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Review

Molecular mechanisms of hepatic apoptosis

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Apoptosis is a prominent feature of liver diseases. Causative factors such as alcohol, viruses, toxic bile acids, fatty acids, drugs, and immune response, can induce apoptotic cell death *via* membrane receptors and intracellular stress. Apoptotic signaling network, including membrane death receptor-mediated cascade, reactive oxygen species (ROS) generation, endoplasmic reticulum (ER) stress, lysosomal permeabilization, and mitochondrial dysfunction, is intermixed each other, but one mechanism may dominate at a particular stage. Mechanisms of hepatic apoptosis are complicated by multiple signaling pathways. The progression of liver disease is affected by the balance between apoptotic and antiapoptotic capabilities. Therapeutic options of liver injury are impacted by the clear understanding toward mechanisms of hepatic apoptosis.
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Facts

- Apoptosis is a typical pathological feature of liver diseases.
- The severity of hepatic apoptosis is varied due to different etiologic factors.
- There are distinct mechanisms to mediate hepatic apoptosis. One mechanism dominates over others at a particular stage.
- Interventions for hepatic apoptosis alleviate liver injury as demonstrated by pre-clinical models and clinical trials. The regulation of hepatic apoptosis affects the progression of liver diseases.

Open Questions

- Multiple signaling pathways form a complicated network to modulate the hepatic apoptosis.
- Molecular targets of hepatic apoptosis and the detail of regulatory mechanisms need to be determined.
- Therapeutic options of liver diseases depend on the correct understanding toward mechanisms of hepatic apoptosis.

Introduction of Hepatic Apoptosis

Liver injury can be caused by different stimuli such as alcohol intake, viral infection, cholestasis, steatosis, drug abuse, and autoimmunity.[1–6] Genetic variability is interacted with environmental factors, which makes liver damage in a variety of severity.[7] In the damaged liver, cell death modes include apoptosis, necrosis, necroptosis, and autophagy. These medical terms have clear definitions in pathology. However, different modes of cell death are intermingled as a continuous process during liver injury. Apoptosis and necrosis are two major types of cell death.[8] They consist in a dynamic spectrum. Apoptosis is an early, chronic, and temperate response subsequent to injury induction, whereas necrosis is an acute and severe reply. Causative factors of liver injury may induce both modes of cell death dependent on the severity of the insult. Apoptotic cells are characterized by energy-dependent biochemical mechanisms and distinct morphological changes.[9,10] Apoptosis shares common cell death machinery, including death receptor-dependent and mitochondria-dependent pathways.[11,12] Dysfunction or dysregulation of the apoptotic program is implicated in a variety of congenital anomalies and pathological conditions.[13,14] Because of differences in etiology, hepatic apoptosis and its pathophysiological role have much discrepancy during liver injury.

Etiology and Mechanisms of Hepatic Apoptosis

Viruses. Hepatotropic viruses such as hepatitis A virus, hepatitis E virus, cytomegalovirus, herpes simplex virus, and Epstein Barr virus often cause acute liver injury,[15,16] whereas hepatitis C virus (HCV) and hepatitis B virus (HBV) lead to chronic liver injury.[17,18] The acute liver injury involves much

