Apoptosis and non-alcoholic fatty liver diseases

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Abstract

The number of patients with nonalcoholic fatty liver diseases (NAFLD) including nonalcoholic steatohepatitis (NASH), has been increasing. NASH causes cirrhosis and hepatocellular carcinoma (HCC) and is one of the most serious health problems in the world. The mechanism through which NASH progresses is still largely unknown. Activation of caspases, Bcl-2 family proteins, and c-Jun N-terminal kinase-induced hepatocyte apoptosis plays a role in the activation of NAFLD/NASH. Apoptotic hepatocytes stimulate immune cells and hepatic stellate cells toward the progression of fibrosis in the liver through the production of inflammasomes and cytokines. Abnormalities in glucose and lipid metabolism as well as microbiota accelerate these processes. The production of reactive oxygen species, oxidative stress, and endoplasmic reticulum stress is also involved. Cell death, including apoptosis, seems very important in the progression of fibrosis in the liver through the production of inflammasomes and cytokines. Abnormalities in glucose and lipid metabolism as well as microbiota accelerate these processes. The production of reactive oxygen species, oxidative stress, and endoplasmic reticulum stress is also involved. Cell death, including apoptosis, seems very important in the progression of fibrosis in the liver through the production of inflammasomes and cytokines. Abnormalities in glucose and lipid metabolism as well as microbiota accelerate these processes. The production of reactive oxygen species, oxidative stress, and endoplasmic reticulum stress is also involved. Cell death, including apoptosis, seems very important in the progression of fibrosis in the liver through the production of inflammasomes and cytokines. Abnormalities in glucose and lipid metabolism as well as microbiota accelerate these processes. 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NAFLD, and the improvement of apoptosis could play a role in controlling the development of NASH. In this review, the association between apoptosis and NAFLD/NASH are discussed. This review could provide their knowledge, which plays a role in seeing the patients with NAFLD/NASH in daily clinical practice.

**Key words:** Apoptosis; Autophagy; c-Jun N-terminal kinase; Nonalcoholic fatty liver diseases; Nonalcoholic steatohepatitis

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Core tip: Nonalcoholic fatty liver diseases (NAFLD), including nonalcoholic steatohepatitis (NASH), are one of the most serious health issues. We searched articles written in English and listed on PubMed for the role of apoptosis in NASH. There are close association between apoptosis and NAFLD/NASH. Several inhibitors of apoptosis have been suggested as potential treatments for NASH, and some are now being tested in clinical trials. Therefore, we should focus on the role of apoptosis in the progression of NAFLD/NASH.

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### INTRODUCTION

The term nonalcoholic fatty liver disease (NAFLD) includes nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). The mechanism by which NASH progresses is still largely unknown. Liver biopsy is an important procedure for the diagnosis of NASH, with a typical case of NASH having hepatocellular steatosis and ballooning, mixed acute and chronic lobular inflammation, and zone 3 perisinusoidal and pericellular fibrosis[1]. These findings have also been observed in the liver of patients with alcoholic steatohepatitis. NAFLD cirrhosis and NAFLD-hepatocellular carcinoma (HCC) are the second leading cause of liver transplants in the USA[2]. Accordingly, there is increasing evidence that HCC can develop in the NASH[3].

In the liver of patients with alcoholic hepatitis, infiltrating polymorphonuclear leukocytes and apoptotic bodies derived from hepatocytes are observed[3]. A combination of environmental factors, host genetics, and gut microbiota can lead to an excess accumulation of fat in the hepatocytes, which can result in lipotoxicity and trigger hepatocyte cell death, liver inflammation, fibrosis, and pathological angiogenesis, resulting in NASH, cirrhosis, and HCC[4]. In this topical review, we will discuss apoptosis, which is involved in the development of NASH.

### APOPTOSIS AND THE ACTIVATION OF CASPASES IN THE PROGRESSION OF NASH

Feldstein et al[5] observed that terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive hepatocytes were significantly increased in the livers of NASH patients, compared to those from patients with alcoholic hepatitis or simple steatosis. Caspases have apoptotic functions as well as non-apoptotic functions. During apoptosis, the caspase cascade shapes the immunogenic properties of apoptosis[6]. Feldstein et al[5] found that active caspases 3 and 7 as well as the strong expression of Fas receptors in NASH specimens were strongly correlated with hepatocyte apoptosis and the progression of NASH. Caspase 3 activation and hepatocyte apoptosis are prominent features of different experimental models of NAFLD as well as human NAFLD and have been shown to be correlated with disease severity[7].

Caspase 3 is known to cleave several cellular substrates including cytokeratin-18 (CK-18), which is the major intermediate fragment protein in the liver[7]. Caspase 3 generated CK-18 fragments are an independent predictor of NASH in patients with suspected NAFLD[7,8]. Traffic-related air pollution has been shown to be associated with CK-18, a marker of hepatocellular apoptosis, in an overweight and obese pediatric population[9]. Mallory-Denk bodies (MDBs) are characteristic of both alcoholic and NASH and discriminate between the relatively benign simple steatosis and the more aggressive NASH. It has been shown that in genetically susceptible mice overexpressing CK-8, consumption of a high-fat diet (HFD) triggered hepatocellular injury, ballooning, apoptosis, inflammation, and MDB development[10].

