochondrial membrane and the decrease in mitochondrial membrane potential result in the loss of oxidative phosphoric acid and the rapid depletion of ATP. Ultimately, cellular homeostasis and ion pump disruption drive cell necrosis (Fig. 1).^{59,60}

NAFLD and NASH

Studies have shown that in response to a high-fat diet (HFD), CypD-knockout mice exhibited reduced triglyceride levels, steatosis, and mitochondrial stress compared with wild-type mice.⁶² Similarly, treatment of HFD-fed mice with the pan-cyclophilin inhibitor CRV431 significantly reduced hepatocyte steatosis, ballooning, and liver fibrosis compared with control mice.⁶³ Further studies have shown that CypD induces MPT by disrupting calcium homeostasis and mediates hepatocyte necrosis.⁶² However, the specific mechanism of necrosis in NAFLD needs further study.

Acetaminophen (APAP)-induced liver injury

APAP is a common cause of drug-induced acute liver failure. Studies have shown that JNK activation plays a role in APAP-induced acute liver failure.⁶⁴ In a mouse model, the inhibition of JNK protected against APAP-induced liver injury.⁶⁵ MPT leads to the opening of mitochondrial pores through interactions with the mitochondrial protein CypD.⁶¹ Inhibiting CypD prevents APAP-induced acute liver injury.⁶⁶ Therefore, necrosis is considered to be the main mode of APAP-induced liver injury. Cytochrome P450-mediated metabolism produces the toxic metabolite N-acetyl-p-benzoquinoneimine (NAPQI). Glutathione (GSH) is required for the detoxification of NAPQI, resulting in GSH depletion. GSH depletion increases oxidative stress and alters calcium homeostasis, leading to MPT. Mitochondrial membrane potential is lost, and ATP depletion ultimately leads to hepatocyte necrosis.⁶⁷

Necroptosis

Unlike necrosis, necroptosis is programmed necrosis mediat-

ed by death receptors.⁶⁸ Necroptosis is also morphologically characterized by cell swelling and plasma membrane rupture. Receptor-interacting protein kinases 1 and 3 (RIPK1, RIPK3) and mixed lineage kinase domain protein (MLKL) play important roles in necroptosis.⁶⁹ Caspase-8 is a hub that mediates cell death, leading to different cell death modes.⁷⁰ The binding of TNF and TNF-R1 provides a binding site for receptor-interacting protein 1 (RIP1). After RIP1 is deubiquitinated by cylindromatosis, caspase-8, RIP1, RIP3, and Fas-associated protein form complex IIa, which activates caspase-8 and mediates apoptosis. A necrosome (complex IIb) consisting of RIP1, RIP3 and MLKL induces necroptosis when the activity of caspase-8 is inhibited (Fig. 1).^{71,72}

NAFLD and NASH

Studies have shown that RIPK3 expression is increased in liver biopsy specimens from NASH patients.⁷³ Increased expression of RIPK3 and pMLKL was found in the livers of HFD-fed mice.⁷⁴ Paradoxically, RIPK3-knockout mice exhibited exacerbated hepatocyte steatosis and liver injury compared with wild-type mice that were fed HFD. A lack of RIP3 exacerbates liver injury in NASH, which may be related to a shift in hepatocytes from necrosis to apoptosis and an increase in the inflammatory response.⁷⁴ RIP3 is a key mediator of necroptosis. Although its overexpression or deletion may cause liver injury in NASH, the way it mediates cell death is different. In addition, in response to methionine–choline-deficient (MCD) conditions, intrahepatic triglyceride levels in caspase-8-knockout or RIP3-knockout mice were significantly increased. Studies have shown that RIP3 activates the JNK pathway in a caspase-8-dependent manner. This pathway promotes the development of NASH.⁷⁵ In addition, inhibition of RIPK1 resulted in downregulation of MLKL and reduced liver injury and liver fibrosis in a HFD-fed mouse model. The same study showed that MLKL knockout increases β -oxidation in fatty liver cells.⁷⁶ Therefore, RIPK1-RIPK3-MLKL-mediated NASH is related to necroptosis, but whether the mechanism involves crosstalk with apoptosis or other modes of cell death needs further study.