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Abbreviations: ROS, reactive oxygen species; CYP2E1, cytochrome P450 2E1; Drat, death resistor ADH domain containing target; ADH, alcohol dehydrogenase; ALDH, acetaldehyde dehydrogenase; ADHr, ADH-related; NAD^+, nicotinamide adenine dinucleotide; ER, endoplasmic reticulum; ATP, adenosine triphosphate; MTP, mitochondrial permeability transition; NASH, non-alcoholic steatohepatitis; UDCA, ursodeoxycholic acid; HNF6, hepatocyte nuclear factor 6; iNOS, inducible NO synthase; IFNγ, interferon-gamma; IL-6, interleukin 6; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; TNFα, tumor necrosis factor alpha; TNFR, tumor necrosis factor receptor; TRAIL, TNF-related apoptosis-inducing ligand; FADD, Fas-associated protein with death domain; RNS, reactive nitrogen species; PUMA, p53-upregulated mediator of apoptosis; UTR, untranslated region; PDC-E2, E2 component of the pyruvate dehydrogenase complex; IAPs, inhibitors of apoptosis proteins; CI/WR, cold ischemia/warm reperfusion
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necrosis, but the chronic infection of HCV and HBV exhibits abundant apoptosis. Only human and chimpanzee hepatocytes are naturally able to support HCV entry, likely due to differences in tropism determinants. A successful HCV infection requires many cellular factors, for example, OCLN, CLDN1, GAGs, SR-BI, LDL-R, and CD81.[19–23] Species-specific entry factors may include ample levels of OCLN and CD81 with large extracellular loop.[24] CLDN1 and SR-BI only expressed in hepatocytes are cell entry tropism determinants.[25,26] HCV replication and production in infected hepatocytes can induce apoptosis and a release of inflammatory cytokines/chemokines, such as tumor necrosis factor alpha (TNFα), TGFβ, interferon-gamma (IFNγ), interleukin 10 (IL-10), IL-12, IL-22, CCL3, CCL4, CXCR3 ligand, IP-10, CCR5 ligands, and RANTES.[27–31] These inflammatory cytokines/chemokines can stimulate hepatocyte apoptosis through different pathways. For instance, TNFα is a classical cytokine and its signaling pathway had been well investigated (Figure 1). Special structures of HCV genome determine particular entry pattern of infectious HCV.[32] HCV genome encodes structural proteins core, E1, E2, and P7 as well as non-structural proteins NS2, NS3, NS4A, NS4B, NS5A, and NS5B. HCV core protein can sensitize TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis(Figure 2).[33,34] The HCV core protein also binds to tumor necrosis factor receptor 1 (TNFR1) to trigger TNFα-induced apoptosis *via* increasing expression of Fas-associated protein with death domain (FADD) and to initiate Fas-induced apoptosis through the formation of the death-inducing signaling complex.[35,36] NS3 protein can additionally sensitize to Fas-induced apoptosis.[37] Expression of E1 and E2 in murine hepatocytes can increase the apoptosis of activated T cells, providing a possible mechanism for immune escape in chronic infection.[38] Moreover, Core, NS3, NS4B, and NS5A are the major immunogenic proteins in chronic HCV infection. An enhanced Fas expression in HCV-infected hepatocytes leads to T cell-mediated apoptosis. Fas-mediated apoptosis shows a much high level as alcohol consumption in hepatitis C patients.[39] There is promising Fas-mediated apoptosis in HCV infection that correlates with the level of serum alanine aminotransferase and histologic grade. The cytokeratin-18 neoantigen (M30), reflecting ongoing hepatocyte apoptosis, is elevated in patients with chronic hepatitis C.[40] The M30 may be a surrogate to replace the repeated liver biopsy. HBV nucleocapsid encloses viral DNA and DNA polymerase.[41] The outer envelope contains embedded proteins that are involved in viral binding of, and entry into susceptible cells. Chronic HBV infection is characterized by certain levels of hepatocyte apoptosis. Hepatic TNFα, TNFR1, and Fas expression (also serum Fas) is enhanced in chronic HBV infection and correlated with degree of liver injury.[42,43] Fas-expressing lymphocytes penetrate areas of apoptotic hepatocytes in patients with fulminant hepatic failure due to HBV. TRAIL expression in NK cells increases in temporal correlation with levels of serum alanine aminotransferase. In clinical, TRAIL receptor 2 is upregulated in livers of patients with chronic HBV.[44] Bax expression in hepatocytes is positively correlated with the number of apoptotic nuclei. HBx protein can upregulate TRAIL-receptor expression and sensitizes hepatocytes to TRAIL toxicity, leading to TRAIL-mediated apoptosis.[45,46] Liver injury is triggered by host immune response to hepatocytes expressing viral proteins. HBV infection regulates the apoptotic machinery to establish persistent infection and evade the immune system. Like HCV, HBV can chronically infect the host as well, eliciting chronic liver injury to fibrosis/cirrhosis and the sequelae of advanced liver disease. Both HBV and HCV mediate adaptive and innate immune responses in liver, which can induce target cell apoptosis, a feature common in these viral liver diseases. The infection of HCV and HBV also is a principal risk factor of hepatocellular carcinoma.[47] A complete understanding toward mechanisms of hepatotropic virus-mediated apoptosis will help to develop therapeutic drugs.

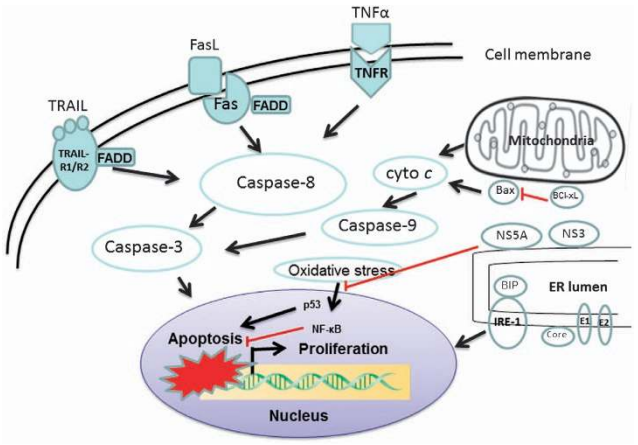

Figure 1 Model of HCV apoptotic signaling pathways. HCV induces infected hepatocytes to apoptosis. Death receptor-mediated extrinsic pathway is enforced by mitochondrial amplification loop. Oxidative and ER stress with apoptosis are also shown, which reflect a potential interaction between the host cell response and apoptosis. Stress pathways are converged at the nucleus and low levels of NF-κB and BCL-xL sensitize hepatocytes to apoptosis. Lines with arrows denote an activating reaction; red lines ending in perpendicular lines denote inhibition of the reaction

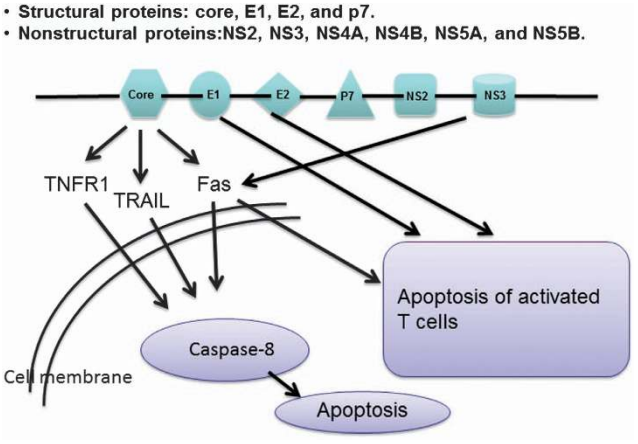

Figure 2 Role of HCV viral proteins in the induction of apoptosis. The positive-stranded genome RNA of HCV encodes structural proteins (core, E1, E2, and p7) and non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B). Special structures of HCV genome determine not only particular entry pattern of infectious HCV, but death mode of infected hepatocytes and immune escape mechanism as well