Inhibition of hepatic apoptosis by pharmacological pan-caspase inhibitor VX-166 may reduce the development of fibrosis in mice with NASH[11,12]. Increases in active caspase 2, active caspase 3, and apoptosis were observed in the livers of patients with NASH[13]. Ballooned hepatocytes in NASH downregulate caspase 9, a pivotal caspase that executes the mitochondrial apoptosis pathway[14]. In rodents, a lack of caspase 8 expression in hepatocytes was shown to reduce the methionine-choline-deficient (MCD)-dependent increases in apoptosis, decreased the expression of pro-inflammatory cytokines, and reduced hepatic infiltration[15]. Caspase 8 may thus be critical for the pathogenesis of NASH.

Caspase 2 is an initiator caspase in lipid-induced cytotoxicity (lipaopoptosis), which plays a role in the pathogenesis of NASH[16]. Caspase 2 plays a role in lipid-induced hepatocyte apoptosis and is related to the production of apoptosis-associated fibrogenic factors[17]. Additionally, liver free coenzyme A content was shown to be reduced in mice with NASH. Decreased hepatic free coenzyme A content was associated with increased...
Liver injury in NASH patients is associated with apoptosis and NF-κB activation even though anti-apoptotic B-cell lymphoma 2 (Bcl-2) is strongly expressed. These changes are caspase-dependent. They are also associated with mitochondrial membrane depolarization and the release of cytochrome c, which activate the mitochondrial apoptosis pathways including activation of the proteins Bcl-2-associated X (Bax) and Bcl-2-interacting mediator of cell death (Bim) [33]. The upregulation of Bax and Bcl-2 expression may also play an important role in apoptosis in NAFLD [39], although it has been reported that NASH patients had significantly lower levels of anti-apoptotic protein Bcl-2 [34]. The degree of apoptosis was inversely correlated with the level of Bcl-2 [26].

Activation of endoplasmic reticulum (ER) stress-associated c-Jun N-terminal kinase (JNK) promotes apoptosis by modifying the expression and function of pro-apoptotic members of the Bcl-2 family such as Bcl-2 homology 3 (BH3) only protein Bim and p53-upregulated modulator of apoptosis (PUMA) [30]. PUMA promotes the activation of Bax and thus mitochondrial outer membrane permeabilization, which leads to the relocation of these pro-apoptotic mediators into cytosol [35]. Cazanave et al. [36] reported that miR-296-5p levels were inversely related to the BH3-only protein PUMA mRNA levels in human liver specimens, and that miR-296-5p regulates PUMA expression during hepatic lipopoapoptosis.

Transglutaminase 2 (TG2), which is induced in the nuclei of ethanol-treated hepatocytes, crosslinks and inactivates the transcription factor, SpI, which results in hepatic apoptosis [37]. In NASH patients, nuclear TG2 and crosslinked SpI formation were elevated. Additionally, activation of apoptosis inducing factor and a release of cytochrome c were observed [37]. Hypoxia, oxidative stress, and lipopoapoptosis could all influence the expression of mitochondrial-encoded NADH dehydrogenase (MT-ND3) in hepatocytes and MT-ND3 may play a role in the progression of hepatic steatosis [28]. Hepatocyte-specific c-Met deletion in hepatocytes was shown to trigger NASH progression. Increased apoptosis was a prominent feature in c-MetΔ(hepa) livers [39]. Intermittent high glucose levels under lipotoxicity could contribute to the development of NAFLD by increasing oxidative stress and hepatocyte apoptosis via changes in mitochondrial permeability and subsequent mitochondrial dysfunction [40].

Bid promotes liver fibrosis coupled with a reduction of inflammation in experimental NASH models. In these models, hepatocyte apoptosis triggered hepatic stellate cell activation as well as liver fibrosis [41]. Increased expression of hepatocellular carcinoma down-regulated mitochondrial carrier protein (HDMCP) was identified in NASH animal models and HFFA-72h cultured LO2 cells. The miR-146-HDMCP-downstream effector pathway is involved in NASH [42]. Collectively, previous studies have demonstrated that apoptosis resulting from mitochondrial injury is associated with the progression of NAFLD and NASH.

**BCL-2 FAMILY MEMBERS AND MITOCHONDRIA IN THE PROGRESSION OF NASH**

Liver injury in NASH patients is associated with apoptosis and NF-κB activation even though anti-apoptotic B-cell lymphoma 2 (Bcl-2) is strongly expressed. These changes are caspase-dependent. They are also associated with mitochondrial membrane depolarization and the release of cytochrome c, which activate the mitochondrial apoptosis pathways including activation of the proteins Bcl-2-associated X (Bax) and Bcl-2-interacting mediator of cell death (Bim) [33]. The upregulation of Bax and Bcl-2 expression may also play an important role in apoptosis in NAFLD [39], although it has been reported that NASH patients had significantly lower levels of anti-apoptotic protein Bcl-2 [34]. The degree of apoptosis was inversely correlated with the level of Bcl-2 [26].

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ACTIVATION OF JNK AND APOPTOSIS IN NASH

Monounsaturated and saturated fatty acids have been shown to induce cellular steatosis, apoptosis, and JNK activation in hepatocytes\(^{33}\). Steatotic hepatocytes from a murine NAFLD model were sensitive to TNF-\(\alpha\)-induced apoptosis via the ASK1–JNK signaling pathway\(^{43}\). Free fatty acids (FFA)-induced ER stress is associated with JNK activation, which has been well documented in human steatosis\(^{35}\). Mixed lineage kinase 3 (MLK) 3 is one of the mitogen-activated protein kinases (MAP3K) that mediate JNK activation in the liver. MLK3 is involved in human NASH through JNK activation\(^{44}\).