ALD

Necroptosis also plays an important role in the occurrence and progression of ALD. Increased expression of RIPK3 and pMLKL was reported in the liver biopsies of ALD patients.^{77,78} Compared with the control group, RIPK3-knockout mice exhibited reduced liver injury and steatosis in response to ethanol induction.⁷⁹ This finding suggests that RIPK3 mediates ethanol-induced liver injury. Furthermore, inhibiting RIPK1 reduced alcohol-induced neutrophil recruitment in mice.⁸⁰ Therefore, RIPK1 mediates inflammatory responses in ALD. Although pMLKL expression was increased in liver biopsies, MLKL knockdown did not alleviate ethanol-induced liver injury compared with that in wild-type mice. This finding suggests that MLKL is not important for ALD.⁷⁸ It is reported that RIP3 expression was reduced in CYP2E1-deficient mice after chronic ethanol feeding, along with a decrease in plasma aspartate aminotransferase (AST) expression. It suggests that CYP2E1 contributes to ethanol-induced RIP3 expression and liver injury. Furthermore, in RIP3-deficient mouse livers, p-JNK was reduced after chronic ethanol feeding.^{79,80} CYP2E1 mediates p-JNK via RIPK3, which promotes liver injury and steatosis in response to ethanol induction. Therefore, CYP2E1-RIPK3-JNK mediates necroptosis, leading to ALD.

Autophagy and autophagy-dependent cell death

Autophagy is considered to be a survival mechanism under

stressful conditions and is essential for maintaining cellular homeostasis. Misfolded or aggregated proteins, damaged organelles, and intracellular pathogens are sequestered within vesicles that become autophagosomes, which in turn function as lysosomes for degradation.^{81,82} Theoretically, autophagy mediates cytoprotective effects, and defects in autophagy accelerate cellular stress, leading to cell death.² However, several studies have shown that cell death does not completely cease when apoptosis is inhibited by caspase inhibitors or apoptosis-related gene knockdown and that autophagy can drive cell death (autophagy-dependent cell death) to compensate.^{83,84} Autophagy-dependent cell death is also regulated.⁸⁵ However, its occurrence is dependent on certain environments and needs to be further explored. The dual nature of autophagy means that we have to distinguish autophagy-dependent cell death from autophagy required for cell survival. At present, studies on autophagy in liver diseases such as viral hepatitis, ALD, NAFLD, and HCC have focused on the protective role of autophagy rather than autophagy-dependent cell death.⁸⁶ Autophagy deficiency is associated with a variety of liver diseases. This review focuses on the mechanisms by which autophagy influences other modes of cell death such as apoptosis and contributes to the development of liver diseases (Fig. 1).

ALD

In general, autophagy activation has a protective effect on ALD. However, the effect of alcohol on autophagy is time- and dose dependent. Acute or low-dose ethanol exposure increases autophagy,⁸⁷ and chronic or high-dose ethanol exposure decreases autophagy. Chronic and high-dose alcohol exposure inhibits autophagy,^{88,89} and inhibiting autophagy results in hepatocyte death.⁸⁸ Crosstalk exists between autophagy and other modes of cell death.⁹⁰ Alcohol causes apoptosis and the release of ROS, and autophagy is activated to remove cellular debris and lipid droplets, thus protecting hepatocytes.^{87,91} Apoptosis promotes cell death by inhibiting autophagy-related proteins.⁹² Studies have shown that inhibiting autophagy enhances cell death and that knocking down autophagy-associated protein 7 (ATG7) leads to liver injury.⁹³ In response to alcohol stimulation, inhibiting autophagy promotes liver injury through apoptosis.⁸⁷

NAFLD and NASH

Reduced levels of autophagy have been observed in patients with NAFLD and in mouse models.^{94,95} It has been shown that inhibiting autophagy in HFD-fed mice promotes apoptosis and increases lipid accumulation in hepatocytes.⁹⁶ Increased JNK signaling and impaired autophagy due to oxidative stress promote NAFLD.⁹⁷ In NAFLD, autophagy protects hepatocytes from death receptor-mediated liver injury. Macroautophagy inhibits apoptosis mediated by the death receptors TNF and Fas,^{98,99} and inhibiting hepatocyte autophagy promotes liver injury by activating caspase-8.¹⁰⁰ Autophagy is a target of NAFLD therapy and exerts hepatoprotective effects by enhancing mitochondrial autophagy.