Alcohol. Long-term use of alcohol in excessive quantities leads to the development of alcoholic liver disease (ALD).[48] Acute and chronic alcohol consumption induces much production of reactive oxygen species (ROS), lowers cellular antioxidant levels, and enhances oxidative stress in the liver tissue.[49] During the body's metabolic reactions, highly reactive ROS is naturally generated in small amounts. However following the exposure of alcohol, massive ROS actively reacts with and damages complex cellular molecules such as lipids, proteins, or nucleotides. Alcohol-induced oxidative stress has a major role in the mechanism by which alcohol causes liver injury. Many signaling molecules take part in alcohol-induced oxidative stress. Cytochrome P450 2E1 (CYP2E1), as an effective generator of ROS, can produce superoxide anion radical, hydrogen peroxide, and powerful oxidants such as the hydroxyl radical in the presence of iron catalysts (Figure 3).[50,51] Although levels of CYP2E1 are elevated under a variety of physiological and pathophysiological conditions, alcohol is a strong inducer to alter a state of oxidative stress through the induction of CYP2E1.[52] Alcohol abuse stimulates an overproduction of ROS and leads to hepatic apoptosis *via* mechanism of oxidative stress. Except for oxidative stress, the impact of alcohol on hepatocytes also includes mitochondrial dysfunction, decreased methylation capacity, endoplasmic reticulum stress, impaired vesicular trafficking, and altered proteasome function.[53] By employing *Drosophila* as a model system, a novel apoptogenic effector *Drat* (death resistor alcohol dehydrogenase (ADH) domain containing target) was identified, which could regulate cell death incited by alcohol challenge as well. *Drat* function was required for alcohol-induced apoptotic cell death.[54] Although ADH and acetaldehyde dehydrogenase (ALDH) influence responses to alcohol, interestingly alcohol-induced cell killing in *Drosophila* model was unaffected by depletion of *ADH* and ADH-related (*ADHr*) alone or in combination. Surprisingly, *ALDH* depletion failed to enhance the apoptogenic effects of alcohol. These results reveal that alcohol itself, rather than its metabolites, triggers apoptotic process in *Drosophila* system. The alcoholic liver injury involves both the parenchymal and non-parenchymal cells in the liver. The occurrence of apoptotic bodies generated from hepatocytes activates Kupffer cells (KCs) (Figure 4).[55] Alcohol treatment results in an enhanced responsiveness of KCs and produces inflammatory cytokines as indicated in alcohol-fed animals. Alcohol exposure also alters the expression of endotoxin receptors and intracellular signaling molecules, which causes both tolerance and sensitization of KCs to endotoxin. Tolerance of KCs may contribute to the impairment of innate immune system in alcoholism, while sensitization to endotoxin enhances progression of alcoholic liver injury. Alcohol awakens KCs to be sensitized by lipopolysaccharides *via* toll-like receptor 4 (TLR4).[56] This sensitization promotes the production of TNFα and ROS. These inflammatory mediators contribute to hepatocyte dysfunction, apoptosis and necrosis of hepatocytes, and the generation of extracellular matrix proteins leading to characteristic fibrosis. Inflammatory and innate immune responses in KCs due to elevated lipopolysaccharide, increased oxidative stress, and profibrogenic factors such as acetaldehyde or lipid peroxidation products contribute to activation of hepatic stellate cells (HSCs).[57] Alcohol exposure can alter the structural integrity of hepatic sinusoidal endothelial cells and activates HSCs. The activated HSCs transform into the collagen-producing cells and the wound-healing response to recurrent liver injury is triggered, which results in excessive accumulation of extracellular matrix proteins, mainly collagen type I. When fiber

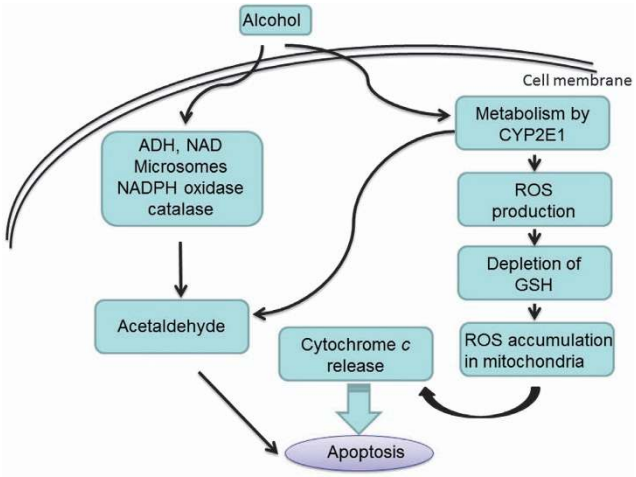

Figure 3 Molecular mechanism of alcohol-mediated hepatocyte apoptosis. Alcohol is metabolized in the liver and can be converted into acetaldehyde. As a toxic substance, the acetaldehyde induces hepatocyte apoptosis. Alcohol breakdown by cytochrome P450 2E1 (CYP2E1) also generates highly reactive molecules known as reactive oxygen species (ROS), particularly after chronic alcohol consumption. ROS can be eliminated by antioxidants such as glutathione (GSH). When cell's antioxidants are depleted and ROS accumulation reaches a critical threshold, mitochondrial damage occurs. This process leads to the release of cytochrome *c* from the mitochondria, which then activates caspases apoptotic pathway