The interplay of p-JNK with mitochondrial Sab (Sh3bp5) leads to impaired respiration, production of reactive oxygen species (ROS), sustained JNK activation, apoptosis in condition of lipotoxicity, and ultimately contributes to the pathogenesis of NASH\(^{45}\). Dramatically reduced expression of cellular repressor of E1A-stimulated genes (CREG) and hyperactivated JNK1 signaling has been observed in the livers of NAFLD patients\(^{46}\). CREG is a robust suppressor of hepatic steatosis and metabolic disorders through its direct interaction with ASK1 and the subsequent inactivation of ASK1-JNK1 signaling\(^{46}\). Thus, JNK signaling pathways play an important role in the apoptosis of NAFLD and NASH.

APOTOPSIS, AND IMMUNE CELLS AND HEPATIC STELLATE CELLS

The complement cascade to clear apoptotic cells and promote liver regeneration is also involved in the progression of NAFLD and NASH\(^{47}\). Distant major histocompatibility complex class I-related chains A and B (MIC A/B) have been identified as ligands for the NK cell receptor G2D (NKG2D) in humans. Compared to controls, patients with NASH displayed increases in NKG2D and MIC A/B mRNA, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-death receptor 5 (DR5), CD95/Fas mRNA, and hepatocyte apoptosis\(^{48}\). These increases suggest that MIC A/B levels also affect the progression of NASH. TRAIL-producing natural killer (NK) cells actively promote a pro-inflammatory environment in the early stages of fatty liver disease, which suggests that this cell compartment may contribute to the progression of NASH\(^{49}\).

Proliferation of hepatic macrophages, and the subsequent production of pro-inflammatory cytokines, initiate inflammatory cascades, orchestrate the activities of transcription factors involved in lipid metabolism/ translocation, and modulate programmed cell death\(^{50}\). The macrophage activation marker-soluble CD163 was independently associated with the apoptosis marker CK-18 in Australian and Italian NAFLD patients\(^{51}\). Furthermore, down-modulation of NF-\(\kappa\)B1 stimulates the progression of NASH in mice by promoting natural killer T (NKT)-cell-mediated responses\(^{52}\). Macrophage scavenger receptors), which play a role in the activation of signal transduction pathways that regulate inflammation, apoptotic cell clearance, chemotraction and angiogenesis, are involved in both the early and advanced stages of NASH\(^{53}\).

Wobser et al\(^{54}\) has demonstrated that human hepatic stellate cells (HSCs) that were incubated with conditioned medium (CM) from steatotic hepatocytes and had fibrogenic activation and were resistant to apoptosis, which is important in the progression of fibrosis in chronic liver diseases\(^{54}\). Myeloperoxidase (MPO), a highly oxidative enzyme secreted by leukocytes, contributes to the activation of HSCs and is a part of a proapoptotic and profibrotic pathway of progression in NASH\(^{55}\). Thus, in patients with NAFLD and NASH, apoptotic hepatocytes stimulate immune cells and HSCs, which contributes to the progression of fibrosis in the liver.

PRO-INFLAMMATORY CYTOKINES AND CHEMOKINES

Feeding tumor necrosis factor (TNF) receptors 1 and 2 double-knock out mice (TNFΔDKO mice) with an MCD-diet for 8 weeks attenuated liver steatosis and fibrosis and also suppressed hepatic induction of TNF-\(\alpha\) vascular cell adhesion molecule 1, and intracellular adhesion molecule 1, compared to wild-type control mice\(^{6}\). These results suggest that blocking the signaling of TNF receptors 1 and 2 is a promising therapeutic target for patients with NASH\(^{56}\). The TNF receptor 1-signaling pathway plays a role in aggravating a state of "simple steatosis" towards a phenotype with "NASH"\(^{57}\). Cyclooxygenase (COX)-2 may promote hepatocellular apoptosis by interacting with TNF-\(\alpha\) and IL6 in rats with NASH\(^{56}\). COX-2 is highly expressed in NASH.

Lipoapoptotic supernatants stimulated monocyte migration to a similar magnitude as monocyte chemotactrant protein, CCL2 (MCP-1)\(^{58}\). The release of pannexin1-dependent pathophysiological eATP in lipoapoptosis can stimulate the migration of human monocytes in NASH\(^{59}\). In cultured Kupffer cells, cholesterol induced the expression of chemotact and inflammatory cytokines (CCL2 and CXCL2, and IL1\(\beta\), TNF and oncostatin M, respectively) and rendered hepatocytes more susceptible to apoptosis\(^{60}\). Lipids, which stimulate DR5, have been shown to induce the release of hepatocyte extracellular vesicles, which contain TRAILs. Lipids also induced the expression of IL1\(\beta\) and IL6 messenger RNAs in bone marrow-derived macrophages in mice\(^{61}\). The C-X-C motif chemokine 10 (CXCL10), which is known to be a pro-inflammation chemokine, was recently shown to play a pivotal role in the pathogenesis of NASH. By binding to its specific receptor CXCR3, CXCL10 recruits activated CXCR3+ T lymphocytes and macrophages to the parenchyma and promotes inflammation, apoptosis, and fibrosis\(^{52}\). The dsRNA receptor Nod-like receptor X1 and NLRP3
inflammasomes may be important in the development of NASH[63]. The function of receptor interacting protein kinase-3 (RIP3)-dependent “necroptosis” in NASH and NASH-induced fibrosis is currently unknown[64]. RIP3-dependent necroptosis controls NASH-induced liver fibrosis[64]. The absence of RIP3, a key mediator of necroptosis, exacerbates HFD-induced liver injury. This exacerbation is associated with increased inflammation and hepatocyte apoptosis as well as early fibrotic responses. These findings indicate that shifts in the mode of hepatocellular death can influence disease progression. Therefore, they may have therapeutic implications because manipulation of hepatocyte cell death pathways is currently considered to be a target for treatment of nonalcoholic fatty liver disease[65]. Thus, inflammasomes and cytokines induce apoptosis and respond to hepatocyte apoptosis in NAFLD and NASH.