HCC

Autophagy has two sides in the occurrence of HCC.¹⁰¹ On the one hand, autophagy plays an inhibitory role in the early stages of tumors by maintaining genome stability, removing damaged mitochondria, and inhibiting malignant transformation of cells.¹⁰² On the other hand, autophagy acts as a tumor promoter during malignancy. Once tumors are formed, autophagy promotes tumor proliferation.¹⁰³

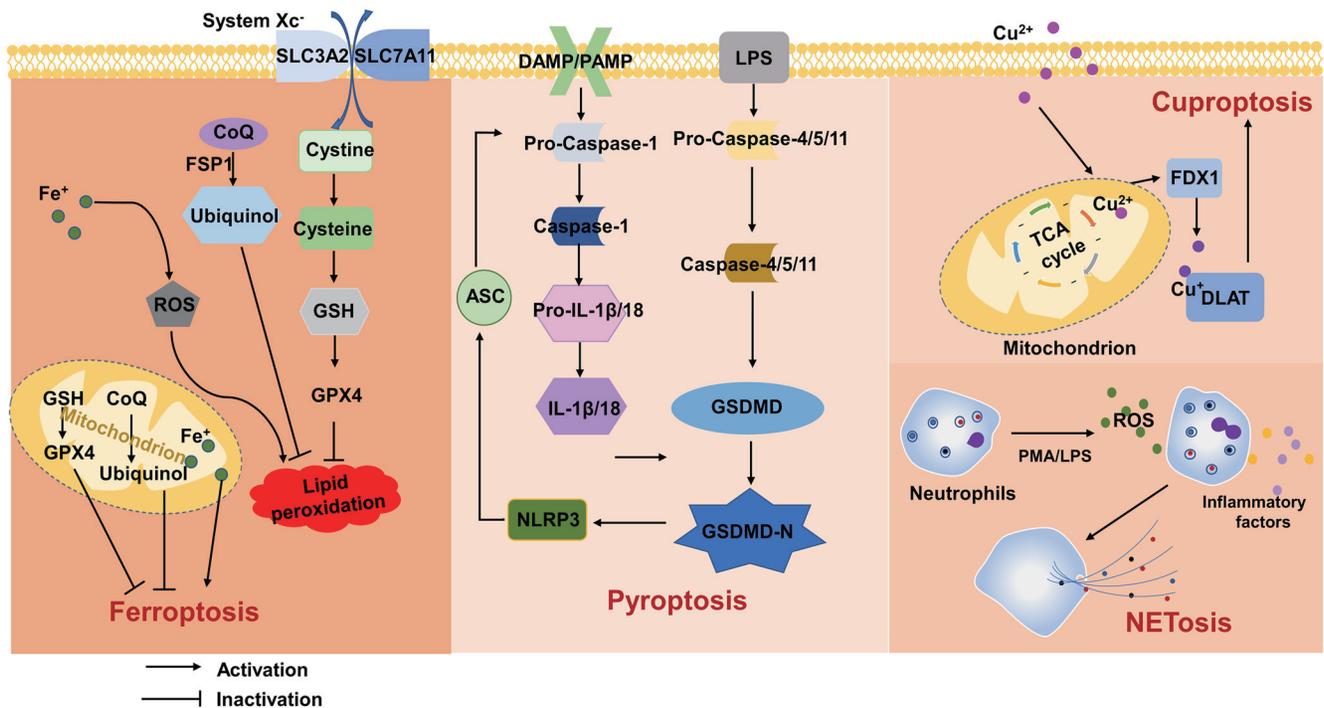


Fig. 2. Mechanisms of ferroptosis, pyroptosis, NETosis and cuproptosis. ASC, apoptosis-associated speck-like protein; CoQ, coenzyme Q; DAMP, damage-associated molecular modes; DLAT, dihydrolipoic acid s-acetyltransferase; FDX1, iron oxidation reduction protein 1; GPX4, glutathione peroxidase 4; GSDMD, gasdermin D; GSH, glutathione; IL, interleukin; LPS, lipopolysaccharide; PAMP, pathogen-associated molecular modes; PMA, phorbol 12-myristate 13-acetate; ROS, reactive oxygen species; SLC3A2, solute carrier family 3 member 2; SLC7A11, solute carrier family 7 member 11; TAC, tricarboxylic acid cycle.