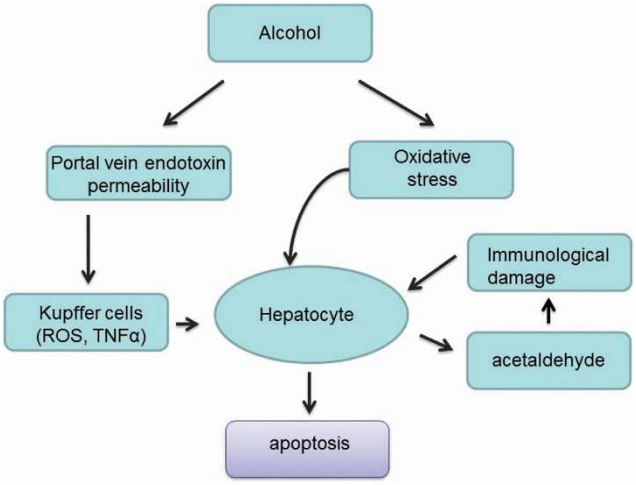

Figure 4 Signaling mechanism of alcoholic liver injury. Oxidative stress is an important mechanism in alcoholic liver disease. Moreover, alcohol increases levels of bacterial endotoxin in the liver, which further activates liver Kupffer cells to produce inflammatory cytokines (e.g., TNFα) and/or chemokines (e.g., IL-8). The acetaldehyde interacts with proteins in the liver, forming compounds called adducts that can additionally activate certain immune cells to produce various cytokines, including interleukins, IFNγ, and TNFα. All of these diverse pathways contribute to the induction of apoptosis and organ damage

synthesis is very active and the decomposition is suppressed, fibrosis will progress. Oppositely, fibrosis can be reversed if the driver, inflammation, is controlled.[58] Hepatocyte apoptosis, inflammation, and fibrosis are apparent features of liver disease in general and of alcoholic liver injury in particular. A comprehensive understanding on ALD mechanisms remains incomplete. The development of therapeutic interventions for ALD is still under way.

Toxic bile acids. The accumulation of bile acids or cholestasis can cause liver dysfunction, cirrhosis, and liver failure.[59] The causes of cholestasis include genetic defects, mechanical aberrations, toxins, and dysregulation in the immune system.[60] Cholestatic animal models such as partial or total bile duct ligation (BDL) can approximately mimic clinical obstructive cholangiopathies, for example, biliary strictures and biliary atresia. Hepatic apoptosis is a routine manifestation of hepatobiliary injury in BDL models.[61] Membrane death receptor-mediated caspase activation is an important pathway during cholestatic liver injury.[62] The mitochondrial pathway is often required to amplify the relatively weak death receptor-induced apoptotic signal in liver cells.[63] Typical apoptosis of cholestatic liver injury is induced through Fas-dependent, ligand-independent aggregation of Fas receptor complex, recruitment of the apoptotic TRAIL receptors, and TNFα cascade (Figure 5).[64–66] Bile acids are normally secreted rapidly from hepatocytes by transporters located in the canalicular membrane. In cholestasis, secretion is impaired, resulting in elevated concentrations of toxic bile acids (TBAs) within hepatocytes. As concentrations of TBA reach a certain threshold, intracellular bile acids modulate vesicular targeting and oligomerization of the Fas receptor, which translocates internal Fas bearing vesicles to the plasma membrane where they self-aggregate in the absence of ligand. Activated Fas receptor complexes on the plasma membrane then cause caspase-8 activation

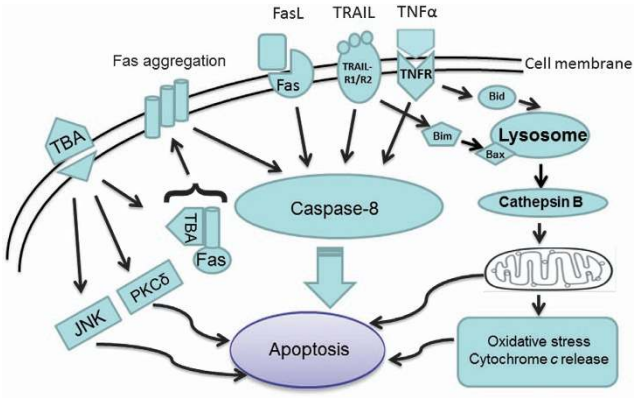

Figure 5 Toxic bile acids (TBAs)-induced hepatocyte apoptosis. In cholestasis, bile secretion is impaired, resulting in elevated concentrations of TBAs within hepatocytes. TBAs can activate the ER stress pathway as well as generation of ROS. TBAs also sensitize hepatocytes to death receptors (Fas, TRAIL, and TNFα)-induced apoptosis. Particularly, TBAs trigger translocation of intracellular Fas bearing vesicles to the plasma membrane where they self-aggregate, activate Fas receptor complexes, and cause caspase apoptotic cascade. In addition, some bile acids also activate PKC-δ and/or JNK1/2 MAP kinase, both of which stimulate pro-apoptotic pathways