**OXIDATIVE STRESS**

It has also been reported that oxidized phosphatidylcholine is localized in apoptotic hepatocytes in the livers of patients with the steatotic disorders, which indicates that oxidized phosphatidylcholine is formed in oxidatively damaged hepatocytes[66]. Transforming growth factor β (TGFβ) may regulate p53/p66shc signaling in both the progression of human NASH and ROS levels and apoptosis[67]. NAFLD patients with reticuloendothelial system (RES) iron have increased TUNEL staining and cellular oxidative stress[68]. RES iron has been shown to be associated with NASH as well as more-severe histologic features[68].

TGFβ signaling activates Smad- and TGFβ-activated kinase 1-dependent signaling and plays a role in regulating cell survival, proliferation, fibrosis, and tumorigenesis. In hepatocytes, TGFβ signaling contributes to hepatocyte death and lipid accumulation through Smad signaling and ROS production, leading to the development of NASH[69]. NOX isoforms, including NOX1, NOX2 and NOX4, and NOX-derived ROS have all been implicated in regulating HSC activation and hepatocyte apoptosis. Both HSC activation and hepatocyte apoptosis are essential steps for the initiation of liver fibrosis and its progression[70]. Mainstream cigarette smoke has been shown to be associated with the degree of oxidative stress and hepatocellular apoptosis in NASH mice[71].

Oxidative stress is central to the pathogenesis of NASH. ROS are characterized by oxidative stress. ROS are generated in several cellular sites and their production is influenced by multi-organ interactions. For fatty liver diseases, mitochondrial dysfunction is the main source of ROS and is closely related to endoplasmic reticulum stress. Both are caused by lipotoxicity and together these three factors form a cycle of progressive organelle damage that results in sterile inflammation and apoptosis[72].

**ER STRESS**

FFAs can increase ER stress, leading to nuclear NF-κB activation and TG2 induction through the pancreatic ER kinase (PERK)-dependent pathways[37,73] CCAAT/enhancer-binding protein homologous protein (CHOP) deficiency has been found to attenuate apoptosis, inflammation, fibrosis, and tumorigenesis in mice who are exposed to fat-loading conditions. This finding indicates CHOP promotes hepatocarcinogenesis in NASH[74]. The overexpression of hypoxia-inducible factor 1α (HIF-1α) has also been shown to blunt upregulation of the ER stress markers, CHOP and chaperone immunoglobulin heavy chain binding protein (GRP78/Bip), while knocking down HIF-1α increases the level of CHOP. These finding indicate that hepatocyte lipotoxicity is associated with decreased HIF-1α expression[75].

MiR-615-3p regulates lipoapoptosis by inhibiting CHOP and may be associated with the pathogenesis of NASH[76]. After exposure to saturated FFA, CHOP has been shown to induce hepatocyte cell apoptosis and inflammatory responses by activating NF-κB through a pathway involving the expression of IL1 receptor associated kinase 2. This activation results in the direct secretion of the cytokines IL8 and TNFα from hepatocytes[77]. Glucagon-like peptide-1 was found to protect against NAFLD by inactivating the ER stress-associated apoptosis pathway[78].

Loss of the unfolded protein response of regulator X-box binding protein 1 enhances injury in both in vivo and in vitro models of fatty liver injury[79]. Hepatocytes in a lipotoxic state ultimately undergo apoptosis through the upregulation of proteins involved in various pathways including PERK, CHOP, JNK, BIM, PUMA, and eventually caspases[80].

