

Mechanism of Hepatocyte Apoptosis

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ABSTRACT: Hepatocyte apoptosis plays important roles in both the removal of external microorganisms and the occurrence and development of liver diseases. Different conditions, such as virus infection, fatty liver disease, hepatic ischemia reperfusion, and drug-induced liver injury, are accompanied by hepatocyte apoptosis. This review summarizes recent research on the mechanism of hepatocyte apoptosis involving the classical extrinsic and intrinsic apoptotic pathways, endoplasmic reticulum stress, and oxidative stress-induced apoptosis. We emphasized the major causes of apoptosis according to the characteristics of different liver diseases. Several concerns regarding future research and clinical application are also raised.

KEYWORDS: hepatocyte apoptosis, viral hepatitis, fatty liver disease, hepatic ischemia reperfusion injury, drug-induced liver injury, signaling pathways

CITATION: Cao et al. Mechanism of Hepatocyte Apoptosis. *Journal of Cell Death* 2016;9:19–29 doi:10.4137/JCD.S39824.

TYPE: Review

RECEIVED: April 1, 2016. **RESUBMITTED:** August 14, 2016. **ACCEPTED FOR PUBLICATION:** August 18, 2016.

ACADEMIC EDITOR: Garry M. Walsh, Editor in Chief

PEER REVIEW: Four peer reviewers contributed to the peer review report. Reviewers' reports totaled 775 words, excluding any confidential comments to the academic editor.

FUNDING: This work was supported by Research Center on Aging and Medicine, Fudan University (13dz2260700 to MJW) and Shanghai Pujiang Program (15PJ1400700 to MJW). The authors confirm that the funder had no influence over the study design, content of the article, or selection of this journal.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

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Paper subject to independent expert single-blind peer review. All editorial decisions made by independent academic editor. Upon submission manuscript was subject to anti-plagiarism scanning. Prior to publication all authors have given signed confirmation of agreement to article publication and compliance with all applicable ethical and legal requirements, including the accuracy of author and contributor information, disclosure of competing interests and funding sources, compliance with ethical requirements relating to human and animal study participants, and compliance with any copyright requirements of third parties. This journal is a member of the Committee on Publication Ethics (COPE).

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Introduction

As the basic unit of life, cells constitute the human body. Their proliferation and death must be tightly regulated to maintain normal organ function. Hepatocytes, the major liver cell type, have a strong ability to regenerate when they face damaging factors, such as ischemia, virus infection, and alcohol. On the other hand, several cell death modalities are noted in hepatocytes, including apoptosis, necroptosis, necrosis, and autophagic-dependent cell death. Cell apoptosis, sometimes called programmed cell death, is a common physiological process that is controlled by multiple genes and involves numerous biological events. Apoptosis is a cellular self-destruction method to remove old and damaged cells to protect cells from external disturbances and maintain homeostasis. Necroptosis, or programmed necrosis, is morphologically characterized by necrotic cell death and involves autophagy.¹ Necrosis is generally uncontrolled and occurs as a passive tissue damage process characterized by cell membrane damage, cell swelling, and cellular disruption leading to inflammation.² Autophagy is an intracellular catabolic process that delivers cytoplasmic components to lysosomes for degradation. Autophagic activity may regulate hepatocyte apoptosis via the mitochondrial pathway.³

Hepatocyte apoptosis plays an important protective role in the removal of external microorganisms by self-destruction. On the other hand, apoptosis also contributes to both chronic and acute liver diseases, such as viral hepatitis, alcoholic and nonalcoholic liver disease, cholestatic disorders, and ischemia

reperfusion injury. Hepatocyte apoptosis plays such an important role in liver injury that understanding the molecular mechanism and regulating factors of how hepatocyte apoptosis proceeds is crucial for the treatment of liver diseases.⁴ Compared with other types of cell death, apoptosis is an energy-requiring process that can be altered. Therefore, apoptosis monitors the scope and extent of cell death, thus protecting the liver from both infection and hepatitis. In acute liver failure patients, caspase activation and apoptosis involve in spontaneous recovery.⁵ Hepatocyte apoptosis also correlates with liver disease severity and participates in the progress of hepatic fibrosis.⁶

This review aims to provide an up-to-date summary of the relationship between hepatocyte apoptosis and different liver diseases and their underlying mechanisms. Readers should keep in mind that liver diseases often involve different modes of cell death and that a change of cell death pathways due to genetic or nongenetic alterations may influence disease progression. For example, hepatocyte necroptosis contributes to high-fat diet-induced liver injury together with the apoptosis pathway. Absence of a key necroptotic mediator, receptor-interacting protein 3 (RIP3), causes aggravated liver inflammation, hepatocyte apoptosis, and fibrosis.⁷

Hepatocyte Apoptotic Pathways

Hepatocyte apoptosis involves two fundamental pathways: the extrinsic pathway, which transmits death signals by the death



receptor (DR), and the intrinsic pathway, which is initiated by intracellular stimuli.