and an apoptotic cascade.[67] TBA induce hepatocyte apoptosis by both Fas-dependent and -independent mechanisms *via* activation of caspases. Another mechanism by which apoptosis occurs beyond bile acid-mediated injury includes overexpression of TNFα and its interlinking with receptors TNFR1 and TNFR2 to activate the caspase pathway in BDL mice.[68] Induction of TRAIL-R2/DR5 expression and apoptosis by toxic GCDC *in vitro* provides new insights into the mechanisms of hepatocyte apoptosis.[69] In support of this mechanism, human cholangiocytes constitutively express TRAIL and apoptosis is significantly elevated in cholangiocytes of human primary sclerosing cholangitis (PSC) and primary biliary cirrhosis (PBC) patients, implicating TRAIL-mediated apoptosis in the progression of chronic cholestatic diseases, particularly PSC.[70] Parenchymal liver injury by BDL and clinical obstructive cholangiopathies all result in hepatic apoptosis and dysfunction. Moreover, hepatic apoptosis can stimulate the profibrotic KCs that amplify the proinflammatory cascade and initiate the fibrogenic response. The early course of BDL is characterized by biliary epithelial proliferation within 24 h of obstruction. This is followed by inflammatory cell infiltrates consisting of mostly polymorphonuclear cells.[71] This is associated with the upregulation of inflammatory cytokines, such as the pro-apoptotic TNFα and mitogenic IL-6. By the second week of biliary obstruction, extracellular matrix proteins such as collagen, laminin, and elastin are deposited in the periductal space and by the third week of obstruction, early evidence of biliary cirrhosis shown as the presence of liver parenchymal nodules and dense portal fibrotic septa is found. The transforming growth factors (TGF-b1/TGF-b2) have been implicated as the dominant soluble mediators for parenchymal fibrosis.[72] The relationship between apoptosis and pathologic hepatic fibrosis has been appreciated in recent years.[73] In bile acid pool, glycochenodeoxycholic acid is cytotoxic and can induce hepatocyte apoptosis as demonstrated by *in vitro* or *in vivo* models, whereas ursodeoxycholic acid (UDCA) is cytoprotective.[67,74] UDCA can stabilize cell membrane of hepatocytes, increases defense against oxidative stress, and inhibits apoptosis. UDCA or its derivative has been utilized in clinical patients to improve liver function in some liver disease.[75] Moreover, the upregulated hepatocyte nuclear factor 6 (HNF6) is liver protective through a mediation of bile transport.[76] A variety of pathways contribute to liver damage and the reparative response, so novel therapies may be developed when targets that mediate these processes are identified.

Fat. Accumulation of fat (or fatty infiltration) in the liver can induce liver injury. Pathological changes of non-alcoholic fatty liver disease (NAFLD) include simple steatosis as well as complex lesions of steatohepatitis with varying degrees of steatosis, inflammation, ballooning degeneration, and fibrosis. Progressive steatohepatitis leads to cirrhosis.[77] Mechanisms of NAFLD are closely linked to abnormalities in insulin response, lipid metabolism and disposition, and chronic inflammatory/oxidative stress response.[78] Obesity, defined as body mass index greater than 30 kg/m^2, is a principal risk factor associated with NAFLD.[79] In obesity, fat laden myocytes and adipocytes become resistant to insulin

signaling. Therefore, it leads to hyperglycemia, hyperlipidemia, and fat deposition in non-adipose tissues such as muscle and liver. Increased intrahepatic fat impairs insulin signaling in the liver and accelerates liver gluconeogenesis. c-Jun amino-terminal kinases (JNKs) can interfere with insulin action in cultured cells and are activated by inflammatory cytokines, free fatty acids (FFAs), and molecules that have been implicated in the development of type II diabetes.[80,81] JNK becomes a potential target for therapeutics. Sensitization to death ligands may also occur independently of JNK activation *in vivo*, which is known to mediate insulin resistance and liver injury in models of dietary obesity. Inflammatory cytokines such as IL-6 and TNFα are produced by monocyte/macrophages and adipocytes.[82] Along with FFAs, they modulate insulin sensitivity by altering the phosphorylation state of insulin receptor substrates. The lipid oxidation response mediated by lipotoxic fat, and mitochondrial, peroxisomal or microsomal CYP2E1 and CYP4A enzymes are increased as well.[83] Excess ROS and lipid peroxidation products are neutralized by free radical scavenger response, or with upregulation of mitochondrial uncoupling protein 2 (UCP-2). UCP-2 protective response to reduce ROS production by limiting mitochondrial adenosine triphosphate (ATP) synthesis, however, depletes ATP production under stress.[84] In the face of diminished antioxidant response, cells are susceptible to mitochondrial damage and cellular apoptotic injury. FFAs induce lipoapoptosis in hepatocytes, which is an obvious feature in non-alcoholic steatohepatitis (NASH).[85] Toxic FFAs can activate the lysosomal pathway of cell death, sensitizing cells to cytokine toxicity. Elevated expression of Fas, TNF receptor, and TRAIL receptor 2 is found in liver biopsy samples.[86,87] Furthermore, FFAs can stimulate the intrinsic apoptosis pathway *via* JNK, which is mediated by intracellular Bim levels and Bax activation, leading to mitochondrial permeabilization, cytochrome *c* release, and caspase activation. Thus, JNK-dependent lipoapoptosis is significantly modulated by proapoptotic protein Bim/Bax activation.[88] In addition, levels of adiponectin are reduced, which increases vulnerability to lipotoxicity and promotes progression from simple steatosis to NASH and even advanced hepatic fibrosis. Blood caspase target M30 is elevated in patients with NASH and serum levels are correlated with hepatic levels. Saturated FFAs induce Bim expression and cell stress, lead to sustained JNK activation, and sensitize hepatocytes to death receptor (Fas and TRAIL)-mediated apoptosis (Figure 6).[87] Hepatocyte apoptosis closely correlates with hepatic inflammation and fibrosis. Inflammatory cytokines from visceral adipose tissue or enteric sources further sensitize the liver to oxidative stress and cellular injury. In particular, TNFα can exacerbate NAFLD by inducing mitochondrial ROS, by activating stress-related kinases that inhibit insulin signaling and promote gluconeogenesis, by suppressing adiponectin anti-lipogenic effect to compound steatosis, and by attenuating the anti-inflammatory effects of adiponectin and PPAR-γ.[89,90] Therefore, secondary inflammation and fibrosis of NAFLD ensue. Hepatic lipid turnover is also regulated by transcription factors ChREBP, SREBP-1c, Foxa2, C/EBPα, and PPAR.[90–93] The dysregulation of microRNAs (miRNAs)