**AUTOPHAGY**

Expression of microtubule associated protein 1 light chain 3α (LC3-II), a hallmark of autophagic flux, was found to be markedly increased in liver specimens from patients with NASH. JNK1 promotes palmitic acid-induced lipoapoptosis, whereas JNK2 activates pro-survival autophagy and inhibits palmitic acid lipotoxicity[81]. Palmitate may induce autophagy by activating the PGC1α pathway in hepatocytes. Autophagy plays a protective role in palmitate-induced apoptosis in hepatocytes[82]. Tumor protein p53 binding protein 2 (ASPP2) is a pro-apoptotic member of the p53 binding protein family that inhibits autophagy[83]. Xie et al[83] reported that ASPP2 may participate in the lipid metabolism of non-alcoholic steatohepatitis. Mitochondrial uncoupling protein 2 (UCP2) also plays a role in the development of NASH[84]. Increasing UCP2 expression in hepatoma cells may contribute to cell autophagy and may inhibit apoptosis as result of fatty acid injury[84]. Cellular degradation of Kelch-like ECH-associated protein 1 through the progress of sequestosome (SQSTM)1/p62-dependent autophagy
activates JNK, upregulates expression of Bim and PUMA, and contributes to hepatocyte apoptosis induced by saturated FFAs. Parkin-mediated mitophagy may mitigate hepatocyte apoptosis, improve mitochondrial quality, and suppress steatosis (lipid accumulation) in animal models of alcoholic fatty liver disease. In rats treated with ethanol-enhanced hepatic mitophagy was associated with Parkin mitochondrial translocation, which was triggered by oxidative mitochondrial DNA damage. Rubicon is overexpressed and plays a pathogenic role in NAFLD by accelerating hepatocellular lipoapoptosis and lipid accumulation and inhibiting autophagy. SirT3 is a nicotinamide adenine dinucleotide-dependent deacetylase that is primarily located inside the mitochondria (SIRT3) negatively regulates autophagy, thereby enhancing the susceptibility of hepatocytes to SFA-induced cytoxicity.

Thus, ROS production, oxidative stress, and ER stress are all known to induce apoptosis. Autophagy modifies the progression of NAFLD and NASH and may have a protective role in hepatocyte apoptosis.

GLUCOSE METABOLISM AND APOPTOSIS

Hepatic insulin signaling is impaired in NASH patients, where downregulation of insulin-sensitive targets is associated with increased apoptosis and fibrogenesis. Hyperinsulinemia has been shown to alter nuclear transcriptional regulators of cholesterol homeostasis. This leads hepatic accumulation of free cholesterol, hepatic injury, and apoptosis in NASH patients.

Fibroblast growth factor (FGF)-21 is highly expressed in the liver and regulates glucose and lipid metabolism in rodents. Concentration of FGF-21 were found to be significantly and independently correlated with hepatic fat content and markers of hepatic apoptosis in obese youth. Another study found that FGF-21 mRNA expression in the human liver increased with steatosis grade and that its serum level is significantly elevated in adult NAFLD patients.

Intrahepatic expression of dipeptidyl peptidase-4 (DPP4) and circulating DPP4 (cDPP4) levels and its enzymatic activity are all increased in NAFLD. Circulating DPP4 activity correlates with measures of hepatocyte apoptosis and fibrosis in NAFLD in patients with type 2 diabetes mellitus and/or obesity. Senescence marker protein-30 is involved in both glucose metabolism disorder and NAFLD.

TRAIL receptor signaling was also found to be involved in the pathogenesis of NASH in mice with a genetic deletion of the TRAIL receptor. Furthermore, patients with NASH had significantly reduced plasma TRAIL concentrations compared to controls, patients with simple steatosis, or obese individuals. TRAIL protects against insulin resistance, NAFLD, and vascular inflammation. Increasing TRAIL levels may be an attractive therapeutic strategy for reducing symptoms of diabetes as well as liver and vascular injuries, which are commonly observed in individuals with NAFLD.

LIPID METABOLISM AND APOPTOSIS

The serine/threonine kinases, glycogen synthase kinase GSK-3α and GSK-3β, can participate in pro-apoptotic signaling during FFA-induced lipoapoptosis. More specifically, saturated fatty acids strongly induce hepatocyte apoptosis. Saturated fatty acids up-regulate the inflammasome in hepatocytes and lead to sensitization to LPS-induced inflammasome activation and inflammatory injury. Saturated fatty acids also induce hepatocyte apoptosis and the activation of caspase 8, which triggers the release of dangerous molecules.

Resistance to lipoapoptosis is, in part, due to an autocrine hedgehog signaling pathway. Farnesoid X receptor is a member of the nuclear receptor superfamily that plays a crucial role in bile acid, cholesterol, lipid, and glucose metabolism as well as apoptosis. It is also involved in the pathogenesis of NASH. The cellular inhibitor of apoptosis proteins 1 and 2 (cIAP-1 and cIAP-2) are potent inhibitors of death receptor-mediated apoptosis. Proteasomal degradation of cIAPs by FFA contributes to hepatocyte lipoapoptosis. Palmitate-induced lipoapoptosis is dependent on calcium-stimulated mitochondrial activation, which induces oxidative stress and hepatic cell lipotoxicity.

Free cholesterol accumulates in NASH patients but not in simple steatosis. Mitochondrial free cholesterol deposition causes hepatocyte apoptosis and necrosis by activating JNK. High-mobility-group-box 1 and toll-like receptor 4 are both involved in this activation mechanism. Cholesterol markedly promoted the apoptosis of steatosis HepG2 cells in vitro, likely through the up-regulation of expression of Bax and caspase 3. Palmitate activation by fatty acid transport protein 4 triggers hepatocellular apoptosis via altered phospholipid composition and steatosis by acylation into complex lipids. These complex lipids are involved in the development of NAFLD. E2F transcription factors are known regulators of the cell cycle, proliferation, apoptosis, and differentiation. E2F1 regulates lipid synthesis and glycosylation and thus contributes to the development of NAFLD.