The extrinsic apoptotic pathway is activated by the binding of the death ligand to DRs on the plasma membrane. Death ligands belong to tumor necrosis factor (TNF) superfamily, including FasL, TNF- α , and TNF-related apoptosis-inducing ligand (TRAIL). Accordingly, DR includes Fas, TNF-receptor 1, and TRAIL-R. The binding of the death ligand to DR induces trimerization and a conformational change of DR. This change recruits cytoplasmic adaptor proteins (such as Fas-associated protein with death domain [FADD]), and the latter recruits apoptosis signaling molecules (such as caspase-8). The DR, adaptor protein, and associated apoptosis signaling molecule form the death-inducing signaling complex (DISC), thus leading to the activation of the effector caspase cascade (which typically involves caspase-3, -6, and -7).⁸⁻¹⁰ Cellular FADD-like interleukin-1 β (IL-1 β) converting enzyme inhibitory protein (c-FLIP) can inhibit DISC and prevent apoptosis.¹¹

The intrinsic apoptosis pathway is characterized by the release of cytochrome c or other caspase-activating factors from the mitochondria intermembrane space into the cytoplasm.¹² This release is mediated by the mitochondrial permeability transition pore. Then, in the cytoplasm, a complex named the apoptosome is formed and activates caspase-9, which further activates the effector caspase cascade.¹³ The mitochondria-mediated intrinsic apoptosis pathway is regulated by Bcl-2 family proteins, including proapoptotic (Bid, Bax, Bak) and antiapoptotic proteins (Bcl-2, Bcl-xL).¹⁴ Bcl-2, Bcl-xL, and other antiapoptotic proteins bind to the outer membrane of the mitochondria, decreasing their permeability and inhibiting the release of cytochrome c into the cytoplasm and the gathering of Bax and Bak. TNF- α binding to its receptor results in the cleavage of Bid into a truncated form and causes Bax oligomerization and insertion into mitochondria, thus initiating apoptosis.¹⁵

In hepatocytes, the apoptotic signals from DR are typically not powerful enough to initiate the effector caspase cascade, so the mitochondria-mediated pathway is generally used to amplify it.^{8,10} This amplification is extremely important for TNF- α -mediated hepatocyte apoptosis.¹⁶ When intrinsic apoptosis pathways are blocked by a caspase-9 inhibitor, TNF- α -induced apoptosis is abolished.¹⁶ In fact, unlike the Fas ligand, TNF- α alone fails to induce liver injury in normal hepatocytes.¹⁷ The concurrent activation of the antiapoptotic factor nuclear factor (NF)-kappa B is probably the underlying mechanism.¹⁸ Rapid activation of NF-kappa B induces c-FLIP expression and weakens caspase-8 activation.¹⁹

Mitochondrial dysfunction can initiate hepatocyte apoptosis, and other intracellular organelles can also trigger apoptosis through the mitochondrial-dependent mechanism.¹⁴ Both TNF- α and TRAIL signaling involve lysosomal protease release into the cytosol and lead to mitochondrial dysfunction.^{20,21} Alterations to calcium homeostasis, protein

overload, or misfolding will induce endoplasmic reticulum (ER) stress. Prolonged ER stress activates Bax and trigger apoptosis.²²⁻²⁴ ER stress is actually the third pathway of apoptosis in addition to the extrinsic and intrinsic pathways.²⁴ Oxidative stress caused by overproduction of reactive oxygen species (ROS) is another apoptosis inducer. Mitochondrial dysfunction seems to precede ROS accumulation.²⁵ Excessive ROS can further cause oxidative damage to mitochondrial DNA (mtDNA), proteins, and phospholipids and induce hepatocyte apoptosis.

Although the caspase cascade plays an essential role in hepatocyte apoptosis, the wide-ranging caspase inhibitor Z-VAD.fmk fails to prevent apoptosis in some experimental settings. Apoptosis induction without caspase cascade activation or when caspases are inhibited is called caspase-independent apoptosis. One example of caspase-independent apoptosis is ROS-induced mitochondrial damage, as mentioned above. A second example is apoptosis induced by the release of apoptosis-inducing factor (AIF) from mitochondria into the cytosol.²⁶ The translocation of AIF from the cytosol to the nucleus directly triggers chromatin condensation and DNA cleavage.²⁷ TNF- α may also cause caspase-independent apoptosis when caspase is inhibited.

In addition to apoptosis, other cell death modalities also participate in hepatocyte death and liver injury through crosstalk and overlap, yielding highly heterogeneous death processes.²⁸ DR activation may also activate necroptosis under certain conditions, such as a specific virus infection, which leads to the interaction between RIP1 and RIP3.²⁹

Activation of similar apoptotic pathways can be observed in different types and phases of liver diseases. On the other hand, each disease exhibits specific features of hepatocyte apoptosis due to the different initiating factors and disease states. In the following sections, the mechanism of hepatocyte apoptosis is discussed based on the types of liver diseases.

Hepatocyte Apoptosis and Viral Hepatitis

Viral hepatitis, a main cause of hepatic cirrhosis, is an infectious disease with high incidence. There are five types of viral hepatitis according to their pathogens: hepatitis A, B, C, D, and E. Virus-induced hepatocyte apoptosis can be caused by the immune response through the release of perforin/granzyme and the expression of specific viral protein products and cell membrane receptors related to apoptosis.³⁰⁻³⁴ Among all types of viral hepatitis, hepatitis B virus (HBV) and hepatitis C virus (HCV) are the most common and serious.