Ferroptosis

Unlike other types of cell death, ferroptosis is an iron-dependent form of cell death accompanied by extensive lipid peroxidation.^{104,105} In addition to the role of iron metabolism in ferroptosis, a growing number of metabolic pathways have been implicated in ferroptosis. These pathways include the GSH/GSH peroxidase 4 (GPX4) axis, guanosine triphosphate cyclohydrolase 1 (GCH1)/tetrahydrobiopterin (BH4)/dihydrofolate reductase (DHFR) axis, dihydroorotate dehydrogenase (DHODH)-dihydroubiquione (CoQH2) axis¹⁰⁶ and F/coenzyme Q (CoQ) axis.^{107,108} Ferroptosis is initiated by intracellular iron accumulation, lipid peroxidation, and the oxidation of phospholipids containing polyunsaturated fatty acids (PUFA-PLs).¹⁰⁹ According to recent findings, the main mechanism of ferroptosis is the ROS-dependent regulation of cell death. The presence of iron in the cytoplasm leads to the formation of ROS through the Fenton reaction. Through this reaction, H₂O₂ is converted to hydroxyl radicals. This process leads to lipid peroxidation, which plays an important role in ferroptosis.¹⁰⁹ The increase in ROS can be regulated by the quenching effect of GPX4.¹¹⁰ In fact, GPX4 prevents iron-independent ROS accumulation, and GSH is a cofactor to produce lipidol (R-OH) from lipid hydroperoxides (R-OOH). This metabolic process has a cytoprotective effect against Fe²⁺-dependent formation and ROS accumulation.^{109,111} Furthermore, System Xc⁻ supports the efficiency of the antioxidant activity of GPX by inhibiting a series of events that trigger a reduction of GSH levels, lipid peroxidation, and consequent ferroptosis.¹⁰⁹ In addition, cytochrome P450 oxidoreductase (POR)-mediated or arachidonic acid lipoygenase (ALOX)-mediated enzymatic reactions have also been shown to promote lipid peroxidation. Iron not only controls the direct peroxidation of PUFA-PLs through the nonenzymatic Fenton

reaction, but also is as an essential cofactor for enzymes involved in lipid peroxidation, such as ALOX and POR, to control ferroptosis.¹¹² A growing number of studies have shown that ferroptosis has an important role in the pathogenesis of liver diseases. Therefore, targeting ferroptosis may provide a promising new therapeutic strategy (Fig. 2).

ALD

Ferroptosis is associated with the development of ALD, and approximately half of patients with ALD exhibit hepatic iron overload.¹¹³ Alcohol metabolism mediates the accumulation of large amounts of acetaldehyde, which causes lipid peroxidation in hepatocytes through mitochondrial GSH depletion and excessive ROS production.⁵ GPX4 protein expression was observed in the hepatocytes of alcohol-treated mice. *In vitro* studies have shown that ferrostatin-1 (Fer-1) partially alleviates the cytotoxicity of alcohol, and Fer-1 almost completely reverses alcoholic liver injury.¹¹⁴ Further studies have shown that Fer-1 significantly attenuates ALD lipid peroxidation and reduces liver injury. Other studies have shown that activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway inhibits ferroptosis and exerts a protective effect against ALD.¹¹⁵ Ferroptosis is a promising therapeutic target for ALD treatment.

NAFLD and NASH

Lipid ROS cause hepatic steatosis through the formation of lipid droplets. A study showed that iron accumulation enhanced lipid ROS levels in MCD-fed mice. Treatment of MCD-fed mice with Fer-1 or liproxstatin-1 significantly improved hepatic steatosis and liver injury.¹¹⁶ GPX4 is most highly expressed in the liver, and GPX4 protects the liver from lipid peroxidation.¹¹⁷ It has been shown that inhibiting GPX4 in

MCD-fed mice promoted NASH through ferroptosis-related effects. Conversely, GPX4 activation inhibited ferroptosis and improved the severity of NASH.¹¹⁸ Thymidine $\beta 4$ delays NAFLD development by upregulating GPX4, which inhibits ferroptosis. In addition, Nrf2 inhibits ferroptosis by promoting antioxidant responses and eliminating lipid ROS accumulation in the liver.¹¹⁹ The activation of Nrf2 in HFD-fed mice delays the occurrence of NAFLD.¹²⁰