Androgen-dependent proapoptotic polycystic ovarian syndrome (PCOS) may directly contribute to NAFLD progression in PCOS patients. A recent study gave a complementary fast food (FF) diet to a NASH mouse model, thus mimicking features of the metabolic syndrome. The study found that miR-21 levels increased in both the liver and muscle and expression of peroxisome proliferator-activated receptor α, a key miR-21 target, was decreased. In a typical model of NASH-associated liver damage, miR-21 ablation results in a progressive decrease in steatosis, inflammation and lipoapoptosis, with a subsequent impairment of fibrosis.
MICROBIOTA AND APOPTOSIS

Intestinal endotoxin [lipopolysaccharide (LPS)] augments liver injury in MCD mice\(^{[109]}\). In a recent study, a group of male C57BL/6 mice were fed with a MCD diet for 17 days, injected with LPS intraperitoneally, and sacrificed 6 h after LPS injection. The study found that LPS upregulated TNF-\(\alpha\) production, which induce hepatocyte apoptosis\(^{[110]}\). Palmitate and lysophosphatidylcholine (LPC) induced upregulation of the p53-upregulated modulator of apoptosis and cell-surface expression of the death receptor TNF-related apoptosis-inducing ligand receptor 2\(^{[111]}\). In part, microbiota may be involved in the progression of NAFLD and NASH through hepatocyte apoptosis.

CONCLUSION

Cell death, including apoptosis, seems important in the progression of NAFLD and NASH (Figure 1). Recently, several inhibitors of apoptosis have been suggested as potential treatments for NASH (Table 1)\(^{[112-124]}\). Clinical trials for the treatment of NASH are currently being conducted\(^{[125]}\) and some are targeting apoptosis in NASH patients. Increased hepatocyte apoptosis may distinguish NASH from NAFLD\(^{[126]}\). Repair responses may play an important role in controlling the disease severities of NASH\(^{[126]}\). We reviewed published articles related to this topic and discussed the importance of apoptosis in NAFLD and NASH.

REFERENCES

1. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999; 94: 2467-2474 [PMID: 10484010 DOI: 10.1111/j.1572-0241.1999.01377.x]

2. Bellentani S. The epidemiology of non-alcoholic fatty liver diseases/nonalcoholic steatohepatitis: The exact role of autophagy in nonalcoholic fatty liver diseases/nonalcoholic steatohepatitis remains unclear. NAFLD: Nonalcoholic fatty liver diseases; NASH: Nonalcoholic steatohepatitis.

Table 1  Inhibitors of apoptosis for nonalcoholic steatohepatitis

| Drug and reagents | Targets | Mechanism of action | Ref. |
|------------------|---------|---------------------|------|
| Triacsin C       | Intracellular long-chain acyl-CoA synthetases (ACSL) | Triacylglycerol (TAG) accumulation into lipid droplets | [112] |
| Ezetimibe        | AMPK phosphorylation | TFEB-mediated activation of autophagy and NLRP3 inflammasome inhibition | [113] |
| Baicalin          | Inhibition of NF-\(\kappa\)B | NF-\(\kappa\)B anti-inflammation signaling pathways | [114] |
| Granulocyte colony stimulating factor (G-CSF) | PISK/Akt | Activation of PISK and Akt pathway | [115] |
| Elafibranor (GFT505) | PPAR\(\alpha/\delta\) | Agonist of PPAR\(\alpha/\delta\) receptors | [116] |
| Isoquercitrin     | Dipeptidyl peptidase-IV (DPP-IV) | Activation of glucagon-like peptide-1 (GLP-1) | [117] |
| Activated carbon N-acetylcysteine (ACNAC) microcapsules | Telomerase | Improved telomerase activity | [118] |
| 3-Acetyl-oleanolic acid (3Ac-OA) | Glucose transporter type 2 (GLUT-2), low-density lipoprotein receptor (LDLR) | AMPK-related pathways | [119] |
| Meretrix oligopeptides (MMO) | NF-\(\kappa\)B | NF-\(\kappa\)B anti-inflammation signaling pathways | [120] |
| Seladelpar (MBX-8025) | Proliferator-activated receptor-\(\delta\) (PPAR-\(\delta\)) | Selective PPAR-\(\delta\) agonist | [121] |
| Resveratrol       | SirT1 | Antioxidant | [122] |
| TBE-31            | NF-E2 p45-related factor 2 (Nrf2) | Regulation of intracellular redox homeostasis | [124] |

Figure 1  Disease progression of nonalcoholic fatty liver diseases and nonalcoholic steatohepatitis. The exact role of autophagy in nonalcoholic fatty liver diseases/nonalcoholic steatohepatitis remains unclear. NAFLD: Nonalcoholic fatty liver diseases; NASH: Nonalcoholic steatohepatitis.
Kanda T et al. Cell death and NASH
disease. Liver Int 2017; 37 Suppl 1: 81-84 [PMID: 28052624 DOI: 10.1111/liv.13299]

Takahashi T, So-Wan T, Kamimura T, Asakura H. Infiltrating polymorphonuclear leukocytes and apoptotic bodies derived from hepatocytes but not from ballooning hepatocytes containing Mallory bodies show nuclear DNA fragmentation in alcoholic hepatitis. Alcohol Clin Exp Res 2000; 24: 68S-73S [PMID: 10803784]

Pevero D, Feldstein AE. Novel Molecular Mechanisms in the Development of Non-Alcoholic Steatohepatitis. Diabetes Metab J 2016; 40: 1-11 [PMID: 26921250 DOI: 10.4093/dmj.2016.40.1.1]