Perforin/granzyme-mediated apoptosis in viral hepatitis. Cytotoxic T lymphocytes (CTL) play crucial roles in the clearance of viral infection by perforin/granzyme B- and Fas/Fas ligand (FasL)-based mechanisms. Liver tissues with more severe inflammation typically exhibit higher Fas expression in the cytoplasm of hepatocytes. When virus-specific T-cells recognize the viral antigen and become activated, they express FasL to convey the apoptotic death signal to Fas-positive hepatocytes.³⁴ Perforin expression by T-cells during

the late acute phase is associated with virus clearance in chimpanzees.³⁵ In the livers of chronic hepatitis B patients, the messenger RNA (mRNA) expression rates of FasL, perforin, and granzyme B were 79.2%, 62.5%, and 33.3%, respectively. The expression correlated with alanine aminotransferase levels, histologic activity index, and apoptosis.³⁶

Inhibition of granzyme B expression might contribute to sustained virus infection. Stimulating hepatocytes with interferon (IFN)- α induces resistance to CTL killing in vitro by upregulating the expression of the granzyme B inhibitor, proteinase inhibitor 9 (PI-9).³⁷ PI-9 expression has also been observed in liver tissue biopsies from patients with chronic HCV infection.³⁷

HBV and apoptosis. HBV infection can result in various outcomes depending on replication of the virus and host immune response. Acute hepatitis B often causes massive hepatocyte damage followed by the clearance of the virus, regaining health in most cases. During acute hepatitis B, DR on the hepatocyte membrane is activated, and significant apoptosis is observed.³⁸ On the contrary, chronic hepatitis B is more complicated. A variety of stages exist in these patients, including the inactive carrier state, chronic hepatitis, cirrhosis, and hepatic decompensation. The progress of chronic HBV infection typically links with different phases of the immune response: first, immune tolerance, followed by immune clearance and low/nonreplication, and finally, immune escape.³⁹ Apoptotic cell death contributes to virus clearance, whereas dysregulated apoptosis facilitates immune escape.

HBV influences the host response by its unique protein products. The HBV genome contains four open-reading frames: preS/S, polymerase, preCore/core, and X. Among them, the HBV X (HBx) gene is the smallest but most important. The HBx protein is a double-edged sword in regulating apoptosis.^{40,41} The overall effect of HBx is to establish and maintain the chronic infection state. Its pro- or antiapoptotic action depends on several factors, such as the different phases of infection, amount of HBx expression, and cellular microenvironment. Activations of survival and proliferation signaling pathways account for its antiapoptotic property, while a change in the mitochondrial membrane potential is important for its apoptotic property. At the early stage of HBV infection, HBx activates AKT and inhibits apoptosis. The balance between virus replication and cell survival may cause the failure to remove infected cells and benefit persistent HBV infection.^{42,43} At the late stages of HBV infection, HBx becomes more proapoptotic and may accelerate HBV-induced liver damage. In addition, Rawat et al³⁰ indicated that HBx works differently in diverse hepatic cells.

The proapoptotic effect of HBx involves the activation of both the extrinsic and the intrinsic pathways. The DR-mediated extrinsic apoptosis pathway is upregulated by HBx by facilitating the activation of caspase-8 by c-FLIP inhibition.¹¹ HBx also dose-dependently increases TRAIL mRNA transcription and protein expression in hepatocytes.⁴⁴

HBx causes a loss of the mitochondrial membrane potential and increases the release of cytochrome c to the cytoplasm from mitochondria.⁴⁵ HBx sensitizes the apoptotic response to oxidative stress and activates caspase-3.^{46,47} Interaction with heat shock protein 60 may also contribute to its proapoptotic effect.⁴⁸

Many studies have reported the antiapoptotic role of HBx. HBx inhibits apoptosis mediated by the Fas-FasL system.⁴¹ This effect may be related to the inhibition of caspase-8 and caspase-3 activation, as well as cytochrome c release.⁴¹ Upregulation of the SAPK/JNK pathway by HBx exerts a survival mechanism in hepatocytes undergoing Fas-mediated apoptosis.⁴¹ Pan et al⁴⁹ suggested that the inhibition of HBx in Fas-mediated apoptosis is related to the activation and translocation of NF-kappa B into the nucleus. This translocation triggers target gene transcription and thus inhibits hepatocyte apoptosis. Serum deprivation, protein kinase C inhibition, or topoisomerase inhibition-induced apoptosis is mitigated by phosphatidylinositol 3-kinase pathway activation in HBx-transformed Chang liver cells.⁵⁰ Increased telomerase activity also plays a role.⁵¹ HBx is antiapoptotic in normal hepatocytes by NF-kappa B activation⁵² and hepatic progenitor cells by Wnt/ β -catenin pathway activation.⁵³

As mentioned above, the seemingly contradictory effect of HBx on apoptosis is the comprehensive outcome of several factors, such as the different phases of HBV infection, NF-kappa B status,⁵² and/or the amount of HBx.⁵⁴ When NF-kappa B is stimulated, HBx inhibits the apoptotic pathways. On the contrary, inhibition of NF-kappa B activation makes HBx proapoptotic.⁵² Zhai et al⁵⁴ reported that moderate HBx overexpression inhibits apoptosis, whereas massive overexpression promotes apoptosis. Interestingly, the amount of HBx affects the subcellular distribution of NF-kappa B. Low HBx expression stimulates nuclear translocation of NF-kappa B; thus, apoptosis is inhibited. In contrast, higher HBx expression relocates a portion of NF-kappa B into the cytoplasm. This relocation antagonizes the antiapoptotic effect of NF-kappa B and leads to enhanced apoptosis.⁵⁴ Knoll et al⁵⁵ found that HBx is proapoptotic in human hepatocellular carcinoma (HCC) cell lines, but antiapoptotic in normal cells. This cell type specificity is due to different interactions between p53 and HBx. The association with Ras activity also plays a role in the functional shift of HBx.⁵⁶