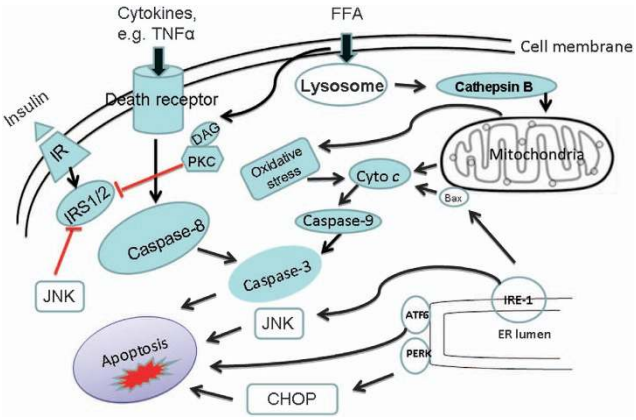

Figure 6 Possible pathways of fat-induced hepatic apoptosis. The distinct mediators of fatty liver disease may include circulating cytokines, adipokines, and free fatty acids (FFAs). Peripheral insulin resistance enhances the delivery of FFAs to the liver, resulting in an imbalance of FFA metabolism and synthesis, thus promoting hepatic steatosis. Steatotic livers increase inflammatory cytokines, ROS, and ER stress, which trigger apoptotic cascade. Insulin sensitivity is further impaired by JNK-mediated phosphorylation of IRS1 and 2. Moreover, adipocytokines such as leptin and adiponectin are regulated by levels of circulating cytokine such as TNFα

contributes to metabolic disorders.[94,95] miR-33a, miR-33b, miR-34a, and miR-122 may be associated with the pathogenesis of NAFLD and its progression to NASH.[94,96] Two miRNAs, miR-34a and miR-296, were involved in the regulation of hepatocyte apoptosis. Particularly, the miR-34a/Sirtuin 1(SIRT1)/p53 proapoptotic pathway in human NAFLD could be suppressed by UDCA.[97] miR-296-5p directly binds to p53-upregulated mediator of apoptosis (*PUMA*) 3′-untranslated region (UTR) and negatively regulates its expression. An altered miR-296-5p could regulate PUMA expression and hepatocyte lipoapoptosis. In NASH patients with increased circulating FFAs, hepatic miR-296 expression was decreased, resulting in an amplification of PUMA with associated lipotoxicity.[98] The future study will pay attention to the relevance of insulin resistance, hyperlipidemia, cytokines, oxidative stress, and adaptive response in obesity pathologies along with novel areas of obesity research such as the role of hepatic transcription factors and target genes in regulating lipid metabolism and fat disposal.

Drugs. Liver is a crucial organ of metabolism and elimination of foreign substances. Liver therefore is a preferred target for drug toxicity.[99] Drug-induced liver injury (DILI) can mimic all forms of acute or chronic liver disease. The pathogenesis of DILI includes cell stress, mitochondrial impairment, and specific immune reactions.[100,101] Current concepts emphasize the central role of mitochondria, of events leading to apoptotic and/or necrotic cell death, and of factors that balance injurious and protective responses. Liver as the central place of detoxification is constantly exposed to cell stress. Cell stress breaks the balance of inflammatory cytokines that promote (e.g., IL-12) or prevent (e.g., IL-4, IL-10, IL-13, MCP-1) injury.[102–104] Consequently, liver cells become more susceptible to lethal effects of TNFα, Fas ligand (FasL), and IFNγ.[105] TNFα and FasL bind to intracellular death receptors. TNF and Fas receptor-associated

death domain proteins will subsequently activate initiator caspase-8. The caspase-8 can start apoptosis through a direct activation of effector caspases-3, 6, and 7. In mitochondria, drugs or their reactive metabolites act on the mitochondrial respiratory chain to cause a series of harmful response, such as ATP depletion, production of ROS, inhibition of β-oxidation, mitochondrial DNA damage, and an increase in the permeability of the mitochondrial membranes (Figure 7).[100,106] As an accumulation of cytosolic ROS and JNK activation more than critical threshold, the mitochondrial damages cause mitochondrial membrane permeabilization, release of cytochrome *c*, and activation of pro-caspase-9. The active pro-caspase-9 subsequently activates executioner caspase-3 that cleaves specific target proteins and results in apoptotic death. If initial injury is so severe that MPT quickly occurs in all mitochondria and rapid ATP depletion, then necrosis will develop. There is no clear-cut to discriminate apoptosis and necrosis. The same hepatotoxin may cause the concomitant occurrence of both death modes, depending on the circumstances including dose and preexisting vulnerability of hepatocytes. The mode of cell death by toxic stimuli is often concentration dependent, with more likely apoptosis at low concentrations and necrosis at high concentrations. For all mechanisms, mitochondria have a central role in chain events leading to apoptotic and/or necrotic cell death. The mitochondrial pathway can also amplify the relatively weak death receptor-induced apoptotic signal in liver. Acetaminophen (N-acetyl-para-aminophenol (APAP), paracetamol)-induced hepatotoxicity remains one of major clinical problems in the field of DILI.[107] APAP could markedly amplify the TRAIL signaling pathway to induce apoptosis in hepatocyte-like cell lines, primary hepatocytes, and liver sinusoidal endothelial cells.[108,109] Especially, TRAIL-induced cell death could be efficiently attenuated by pan-caspase inhibitor Z-VAD-fmk.[109] TRAIL- or Bim-deficient mice were substantially protected from APAP-induced liver damage.[108] Toll-like receptor 3 (TLR3, CD283) also contributed to APAP-induced liver damage.[107] The activation of TLR3 was required for APAP-induced liver failure as proven in TLR3-deficient mice. The TLR3 activation could stimulate the TNFα production and the expression of phosphorylated JNK in APAP-injured livers. Future perspectives will focus on the understanding of pathogenetic mechanisms of DILI. There are no pathognomonic indicators for DILI, even if liver biopsy is not diagnostic.[110] New marker for apoptosis such as the monoclonal M30 antibody, recognizing a caspase-generated cytokeratin-18 neoantigen, may be useful in the early diagnosis of DILI.[111] JNK acts on acetaminophen metabolism to induce hepatotoxicity. JNK inhibitor is thus used in clinical patients with acetaminophen-induced liver injury.[112] Caspase inhibitors block apotpotic cascade to reduce liver injury. Caspase inhibitors may be applied in the treatment of DILI. Anti-apoptotic role of inhibitors of apoptosis proteins (IAPs) or Bcl-2 proteins makes them potentially to be utilized in clinical practice of DILI.[113] Although UDCA has a pan-antiapoptotic role through the modulation of MPT, its use in the treatment of DILI patients is still to be determined. Actually, inhibition of apoptosis is becoming a new strategy to develop therapeutics of DILI.