APAP-induced liver injury

A study showed that ferroptosis occurred in APAP-induced liver injury, and Fer-1 provided modest protection against APAP-induced primary hepatocyte death in mice.¹²¹ Ferroptosis occurred in a mouse model of APAP-induced acute liver failure with elevated lipid peroxide derived from n-6 PUFAs. In addition, the ferroptosis inhibitor UAMC-3203 and the VDAC1 oligomerization inhibitor VBIT-12 reduced ferroptosis in APAP-induced liver injury animal models by preserving mitochondrial function.¹²² These studies suggest that ferroptosis may serve as a therapeutic target for APAP-induced liver injury.

Hepatic fibrosis and cirrhosis

Ferroptosis is a double-edged sword in liver fibrosis. When ferroptosis targets activated HSCs, ferroptosis suppresses liver fibrosis.¹²³ HBx promotes the development of liver fibrosis by inhibiting ferroptosis. Chrysophanol promotes ferroptosis by downregulating GPX4 and SLC7A11 (a key part of System Xc⁻) levels. Thus, chrysophanol attenuates the activation of HSCs and improves liver fibrosis.¹²⁴ Erastin/sorafenib alleviates liver fibrosis in mice by inducing HSC ferroptosis.¹²⁵ Magnesium isoglycyrrhizinate promotes HSC ferroptosis by regulating heme oxygenase 1, which significantly reduces liver fibrosis and attenuates liver injury.¹²⁶ P53 plays a crucial role in the induction of ferroptosis.¹²⁷ Artemether, a derivative of artemisinin, reduced liver fibrosis in a mouse model of liver fibrosis. Knockdown of P53 exacerbated liver fibrosis by blocking artemether-induced HSC ferroptosis, which in turn exacerbated liver fibrosis.¹²⁸ Hepatocyte ferroptosis leads to liver fibrosis. Studies have shown that increased hepatocyte ferroptosis promotes the occurrence of liver fibrosis. Liver fibrosis was reversed when Fer-1 inhibited hepatocyte ferroptosis.¹²⁹ It is important to explore the role of ferroptosis in the development of hepatic fibrosis.

HCC

The role of ferroptosis in HCC is complex. On the one hand, ferroptosis associated with iron overload is involved in the progression of different types of liver diseases, which promotes the development of HCC.¹³⁰ On the other hand, ferroptosis inhibits HCC cell proliferation. PUFAs reduce HCC growth by inhibiting β -catenin and cyclooxygenase 2 (COX-2).¹³¹ Studies have shown that ferroptosis regulators GPX4 is overexpressed in HCC.¹³² Therefore, induction of ferroptosis may be a promising therapeutic strategy for HCC.

Pyroptosis

Pyroptosis is a type of programmed cell death that is usually induced in cells in the innate immune system.¹³³ Pyroptosis is activated by the presence of pathogen-associated molecular patterns (PAMPs) or cell-derived damage-associated molecular patterns (DAMPs). There are two main molecular mechanisms of pyroptosis, the classic caspase-1-dependent pathway and the noncaspase-1-dependent pathway.^{134,135} In caspase-1-dependent pyroptosis, the activation of inflam-

masome receptors such as NLRP1, NLRP3, NLRC4, AIM2 by PAMPs or DAMPs triggers the recruitment of the adapter proteins ASC and caspase-1 to form a macromolecular complex in which caspase-1 is activated. Activated caspase-1 directly cleaves gasdermin D (GSDMD), and the precursor cytokines pro-interleukin (IL)1 β and pro-IL18, leading to pyroptosis and the maturation of IL1 β and IL18. The cleaved gasdermin D N-terminal fragment (GSDMD-NT) forms pores in the cell membrane and mediates the release of cytoplasmic contents.^{135,136} During noncaspase-1-dependent cell pyroptosis, caspase-4 or caspase-5 in human cells or caspase-11 in murine cells recognize lipopolysaccharide (LPS) in the cytosol.¹³⁷ These caspases directly cleave GSDMD and induce pyroptosis.¹³⁵⁻¹³⁷ Recent studies have shown that pyroptosis plays a role in various liver diseases, such as viral hepatitis, ALD,²⁶ NAFLD,¹³⁸ liver fibrosis, and liver failure.¹³⁹ This review focuses on the mechanism of pyroptosis in liver diseases (Fig. 2).