Throughout the HBV genome, there are naturally occurring mutations that may favor virus replication and alter apoptosis.⁵⁷ An HBx variant, carboxyl-terminus-truncated HBx, is frequently found in HCC. This tumorigenic variant downregulates growth arrest-specific 2-induced p53-mediated apoptosis.⁵⁸

Factors other than the products of the HBV genome may influence the outcome of HBV infection. Cellular inhibitor of apoptosis proteins are endogenous inhibitors of apoptosis. They favor viral persistence by weakening TNF signaling and restrict the clearance of infected cells.⁵⁹



HCV and apoptosis. HCV is a leading cause of chronic viral hepatitis. HCV infection may result in progressive liver fibrosis, cirrhosis, and HCC. Hepatocyte apoptosis plays a major role in disease progression. In acute HCV infection, intensive hepatocyte apoptosis helps to eliminate the virus. In chronic HCV, hepatocyte apoptosis is accompanied by cell proliferation and ensures persistent infection, as observed in chronic HBV patients.⁶⁰ An important difference is noted between HBV and HCV infection: HCV-induced apoptosis is not strongly correlated with the amount of virus but is rather immune-oriented.⁶¹ By studying liver biopsy specimens from chronic HCV patients, hepatocyte apoptosis correlates with histology grading and infiltration of CD8-positive immune cells.⁶² Hepatocyte apoptosis is elevated in HCV patients with fibrotic liver damage, as evidenced by the increased serum caspase activity.⁶³

The HCV genome encodes a pre-polyprotein, which can be cleaved into three independent structural proteins (core protein, E1, and E2) as well as six nonstructural proteins.⁶⁴ Among them, the core protein is the most important structural protein because it regulates hepatocyte apoptosis. The HCV core protein may be either pro- or antiapoptotic. This inconsistency derives from several influencing factors: genetic heterogeneity caused quasi-species HCV core proteins, activation of different signaling pathways, and diverse experimental settings.⁶⁰ Similar as the dual regulatory role of HBx in hepatocyte apoptosis, activations of survival and proliferation signaling pathways are responsible for its antiapoptotic property, while a change in the mitochondrial membrane potential is usually seen when HCV core appears to be apoptotic.

The antiapoptotic effect of the HCV core protein correlates with its ability to enhance NF-kappa B expression.⁶⁵ The core protein blocks TNF- α -mediated apoptosis signaling by sustaining the expression of c-FLIP, thus inhibiting caspase-8 activation.⁶⁶ The core protein also inhibits Fas-mediated apoptosis by inhibiting the release of cytochrome c and activating caspase-9, -3, and -7.⁶⁷ The direct interaction between the core protein and DNA-binding domain of Smad3 results in the inhibition of the transforming growth factor- β -induced apoptotic pathway.⁶⁸ Moreover, the HCV core protein downregulates p21 and inhibits curcumin-induced apoptosis by microRNA-345 targeting human hepatoma cells.⁶⁹ Several studies note that tumor suppressor p53 may be a target of the core. The proapoptotic effect of ROS is abolished when the p14-MDM2 (mouse double minute 2)-p53 pathway is repressed by the core through p14 promoter hypermethylation.⁷⁰ Elevated sirt1 expression is another mechanism of p53 attenuation.⁷¹

The HCV core protein can also be proapoptotic. It augments TRAIL-mediated apoptosis by enhancing Bid cleavage and activating the mitochondria apoptosis signaling pathway.⁷² Core enhances the signal transduction of Fas and TNF-R1 by interacting with them.⁷³ Researchers found that Core connects with the death domain of FADD⁷³ and enhances

FADD-mediated apoptosis.⁷⁴ In terms of TNF- α signaling, Core does not directly interact with the death domain of TNF-R1-associated death domain protein (TRADD) but rather interferes with the binding of TRADD to TNF-R1 and inhibits signaling transduction.⁷⁴ Furthermore, Core disrupts mitochondria function by direct physical interactions⁷⁵ and indirect ROS production.⁷⁶ Based on these findings, Core-induced apoptosis is mitochondria dependent.⁶¹

Another two structural proteins, E1 and E2, also influence hepatocyte apoptosis. E1 increases apoptosis mainly through its C-terminal transmembrane domain.⁷⁷ This hydrophobic region alters membrane permeability and activates apoptosis.⁷⁷ E2 inhibits DR-induced apoptosis in hepatoma cells by inhibiting the release of mitochondrial cytochrome c.⁷⁸ However, another group observed its proapoptotic effect with the mechanism of inducing mitochondria-related and caspase-dependent apoptosis in the same cell line.⁷⁹ A study also demonstrated that the proapoptotic effect of the nonstructural protein of HCV by causing mitochondrial damage.⁸⁰

In addition to viral hepatitis, noninfectious liver diseases also threaten the health of human beings. Some of these diseases are quite common in civilized society. Next, we discuss the mechanism of hepatocyte apoptosis in three noninfectious liver diseases, including fatty liver disease due to either metabolic disorders or alcohol abuse, hepatic ischemia reperfusion injury (HIRI) that typically occurs during surgery, and drug-induced liver injury (DILI) after medication.