Feldstein AE, Canbay A, Angelo P, Taniat M, Burgart LJ, Lindor KD, Gores GJ. Hepatocyte apoptosis and fis expression are prominent features of human nonalcoholic steatohepatitis. Gastroenterology 2003; 125: 437-443 [PMID: 12891546]

McArthur K, Kile BT. Apoptotic Caspases: Multiple or Mistaken Identities? Trends Cell Biol 2018; 28: 475-493 [PMID: 29551258 DOI: 10.1016/j.tcb.2018.02.003]

Wieckowska A, Zein NN, Yerian LM, Lopez AR, McCullough AJ, Feldstein AE. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. Hepatology 2006; 44: 27-33 [PMID: 16799979]

Feldstein AE, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokertatin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. Hepatology 2009; 50: 1072-1078 [PMID: 19585618 DOI: 10.1002/hep.23050]

Hsieh S, Leaderer BP, Feldstein AE, Santoro N, McKay LA, Caprio S, McConnell R. Traffic-related air pollution associations with cytokeratin-18, a marker of hepatocellular apoptosis, in an obese and overweight paediatric population. Pediatr Obes 2018; 13: 342-347 [PMID: 28730729 DOI: 10.1111/jojo.12228]

Kucukoglu O, Guldenen N, Chen Y, Usachov V, El-Heliebi A, Haybaeck J, Denk H, Trautwein C, Strnad P. High-fat diet triggers cell death and NASH biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. J Hepatol 2014; 60: 169-178 [PMID: 24519272 DOI: 10.1002/hep.24762]

Witek RP, Stone WC, Karaca FG, Syn WK, Pereira TA, Agboula KM, Omenetti A, Jung Y, Teaberry V, Choi SS, Guy CD, Pollard J, Charlton P, Diehl AM. Pan-caspase inhibitor VX-166 reduces fibrosis in an animal model of nonalcoholic steatohepatitis. J Hepatol 2009; 50: 1421-1430 [PMID: 19676126 DOI: 10.1016/ hep.23167]

Anstee QM, Concas D, Kudo H, Levene A, Pollard J, Thomas HC, Thursz MR, Goldin RD. Impact of pan-caspase inhibitor VX-166 on liver disease. J Hepatol 2010; 53: 542-550 [PMID: 20557969 DOI: 10.1016/j.jhep.2010.03.016]

Ferreira DM, Castro RE, Machado MV, Evangelista T, Silvestre A, Costa A, Coutinho J, Carepa F, Cortez-Pinto H, Rodrigues CM. Apoptosis and insulin resistance in liver and peripheral tissues of morbidly obese patients is associated with different stages of non-alcoholic fatty liver disease. Diabetologia 2011; 54: 1788-1798 [PMID: 21455726 DOI: 10.1007/s00125-011-2130-8]

Kakisaka K, Cazanave SC, Werneburg NW, Razumilava N, Mertens JC, Bronk SF, Gores GJ. A hedgehog survival pathway regulates hepatic apoptosis and hepatocytes. J Biol Chem 2010; 285: 844-851 [PMID: 20303062 DOI: 10.1074/jbc.M109.043740]

Johnson ES, Lindblom KR, Robeson A, Stevens RD, Ilkayeva OR, Newgard CB, Kornbluth S, Andersen JL. Metabolomic profiling reveals a role for caspase-2 in lipopapostasis. J Biol Chem 2013; 288: 14463-14475 [PMID: 23553630 DOI: 10.1074/jbc.M112.437210]

Machado MV, Michelotti GA, Pereira Tde A, Boursier J, Kruger L, Swiderska-Syn M, Karaca G, Xie G, Guy CD, Bohine B, Lindblom KR, Johnson E, Kornbluth S, Diehl AM. Reduced lipopapostasis, hedgehog pathway activation and fibrosis in caspase-2 deficient mice with non-alcoholic steatohepatitis. Gut 2015; 64: 1148-1157 [PMID: 25053716 DOI: 10.1136/gutjnl-2014-307682]

Li CP, Ji HH, Hu SY, Li P, Zhong XL. Roles of Fas/FasL, Bel-2-Bax, and Caspase-8 in rat nonalcoholic fatty liver disease pathogenesis. Genet Mol Res 2014; 13: 3991-3999 [PMID: 24938610 DOI: 10.4238/2014.May.23.10]

Rogers AB, Weiss RS, Mauer KJ. The DNA damage checkpoint protein ATM promotes hepatocellular apoptosis and fibrosis in a mouse model of non-alcoholic fatty liver disease. Cell Cycle 2015; 14: e2091 [PMID: 26866272 DOI: 10.1083/ cddis.2016.12]

Lee SJ, Yoo JD, Choi SY, Kwon OS. The expression and secretion of vimentin in the progression of non-alcoholic steatohepatitis. J Hepatol 2016; 64: 85-94 [PMID: 25448926 DOI: 10.1016/j.jhep.2016.02.008]

Koyama N, Yamazaki T, Kanetsuki Y, Hirota J, Asai T, Mitsumoto M112.437210 Kanda T et al. Cell death and NASH
hepatocyte lipotoxicity. Inhibition of mitochondrial respiration in palmitic acid induced hepatocyte lipotoxicity. J Biol Chem 2006; 281: 12009-12011 [PMID: 16505490]