Viral hepatitis

Pyroptosis is associated with viral hepatitis. Previous studies have suggested that in patients with chronic hepatitis B, HBcAg induces IL18 secretion by inducing caspase-1.¹⁴⁰ HBcAg inhibits LPS-induced activation of the NLRP3 inflammasome and IL1 β production in two ways: direct inhibition by inhibiting NF- κ B phosphorylation and through inhibition of ROS production, which inhibits caspase-1 activation and IL1 β maturation.¹⁴¹ Furthermore, in patients with chronic HCV infection, HCV RNA directly induces NLRP3 inflammasome activation in infected hepatocytes.¹⁴² In KCs, IL1 β production via the NLRP3 inflammasome promotes HCV virus amplification,¹⁴³ and the activation of natural killer cells inhibits HCV replication.¹⁴⁴ Although the mechanisms of pyroptosis in viral hepatitis need to be further explored, inhibiting NLRP3 or IL1 β activity is an effective strategy.

ALD

In ALD, IL1 β promotes hepatic steatosis and liver injury. The upregulation of caspase-1 and inflammasome activation in KCs increases IL1 β . However, recombinant IL1Ra blocks IL1 signaling *in vivo*.¹⁴⁵ In an alcohol-induced mouse model, alcohol promoted TXNIP overexpression and NLRP3 activation, which induced hepatocyte pyroptosis.¹⁴⁶ In addition, a non-caspase-1-dependent pathway induced pyroptosis in ALD. The activation of caspase-11 in alcoholic hepatitis mice and caspase-4 in alcoholic hepatitis patients promotes pyroptosis mediated by GSDMD.¹⁴⁷

NAFLD and NASH

Compared with other liver diseases, pyroptosis has been most studied in NAFLD/NASH. GSDMD-N expression is significantly upregulated in the liver biopsies of NASH patients.¹⁴⁸ Similarly, GSDMD expression was increased in an MCD-fed mouse model. GSDMD-knockout mice have less steatosis than wild-type mice.¹⁴⁸ Further studies have shown that pyroptosis driven by GSDMD-N increased the severity of steatohepatitis in mouse models.¹⁴⁸ NLRP3 knockdown attenuated liver injury in a mouse model of NAFLD.¹⁴⁹ In contrast, NLRP3 activation promoted the development of NASH or NAFLD through hepatocyte pyroptosis.¹⁵⁰ The selective NLRP3 inhibitor MCC950 was used in a steatohepatitis model constructed by HFD and MCD feeding and resulted in reduced NF κ B activation and reduced liver inflammation.¹⁵¹ MCC950 inhibited inflammation and liver fibrosis in NAFLD mouse models by reducing the expression of caspase-1, IL6, and IL1 β .¹⁵² Inhibiting NLRP3 and GSDMD significantly reduces

inflammation and fibrosis and ultimately alleviates NAFLD by regulating the pyroptosis signaling pathway.¹⁵³

Liver fibrosis

Pyroptosis is also associated with liver fibrosis.¹⁴⁸ Activated HSCs promote collagen deposition through pyroptosis.¹⁵⁴ IL1 β promotes the proliferation of HSCs in rats, which in turn enhances the expression of collagen and TGF β and induces liver fibrosis.¹⁵⁵ In addition, eosinophils promote liver fibrosis by inducing the secretion of IL1 β and IL18, which leads to pyroptosis. However, a caspase-1 inhibitor can reverse liver fibrosis.¹⁵⁶ Studies have shown that GSDMD promotes the progression of liver fibrosis in MCD-fed mice.¹⁴⁸ Excessive activation of NLRP3 exacerbates liver inflammation and liver fibrosis. NLRP3 knockdown protects the liver from drug-induced liver fibrosis.¹⁵⁷