Hepatocyte Apoptosis and Fatty Liver Disease

Hepatocyte steatosis, the accumulation of fat inside the cell, is a typical pathological change observed in fatty liver diseases. The biochemical changes in the pathogenesis of fatty liver include reduced fatty acids oxidation, increased fatty acids transportation into the liver, and enhanced fatty acid synthesis.⁸¹ According to the etiology, fatty liver disease is typically divided into two categories: alcoholic liver diseases (ALDs) and nonalcoholic fatty liver diseases (NAFLDs). Both of these conditions exhibit remarkable hepatocyte apoptosis.⁸² On the other hand, there is a synergistic effect between non-alcoholic and alcoholic fatty liver injury.⁸³ Although steatosis marks the onset of fatty liver disease, it may progress to fibrosis, cirrhosis, and HCC.⁸⁴ Both intrinsic and extrinsic factors are involved in steatosis-induced apoptosis. Among all the apoptotic pathways, oxidative stress is of great importance in both ALDs and NAFLDs. Metabolic alterations, such as a high-fat state, inhibit the activity of electron transport chain complexes and elongate the life of electron transport intermediates, thus facilitating ROS generation.⁸¹

Bacterial endotoxins absorbed from the gastrointestinal tract into portal circulation are increased in ALD patients, suggesting the influence of the gut-liver axis in ALD. Both ethanol^{85,86} and a high-fat diet⁸⁷ can disrupt the intestinal epithelial barrier and increase intestinal permeability. Lipopolysaccharide (LPS) is a bacterial endotoxin that can be

absorbed and enters the liver. LPS activates Toll-like receptor 4 and augments the secretion of TNF- α by the liver and thus promotes hepatocyte apoptosis.⁸⁸ The administration of *Lactobacillus johnsonii* can lower intestinal permeability and thus reduces serum LPS levels in NAFLD mice.⁸⁹

Alcoholic liver diseases. Excess alcohol intake increases the risk of ALD. Long-term drinking typically results in ALD with obvious damage to liver function and distinct increases in hepatocyte apoptosis.⁹⁰ An in vivo rat model showed that hepatic apoptosis increased over the duration of alcohol intake.²² The number of apoptotic cells in liver specimens from ALD patients is highest in grade 4 steatohepatitis compared with lower grades.⁹⁰ Although the detailed mechanism of hepatocyte apoptosis during ALD is diverse, it is widely accepted that overproduction of ROS is the major cause of alcoholic hepatocyte injury through the mechanism of mitochondrial damage and ER stress.^{91,92}

Alcohol and its metabolites cause lipid peroxide to induce oxidative stress. The later not only exhausts mitochondrial antioxidant defender glutathione (GSH) but also activates Fas/FasL and the downstream apoptotic signaling pathway.^{93,94} Hepatocyte Fas expression was moderate to strong in ALD patients compared with only minimal Fas expression in control groups.⁹⁰ GSH depletion sensitizes hepatocytes to TNF- α -induced cell death.⁹⁵ Redundant ROS is harmful to the function of mitochondria by damaging the respiratory chain and releasing the pro-oxidant, proapoptotic protein cytochrome P450 2E1.⁹⁶

Alcohol induces ER stress in hepatocytes.^{97,98} Alcohol feeding elevates mRNA levels of ER chaperones and caspase-12.⁹⁹ Caspase-12 is a key element in ER stress-related apoptosis.⁹⁸ Chronic alcohol feeding reduces the activity of cystathionine beta-synthase and results in hyperhomocysteinemia. The latter induces ER stress and activates caspase-12.²² ER stress is also involved in the development of ALD. IFN regulatory factor 3, a transcription factor that regulates the innate immune response, links ER stress with the proapoptotic protein Bax and contributes to hepatocyte apoptosis.²³

In addition to oxidative stress and ER stress, inhibition of survival genes, calcium overload, and the immune response are involved in ALD-induced apoptosis. Alcohol enhances nuclear translocation and activation of transglutaminase 2 and inactivates the transcription factor Sp1, thus inhibiting the expression of the survival gene c-Met.¹⁰⁰ Alcohol elevates store-operated Ca²⁺ entry and leads to intracellular Ca²⁺ overload and apoptosis.¹⁰¹ Alcohol also causes accumulation of secretory IgM in the liver, which activates complement, increases the expression of inflammatory cytokines, and leads to bid-dependent apoptosis.¹⁰²

Based on the above information, appropriate treatment for ALD includes alimentary and drug therapy. Acetylcysteine indirectly increases the content of GSH in hepatocyte and is helpful to clear apoptosis-inducing radicals.¹⁰³ The results from clinical trials support the use of TNF- α inhibitors, such as infliximab and pentoxifylline. These inhibitors significantly

inhibit apoptosis caused by the TNF- α signal.^{104,105} The administration of *S*-Adenosylmethionine may be a potential therapy for ALD. The mechanism involves reduced expression of TNF- α and increased expression of the anti-inflammatory cytokine IL-10.¹⁰⁶

Nonalcoholic fatty liver disease. High-fat food and metabolic disorders endangered people worldwide with NAFLD. This disease refers to pathological changes, including nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis. Active caspase-3 and -7 and strong Fas expression is present in specimens from NASH patients. In addition, the number of apoptotic hepatocytes is significantly increased and correlates well with disease severity.¹⁰⁷ Furthermore, there is a positive correlation between hepatocyte apoptosis and hepatic fibrosis.¹⁰⁷ Three risk factors inseparably correlate with hepatocyte apoptosis in NAFLD. The first risk factor is dysregulated hepatic lipid accumulation. The second risk factor is cellular stress derived from oxidative, metabolic, and cytokine alterations. The third risk factor is mitochondrial dysfunction.¹⁰⁸ Increased plasma caspase-3 generates cytokeratin-18 fragments and soluble Fas. An elevated circulating level of these two biomarkers is observed in NASH patients and correlates well with liver histopathology.¹⁰⁹