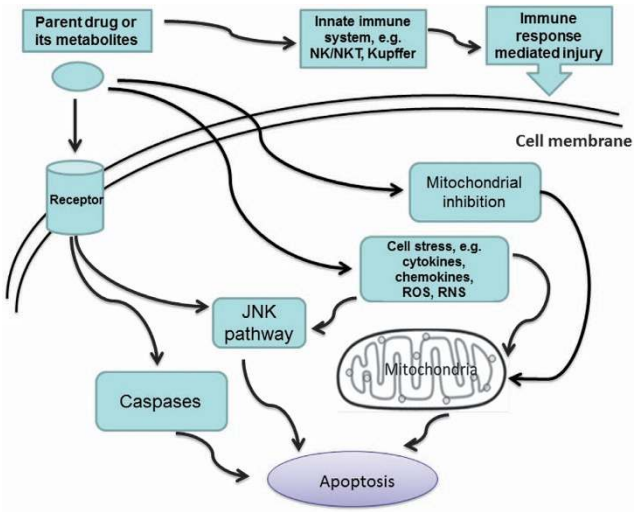

Figure 7 Potential mechanisms for drug-induced hepatic apoptosis. Pathogenesis of drug-induced liver injury (DILI) includes cell stress, mitochondrial impairment, and specific immune reactions. Parent drugs or their reactive metabolites can cause cell stress to produce cytokines, chemokines, ROS, and reactive nitrogen species (RNS). Current concepts emphasize the central role of mitochondria, which leads to apoptotic and/or necrotic cell death. Hepatocytes, Kupffer cells, and even endothelial cells all participate in DILI. Inflammatory mediators of innate immune system determine the outcome of DILI

Immunologic factors. Immune-mediated mechanism can be either an independent factor or an interactive factor to trigger the pathogenesis of liver injury. For instance, HCV persistent infection or exposure of self-antigen uncovered by the viral infection can stimulate immune reaction. The mechanisms that lead to the establishment of immune evasion and persistent infection are complex and beyond the scope of this review. The immune response has also involved hepatobiliary diseases such as PBC, PSC, and autoimmune hepatitis.[114] These diseases can also cause an accumulation of TBAs that exacerbate hepatic apoptosis. PBC is characterized by inflammatory destruction of small and medium size intrahepatic bile ducts, eventually leading to liver failure. PBC can be initiated by mechanisms that lead to loss of tolerance toward mitochondrial antigens. A proposed theory contains that (i) pre-existing genetic condition perpetuates the destruction of the biliary epithelium by the immune system; (ii) the modifications of mitochondrial autoantigens by infectious agents and/or xenobiotics; (iii) the unique apoptotic features of biliary epithelial cells (BECs) (Figure 8).[115] Autoimmune characteristics of PBC ensue from a multi-lineage loss of tolerance to PDC-E2 (E2 component of the pyruvate dehydrogenase complex). BECs handle PDC-E2 and attract immune attack by virtue of the unique biochemical mechanisms.[116] The apoptosis of BECs in PBC is secondary to the invasion of inflammatory cells. BECs have ability to phagocyte apoptotic BECs and present mitochondrial derived self-peptides.[117] The apoptosis of BECs may be a potential source of 'neo-antigens' that are responsible for activating autoreactive lymphocytes, as many autoantigens are selectively modified during apoptosis and may facilitate molecular mimicry or autoimmunity. PSC is

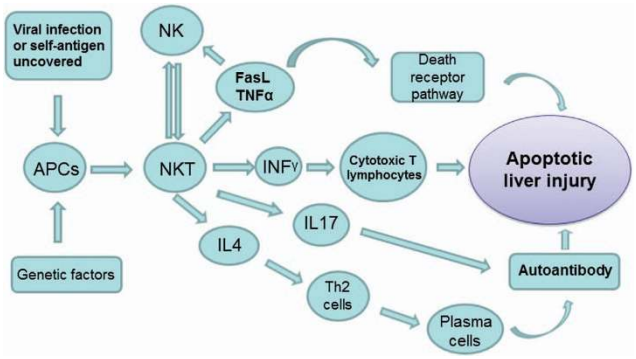

Figure 8 Pathogenesis of immune-mediated apoptotic liver injury. The liver has big population of Kupffer cells, dendritic cells, NK cells, and NKT cells. An innate immune response is initiated when antigens are presented by antigen-presenting cells (APCs) which then activate, directly and/or indirectly, NKT cells and other innate immune cells. The apoptosis of biliary epithelial cells (BECs) is a potential source of 'neo-antigens' that may be responsible for facilitating molecular mimicry or autoimmunity. NKT cells upregulate FasL on their surface which binds to the Fas receptor expressed on target hepatocytes, leading to apoptosis. Activation of NKT cells can also indirectly induce hepatocyte apoptosis through the release of cytokines, including IFNγ and TNFα. Th2 responses are also induced due to the presence of IL-4, which then promote maturation of B cells into plasma cells for the production of autoantibodies. Furthermore, IL-17 family has been linked to many immune/autoimmune-related diseases