NETosis

It has been shown that activated neutrophils capture and kill pathogens by releasing neutrophil extracellular traps (NETs), which consist of depolymerized chromatin and intracellular granule proteins, to the outside of the cell. This novel form of inflammatory cell death, which is accompanied by neutrophil death during the formation of NETs, is known as NETosis.¹⁵⁸ NETosis is a specific form of programmed cell death in which NADPH oxidase is activated when the concentration of Ca²⁺ in the cytoplasm increases, leading to the massive production of ROS and ultimately inducing NETosis.¹⁵⁹ ROS trigger the release of NE into the cytoplasm, where myeloperoxidase (MPO) activates its protein hydrolytic activity, ultimately leading to chromatin decondensation and disruption of the nuclear membrane.¹⁶⁰ Although NETs do not cause hepatocyte death, they are involved in the pathogenesis of various chronic liver diseases, such as autoimmune liver disease, NASH, and HCC (Fig. 2).¹⁶¹

Viral hepatitis

Studies have shown that HBV C protein (HBc) and HBV E protein (HBe) caused lower cfDNA levels in patients with chronic hepatitis B infection. It shows that HBc and HBe proteins inhibit NETs release. Further studies revealed that HBc and HBe proteins reduce neutrophil autophagic activity and inhibit ROS production by enhancing mTOR activity. In turn, the inhibition of NETs release ultimately delays the elimination of HBV. It makes HBV to evade the immune system and eventually leads to the establishment of persistent infection in this way.¹⁶²

NAFLD and NASH

Studies have shown that serum neutrophil levels are significantly elevated in patients with NASH. NETs were detected in MCD/HF diet-fed mouse models. The removal of NETs significantly reduced liver injury in MCD/HF diet-fed mouse models.¹⁶³ *In vivo* and *in vitro* experiments revealed that FFAs stimulated the formation of NETs in a mouse model of NASH. Inhibiting NETs reduces the production of inflammatory cytokines and inflammatory cell infiltration, which in turn slows the progression of NASH.¹⁶⁴ Other studies have shown a positive correlation between serum levels of NETs markers (MPO-DNA complexes) and sphingosine kinase 1 (S1P rate-limiting enzyme) levels in damaged livers in a mouse model of NASH. *In vitro*, S1P receptor 2 (S1PR2) is involved in regulating the transition of neutrophils from apoptosis to NETosis. Knockdown of S1PR2 in MCD/HF diet-fed mice significantly inhibited NETs formation in damaged liver tissue, which in turn reduced liver inflammation and fibrosis.¹⁶³

Cuproptosis

Copper is an important catalytic cofactor involved in oxygen metabolism and energy conversion.¹⁶⁵ Changes in the concentration of copper cause significant damage to cells.¹⁶⁶ Cuproptosis is a novel type of cell death, and studies have shown that intracellular copper accumulation leads to the aggregation of mitochondrial lipoproteins and protein destabilization.¹⁶⁷ In addition, copper ions regulate cell death by targeting lipidylated mitochondrial tricarboxylic acid cycle (TAC) proteins.¹⁶⁸ Further studies revealed that iron oxidation reduction protein 1 (FDX1) encodes a reductase that reduces Cu²⁺ to Cu⁺. In contrast, dihydrolipoic acid S-acetyltransferase (DLAT), a protein target of lipid acylation, is involved in the TAC cycle. FDX1 promotes DLAT lipid acylation, Cu⁺ enhancement of lipid acylated protein aggregation and the reduction of iron-sulfur cluster proteins, thereby promoting cell death.^{168,169} The liver is an important organ for regulating energy metabolism in the body.¹⁷⁰ Impaired energy metabolism is associated with the exacerbation of acute liver failure. Studies have shown that an imbalance in copper metabolism affects normal liver metabolism.^{171,172} Thus, cuproptosis may be a potential cell death mode in liver diseases, which can be used as a new research direction and therapeutic target (Fig. 2).

NAFLD and NASH

Copper and lipid metabolism have a complex relationship, and increased cellular copper may downregulate lipid and lipogenesis genes.¹⁷³ Study carried out transcriptome analysis on the liver tissues of HFD-induced NAFLD mice and normal mice. Gene set variation analysis results showed that the cuproptosis was aberrantly activated in NAFLD. Among them, DLD and PDHB may be potential hub genes for the diagnosis and treatment of NAFLD.¹⁷⁴

Targeting cell death in liver diseases' application

Currently, some preclinical or clinical studies has provided the basis to transfer cell death-based therapies for liver diseases to the clinic. In this section, we summarize the involvement of cell death in liver diseases.