The progression of NAFLD is explained by the “two-hit theory”. The first strike is dysregulated hepatic lipid accumulation and steatosis caused by metabolic alterations; the second strike is mitochondrial dysfunction caused by metabolic, oxidative, and cytokine stress.¹¹⁰ Antioxidants, such as vitamin E and betaine, reduce the level of transaminase by mitigating cellular oxidative stress and inhibiting hepatocyte apoptosis.⁸¹ Cytokines, such as TNF- α , IFN- α , IL-4, and IL-13, activate their downstream pathways and lead to hepatocyte apoptosis.^{108,111}

Mitochondrial dysfunction plays a key role in hepatocyte apoptosis of NAFLD.⁸¹ It disrupts the balance of fat metabolism in hepatocytes, induces oxidative stress, and increases ROS production. ROS overproduction may damage mitochondrial proteins, phospholipids, and mtDNA.^{112,113} Hepatocyte mtDNA depletion leads to the decreased expression of polypeptides encoded by mtDNA and increases mitochondrial dysfunction.^{81,114}

ER stress is also involved in apoptosis in NAFLD.¹¹⁵ Unsaturated fatty acid treatment reverses ER stress and inhibits apoptosis.¹¹⁶

Hepatocyte Apoptosis and Hepatic Ischemia Reperfusion Injury (HIRI)

As a biochemical factory, normal liver function is highly dependent on the oxygen supply. Reduced oxygen availability often occurs among inpatients during liver surgery, such as liver transplantation or hepatic lobectomy. These situations may block blood flow to the hepatic pedicle and induce HIRI.

HIRI is a key factor of postoperative liver dysfunction (eg, nonfunctional primary liver transplantation and liver



failure after liver surgery).¹¹⁷ The HIRI rodent model is produced from 1-hour portal triad clamping followed by 24-hour reperfusion. Hepatocyte apoptosis is significantly increased in the HIRI group compared with sham-operated group, and this trend worsens at later time points (55-fold increased after 4-hour reperfusion and 200-fold increased after 24-hour reperfusion compared with sham).¹¹⁸ To date, the mechanism by which HIRI causes apoptosis and ultimately leads to liver dysfunction is not clear. Interaction among several types of liver cells, such as hepatocytes, Kupffer cells, and neutrophils contribute to the progression of HIRI.¹¹⁹ In terms of hepatocyte apoptosis during HIRI, several changes participate in this process, including the production of oxygen-free radicals, calcium overload, changes in mitochondrial permeability, and cytokine- and apoptosis-related gene expression.^{117,120,121} Ca^{2+} overload, anaerobic metabolism, acidosis, and oxidative stress collectively trigger hepatic apoptosis during HIRI. Intracellular Ca^{2+} overload activates Ca^{2+} -dependent enzymes and ultimately leads to cell death.¹²² Oxidative stress causes mitochondrial dysfunction and lipid peroxidation, promoting apoptosis through induction of reactive molecules, such as ROS.¹²³ Antioxidants, such as vitamin E derivatives, inhibit hepatocyte apoptosis in HIRI by inhibiting the production of oxygenic free radicals.¹²⁴ In addition, changes in the metabolic pattern from aerobic to anaerobic inhibit the redox reaction inside hepatocytes and result in significant intercellular ATP depletion. A shortage of energy currency will lead to mitochondrial damage and microcirculation failure. In addition, enhanced anaerobic glycolysis decreases intercellular pH and leads to acidosis-related apoptosis.¹²⁵

Both ischemic pre- and postconditioning protect hepatocytes from apoptosis after reperfusion.¹¹⁸ Pre- and postconditioning share some similar mechanisms of protection.¹¹⁷ Enhanced antioxidant capacity, activation of p38 pathway, and nitric oxide generation may participate in this protection.^{117,121}

Hepatocyte Apoptosis and Drug-Induced Liver Injury (DILI)

Most drugs are metabolized by the liver and kidney; thus, the liver is susceptible to DILI. Different types of drugs can cause mild to severe liver injury. According to the data provided by Drug-Induced Liver Injury Network, the estimated global annual DILI incidence rate is 13.9–24.0 per million people.

The etiology of DILI differs among races and regions. According to a study that includes 24,112 cases in China, the leading drugs that cause DILI rank as follows: antituberculosis medicine (31.3%), complementary and alternative medicines (18.6%), anti-infectious drugs (9.7%), nonsteroidal anti-inflammatory drugs (NSAIDs, 7.6%), and antitumor drugs (4.7%).¹²⁶ In a French population-based study, anti-infectious drugs (25%) are the primary cause of DILI, followed by psychotropic (22.5%) and hypolipidemic drugs (12.5%).¹²⁷ In a Spanish population, NSAIDs (36.4%), analgesics (26.6%), and antibiotics (22.9%) are the top causes of DILI.¹²⁸ On the

other hand, DILI is likely to be more severe in patients who suffer from preexisting liver diseases, such as viral hepatitis and fatty liver diseases.¹²⁹

Various types of drugs cause liver injury through diverse pathways. This characteristic of DILI exacerbates the difficulty of prevention and treatment. DILI is derived from three sources: 1) direct hepatocyte injury; 2) exacerbation of liver disease, especially hepatitis; and 3) a preexisting liver disease that alters the pharmacokinetics of drugs by typically prolonging the effective time of drugs, thus enhancing the toxicity.¹³⁰ Through liver metabolism, drugs can elicit electrophilic products. These products will cause damage to the membrane of cell, mitochondria, and microsome via covalent binding.¹³¹