another chronic liver disorder shown by inflammatory destruction of small intrahepatic bile ducts as well as extrahepatic bile ducts.[118] PSC is strongly related to inflammatory bowel disease, colorectal cancer, osteoporosis, and cholangiocarcinoma.[119–121] The role of specific gene deficiencies in the susceptibility to immune-mediated cholangiopathies is highlighted. Effector mechanisms of adaptive immunity lead to bile duct epithelial apoptosis. PSC as an immune-mediated liver disease is associated with apoptotic death as reflected by programmed cell death ligands and circulating apoptotic markers. In general, apoptosis can be initiated *via* an extrinsic pathway that is triggered by engagement of death receptors on the cell surface, or *via* an intrinsic pathway that is induced by mitochondrial injury and is influenced by members of the Bcl-2 family. In both pathways, effector caspases are finally activated, which cleave and degrade cell structures, resulting in the release of apoptotic products into the circulation. New diagnostic tests can detect these apoptotic markers that include nucleosomes, caspases, cytokeratin fragments, macrophage migration inhibitory factor, soluble intracellular adhesion molecule, natural killer cells group 2D, and programmed death ligands such as Fas and Fas ligands.[122] Concanavalin A (Con A)-induced liver injury is an experimental model of immune-mediated liver diseases.[123] Con A has high affinity toward the hepatic sinus. After Con A injection, high levels of the cytokines IL-2, TNFα, IFNγ, IL-6, GM-CSF, and IL-1 are found, which result in the activation of CD4 cells.[124] Con A-induced cytokines such as TNFα and IFNγ have direct implications for the induction of a broad hepatic apoptosis, which mimics clinical fulminant hepatic failure. Non-peptide caspase inhibitor not only protected hepatocytes from the fatal apoptogenic effect of Con A, but also suppressed the elevation of AST and ALT and IL-1 beta concentration.[125] Inducible NO synthase (iNOS) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) also involve Con A-induced autoimmune liver injury.[126,127] Immunopathogenesis and apoptotic mechanisms are crucial to develop therapeutic approaches for immune-mediated liver diseases.

Summary and Future Study

Mechanisms of hepatic apoptosis are scrutinized here. It is worth noting that (i) apoptosis cannot be thought of as an exclusive mechanism. Apoptosis is actually intermingled with other cell death modes such as necroptosis and necrosis. For instance, TRAIL-induced apoptosis could be switched into regulated necrosis (or necroptosis) in human HT29 and HepG2 cells under acidic extracellular condition. The role of TRAIL as inducer of necroptosis had also been shown in a mouse model of Con A-induced hepatitis, where death of hepatocytes was dependent on TRAIL and natural killer T cells.[128] Inhibition of apoptosis is not sufficient to prevent liver injury in some models. The role of necrosis may be more than that of apoptosis in cholestatic livers;[129] (ii) multiple signaling pathways should be considered even in single causative factor (e.g., virus). Viral infection can activate an apoptotic network, including death receptor-mediated cascade, ER stress, lysosomal permeabilization, ROS generation, and mitochondrial dysfunction. All of these mechanisms are intermixed each other. Probably, one mechanism dominates in a particular stage. For example, lysosomal involvement in cell death is an early event before mitochondrial permeabilization or caspase activation. Mitochondrial dysfunction is a prerequisite for hepatocyte apoptosis; (iii) pathophysiologic role of apoptosis is sophisticated by its contradictory facts. Current data indicate that blocking hepatic apoptosis (e.g., deletion of caspase-8) may also trigger an increased liver necrosis or necroptosis.[130,131] A lack of apoptosis or enhanced liver apoptosis may both result in hepatocellular cancer depending on the tissue environment.[132,133]

There are expectable perspectives for therapeutics of liver diseases. Inhibition of hepatic apoptosis can reverse or delay the progression of liver diseases, which had been demonstrated by pre-clinical models and clinical trials. The hydrophilic bile salt UDCA inhibited TBA-induced apoptosis. UDCA and its derivative have been using for a long time to treat cholestatic liver diseases, for example, PBC and PSC.[74] Caspase inhibitors may be a promising treatment option in patients with liver diseases. Caspase inhibitor GS-9450 induced a significant reduction in ALT level in NASH patients.[134] PF-03491390 treatment significantly diminished serum AST and ALT levels in patients with chronic HCV infection.[135] When IDN-6556 was administered in cold storage and flush solutions, the IDN-6556 could provide local therapeutic protection against cold ischemia/warm reperfusion (CI/WR)-mediated apoptotic injury during liver transplantation.[136] Oral IDN-6556 considerably lowered aminotransferase activity in HCV patients.[137] miRNAs have recently been found to represent another crucial regulatory layer overlaying and intersecting with transcriptional control mechanisms. The expression of miR-34a was markedly increased in alcohol-exposed liver or human primary hepatocytes.[138] miR-122 level correlated with scale of serum lipids in

NAFLD patients. In both HCV infection and NAFLD patient groups, serum levels of miR-122 and miR-34a correlated with liver enzyme levels, fibrosis stage, and inflammation activity.[96]

In summary, apoptosis has been obviously recognized as a prominent pathogenesis of liver diseases. Full details of apoptosis-related signaling network need to be clarified in future study. Especially, profiles of apoptotic/antiapoptotic regulatory targets and clinical use of these targets will be explored. The study on hepatic apoptosis can develop novel approaches for the treatment of liver diseases.

Conflict of Interest

The author declares no conflict of interest.

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