Targeting apoptosis in liver diseases

Clinical trials have shown that inhibition of hepatocyte apoptosis delays the progression of liver disease. Pan-caspase inhibitors PF-03491390 improved liver function in patients with chronic HCV infection.¹⁷⁵ The selective caspase inhibitor GS-9450 significantly reduced alanine transaminase (ALT) levels in NASH patients by reducing hepatocyte apoptosis.¹⁷⁶ Besides, as small noncoding RNAs, miRNAs are highly stable and easily detected in the circulation compared with traditional biomarkers. Studies have shown that many miRNAs are specifically expressed in hepatocytes. MiR-34a/SIRT1/p53 leads to NASH by promoting apoptosis of hepatocytes. It is reported that miR-296-5p levels are inversely correlated with PUMA (pro-apoptotic protein) mRNA levels in human liver samples. In NASH patients, hepatic miR-296 expression was decreased, PUMA was increased, and hepatic steatosis was observed.¹⁷⁷ MiR-34a and miR-296 play a role in NAFLD by regulating hepatocyte apoptosis. Sorafenib is approved for the first-line treatment of advanced liver cancer, and its main antitumor activity is related to caspase activation, Bax and Bak activation.⁵⁷

Targeting necrosis in liver diseases

Clinical studies have shown that glycyrrhizin, as an HMGB1 inhibitor, reduces serum ALT levels in patients with HCV in-

fection and improves hepatic necroinflammation and fibrosis. Glycyrrhizin can be used as an adjuvant drug in viral hepatitis.¹⁷⁸

Targeting ferroptosis in liver diseases

Targeting ferroptosis therapy plays important role in non-viral liver diseases.¹⁷⁹ Preclinical studies have found that Fer-1 improves ALD and NAFLD by inhibiting ferroptosis in hepatocytes.^{108,114} As a ferroptosis inducer, erastin improves liver fibrosis by promoting ferroptosis by inhibiting the system Xc⁻.¹²⁵ In addition to apoptosis inducers, sorafenib can also induce a new type of programmed cell death, ferroptosis. However, it shows that inhibition of ferroptosis mediates sorafenib resistance in HCC. Studies have shown that solute carrier family 27 member 5 (SLC27A5) is downregulated in sorafenib-resistant HCC. Loss of SLC27A5 enhances GSH reductase (GSR) expression in a Nrf2-dependent manner, thereby maintaining GSH homeostasis and it promotes HCC by inhibiting ferroptosis.¹⁸⁰ It provides a potential therapeutic strategy for overcoming sorafenib resistance.

Targeting pyroptosis in liver diseases

Inhibition of NLRP3 or its downstream signaling pathways (e.g., caspases and IL1) is the main strategy for the treatment of pyroptosis. Animal experiments have shown that taurine or the ER stress inhibitor Tudaka relieves nonalcoholic fatty liver/NASH by inhibiting the activity of NLRP3 inflammasome.^{150,181} A pan-caspase inhibitor, IDN 6556, reduced liver injury and fibrosis in a mouse model of NASH. Clinical trials have shown that pan-caspase inhibitors IDN 6556 or PF-034 reduce serum AST and ALT levels in patients with chronic hepatitis C.^{175,182}

Future directions and therapeutic implications

Cell death is central to the study of the development and progression of liver diseases. In recent years, cell death in liver diseases has been intensively studied and new cell death modes that enhance our understanding of the pathophysiology of liver diseases have been discovered. In this review, it was found that different cell death modes occur simultaneously in certain liver diseases. Therefore, the various cell death modes are not independent or compartmentalized, and different cell death modes crosstalk with each other. The activation or inhibition of one cell death mode may activate other cell death modes. Future studies should focus on exploring the regulatory relationship between various cell death response pathways in the liver. Moreover, the application of cell death in liver diseases is mostly in the preclinical stage. We should aim to translate cell death into clinical applications and predict the onset and regression of liver diseases using hepatocyte death-related biomarkers as early as possible. That will provide a basis for the diagnosis, monitoring and treatment of liver diseases in the future and could help to block liver diseases in the initial stage or delay the progression of liver disease and improve the cure rate.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Conceptualization of the study (YW, ZG), performance of the methodology and investigation, writing of the original draft of the manuscript (YW), visualization of the data and figure generation (JG), writing, review and editing of the manuscript (CS, YZ), and funding acquisition and supervision (ZG). All authors read and approved the final manuscript.

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