Most DILI is dose dependent. A daily dosage greater than 50 or 100 mg of oral medication is associated with a higher risk for DILI.^{132,133} In addition to the daily dosage, the “total dose of drug” should also be taken into consideration. Prolonged and continuous administration in the low “therapeutic” range may lead to severe liver damage.¹³⁴ Dose-dependent liver injury may be due to the production of active metabolites, which exhaust cellular GSH and lead to lipid peroxidation and mitochondrial damage. ER stress is also believed to play a role in DILI.¹³⁵ Here, we list three categories of drugs that are common causes of DILI and discuss their role in hepatocyte apoptosis.

Antituberculosis drugs. DILI is a common side effect of antituberculosis drugs (ATDs).¹³⁶ Approximately 1%–18% of ATD-treated patients will develop DILI.^{129,137,138} Liver toxicity of first-line ATD, especially isoniazid, is mostly studied. Specifically, 5 mg/kg isoniazid per os daily for 9 months induces hepatitis at the overall rate of 1% and is age related.¹³⁸ In vitro experiments indicate that 24-hour exposure to isoniazid dose dependently (13, 26, and 52 mM) caused cytotoxicity in HepG2 cells.¹³⁹ In vivo data also indicate the likelihood to induce hepatotoxicity by oral administration of isoniazid and rifampicin (200 mg/kg daily) for 30 days in rats.¹⁴⁰ Combined administration of isoniazid (50 mg/kg subcutaneously), rifampicin (250 mg/kg intragastrically), and pyrazinamide (45 mg/kg intragastrically) for 14 days in rats significantly induced hepatocyte apoptosis.¹⁴¹ The mechanism of ATD-induced DILI involves both the external apoptosis pathway mediated by the cell surface receptor Fas and the internal apoptotic pathway, which is p53-dependent. Simultaneously, the level of antiapoptotic Bcl-2 protein was reduced.¹⁴¹ Decreased intracellular antioxidants (eg, superoxide dismutase) and elevated ROS generation also play a role.¹³⁹

Complementary and alternative medicines. Although herb medicine is not chemically synthesized, components, such as alkaloids, glycosides, toxic proteins, and metals may still cause liver toxicity.¹⁴² The clinical manifestation includes both acute and chronic toxicity. Although acute toxicity is typically caused by overdose or intravenous administration, chronic toxicity is more likely due to long-term usage-related accumulative toxicity.¹⁴² The property of an herb medicine is

typically unique and complicated, thus making it difficult to determine the “typical mechanism” of its liver toxicity. Some drugs or their metabolites can cause directly injury to hepatocytes through toxic effects, whereas others initiate an immune response. Overdose is a common cause of herb medicine-induced liver toxicity. For example, 3–9 g of Gardenia is typically safe, whereas 30 g or more may induce liver injury.¹⁴² In fact, the degree of injury not only depends on the type and dose of drugs but also on the processing procedures. For example, *Euphorbia kansui* is used to treat edema, but it also shows liver toxicity at doses of 2.97 and 5.94 mg/mL to LO2 hepatocytes. Stir-baking with vinegar reduces its toxicity by reducing the levels of terpenoids and inhibiting the intrinsic apoptosis pathway.¹⁴³

Antitumor drugs. Antitumor drugs are a large family of drugs that are involved in diverse molecular mechanisms. Among them, cytotoxic antitumor drugs are often reported to have liver toxicity. The side effects of antitumor drugs typically occur within 1–4 weeks from the beginning of chemotherapy. Alkylating agents such as Cytoxan; antimetabolites, such as methotrexate; DNA-damaging antibiotics, such as mitomycin; mitosis inhibitors, such as vindesine; and tyrosine kinase inhibitors (TKI), such as epithelial growth factor receptor TKI, induce DILI. Approximately 10% of patients developed DILI after docetaxel (mitosis inhibitor) treatment.¹⁴⁴ ROS produced by antitumor drugs is an important mechanism to induce tumor cell death due to hepatocyte toxicity, especially in steatotic livers.¹⁴⁵ Repeated use of

10 µg/mL nedaplatin causes significant apoptosis by reducing Bcl-2 expression, enhancing p53 expression, and increasing ROS production and mitochondria damage in cultured normal hepatic cell lines.¹⁴⁶ Interestingly, when the antioxidant dihydromyricetin was administered together with nedaplatin, normal hepatocytes were protected from DILI, and the effect of nedaplatin on tumor cells was enhanced.¹⁴⁶

Conclusion and Predictions

Hepatocyte injury is a complicated biological process that is mediated by various factors and may lead to hepatocyte apoptosis. In this review, we provide a general idea regarding apoptotic pathways in hepatocytes as well as unique features in particular liver diseases. The common apoptotic pathways in hepatocytes include fundamental extrinsic and intrinsic pathways, ER stress, and oxidative stress-mediated signaling. Four types of liver diseases accompanied by significant hepatocyte apoptosis are discussed here: viral hepatitis, steatotic liver, ischemia reperfusion injury, and drug-induced injury. Figure 1 summarizes the apoptotic pathways in hepatocytes, as well as the influence of viral and noninfectious factors in this process.

Studies of the mechanism of hepatocyte apoptosis in different liver diseases are of great value for providing a better strategy to prevent and treat liver diseases. In the past decades, scientists have identified numerous apoptosis-related genes and signaling transduction pathways. However, some mysteries are still waiting to be unveiled. One of the most important questions is how to adjust the expression of apoptotic genes

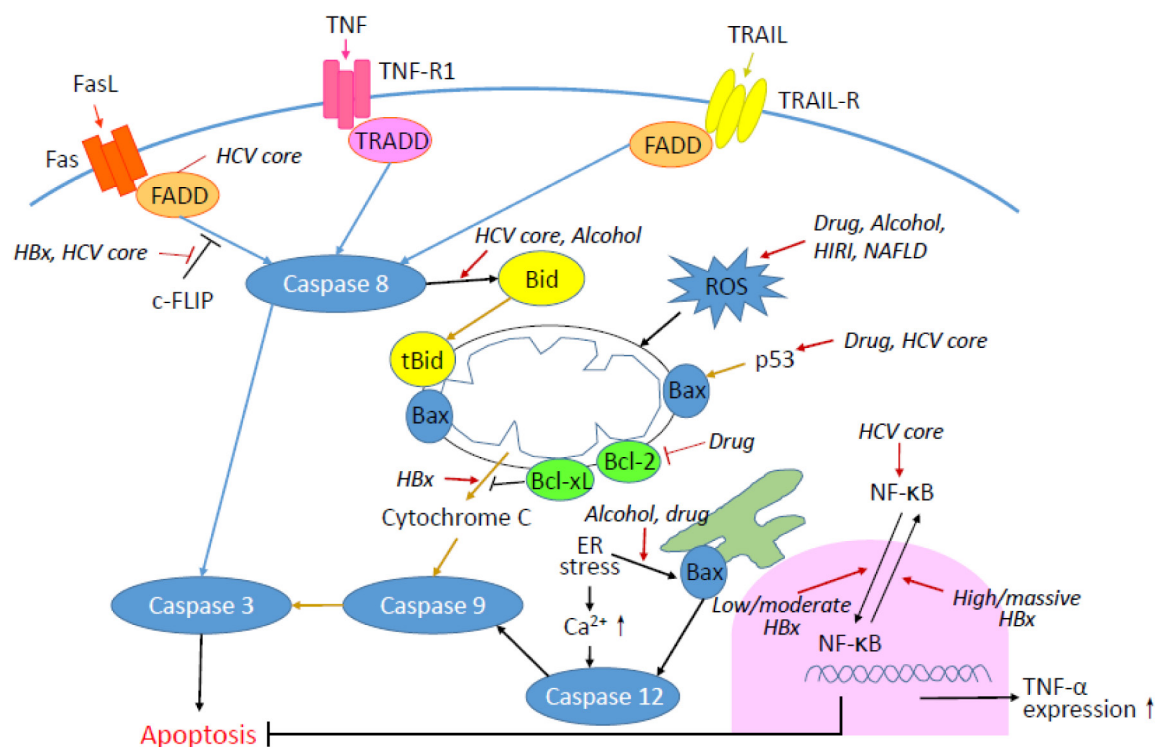


Figure 1. The schematic diagram of apoptotic pathways in hepatocytes. Blue lines indicate extrinsic pathways, whereas light brown lines indicate intrinsic pathways. The influence of virus infection, alcohol, fat, ischemia reperfusion, and drug on hepatocyte apoptosis is also indicated by italics and red arrows.



and pathways to prevent or alleviate liver diseases. Hepatic apoptosis intervention is a fascinating yet challenging journey to delay liver disease progression and reduce its morbidity. Several concerns need to be carefully investigated to accelerate both preclinical and clinical studies.

First, more accurate and dynamic methods of monitoring in vivo apoptosis must be explored. Gold standard, the classical diagnosis of liver damage, depends on a histological evaluation from biopsy samples. Most of our current knowledge about in vivo hepatocyte apoptosis is derived from this method. However, it is difficult to obtain dynamic data safely from invasive procedures, and sampling error is another major concern. Noninvasive evaluations, such as imaging techniques and serum biomarkers, are now widely used to estimate the level of liver injury. High-resolution imaging, specific biomarkers, and other new indicators that correlate with the onset, progression, and location of apoptosis must be explored.

Second, the switch among different modalities of cell death deserves intensive study. Based on our current understanding, liver injury includes several types of cell death: apoptosis, necroptosis, necrosis, and autophagic-dependent cell death. With methodological advancement, more death modalities may be identified. On the one hand, scientists should study the characteristics of different death modalities; on the other hand, understanding the switch mechanism among them and manipulating these switches are of great value to conquer the illness. For example, to eliminate intruding enemies, such as microorganisms and harmful metabolites, with minimal impact to hepatocytes, it is essential to understand the “on and off” mechanism of hepatocyte apoptosis and the transformation between apoptosis and other death modalities.

Third, findings from animal experiments must be cautiously evaluated and interpreted. The gap between animal models and clinical cases must be always kept in mind. Physiology and pathophysiology dissimilarities derived from metabolic enzymes, absorption profiles, and concomitant diseases all contribute to this gap. Furthermore, given that metabolic, genetic, and health largely influence the outcome of liver injury, precision medicine is very likely to be the future direction of hepatology. To accurately define the network controlling hepatocyte apoptosis, more time and work are needed.

Author Contributions

Wrote the manuscript: LC, XBQ, and MJW. Revised the manuscript: WJZ, XOY, and MJW. All the authors approved the final version of manuscript.

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