El Bassat H, Zidaa DH, Hasby EA, Nagy H, Abo Ryia MH. Apoptotic and anti-apoptotic seromarkers for assessment of disease severity of non-alcoholic steatohepatitis. Arab J Gastroenterol 2014; 15: 6-11 [PMID: 24630506 DOI: 10.1016/j.ajg.2014.01.009]

Ibrahim SH, Akazawa Y, Cazanave SC, Bronk SF, Elmi NA, Mott JL, Elmi NA, Bronk SF, Masuoka HC, De Mollerat Du Jeu X, Johnson CD, Nektaria A, Wobser H, Nakatsu G, Zhang X, Yu J. Pathophysiologicial mechanisms and therapeutic potentials of macrophages in non-alcoholic steatohepatitis. Expert Opin Ther Targets 2016; 20: 615-626 [PMID: 26609894 DOI: 10.1517/14728222.2016.1125883]
and lipid accumulation in nonalcoholic fatty liver disease in mice. *Hepatology* 2016; 64: 1994-2014 [PMID: 27637015 DOI: 10.1002/hep.28820]

88 Li S, Dufu X, Ning H, Song Q, Wei W, Zhang X, Shen C, Li J, Sun C, Song Z. Sirtuin 3 acts as a negative regulator of autophagy dictating hepatocyte susceptibility to lipotoxicity. *Hepatology* 2017; 66: 936-952 [PMID: 28437863 DOI: 10.1002/hep.29229]

89 García-Monzón C, Lo Iacono O, Mayoral R, González-Rodríguez A, Miquelena-Colina ME, Lozano-Rodríguez T, García-Pozo L, Vargas-Castrillón J, Casado M, Boscá L, Valverde AM, Martín-Sanz P. Hepatic insulin resistance is associated with increased apoptosis and fibrogenesis in nonalcoholic steatohepatitis and chronic hepatitis C. *J Hepatol* 2011; 54: 142-152 [PMID: 20888662 DOI: 10.1016/j.jhep.2010.06.021]

90 Van Rooyen DM, Larter CZ, Haigh WG, Yeh MM, Ioannou G, Kuver R, Lee SP, Teoh NC, Farrell GC. Hepatic free cholesterol accumulates in obese, diabetic mice and causes nonalcoholic steatohepatitis. *Gastroenterology* 2011; 141: 1393-1403, e1-1403.e5 [PMID: 21703998 DOI: 10.1053/j.gastro.2011.06.040]

91 Giannini C, Feldstein AE, Santoro N, Kim G, Kursawe R, Pierport B, Capeo S. Circulating levels of FGF-21 in obese youth: associations with liver fat content and markers of liver damage. *J Clin Endocrinol Metab* 2013; 98: 2903-3000 [PMID: 23626603 DOI: 10.1210/jc.2013-1250]

92 Li H, Fang Q, Gao F, Fan J, Zhou J, Wang X, Zhang H, Pan X, Bao Y, Xiang K, Xu A, Jia W. Fibroblast growth factor 21 levels are increased in nonalcoholic fatty liver disease patients and are correlated with hepatic triglyceride. *J Hepatol* 2010; 53: 934-940 [PMID: 20675007 DOI: 10.1016/j.jhep.2010.05.018]

93 Williams KH, Vieira De Ribeiro AJ, Prakoso E, Harith HH, Genner SW, Dang L, Cogger VC, Cortez-Pinto H. Baicalin attenuates non-alcoholic steatohepatitis by suppressing NLRP3 inflammasome inhibition. *J Hepatol* 2017; 66: 1767-1781 [PMID: 28918598 DOI: 10.1002/hep.29229]

94 Kondo Y, Ishigami A. Involvement of senescence marker protein-30 in glucose metabolism disorder and non-alcoholic fatty liver disease. *Geriatr Gerontol Int* 2016; 16 Suppl 1: 4-16 [PMID: 27018279 DOI: 10.1111/geri.12722]

95 Idrissova L, Malhi H, Werneburg NW, LeBrasseur NK, Bronk SF, Fingas C, Tchkoni T, Pirikashvila T, White TA, Stout MB, Hirsova P, Krishnan A, Liedtke C, Trautwein C, Finmborg N, El-Deisy WS, Kirkland JL, Gores GJ. TRAIL receptor deletion in mice suppresses the inflammation of nutrient excess. *J Clin Exp Pathol* 2013; 5: 809-819 [PMID: 25350590 DOI: 10.1186/1479-5876-15-819]

96 Carland SP, Harith HH, Genner SW, Dang L, Cogger VC, Vellozzi M, Di Bartolo BA, Thomas SR, Adams LA, Kavurma MM. Non-alcoholic fatty liver disease, vascular inflammation and insulin resistance are exacerbated by TRAIL receptor deletion in mice. *Sci Rep* 2017; 7: 1898 [PMID: 28507343 DOI: 10.1038/s41598-017-01221-4]

97 Machado MV, Cortez-Pinto H. Cell death and nonalcoholic steatohepatitis: what is ballooning relevant? *Expert Rev Gastroenterol Hepatol* 2011; 5: 213-222 [PMID: 21476916 DOI: 10.1586/egh.11.6]

98 CsaK T, Ganz M, Pesipis J, Kodyš K, Doležalová A, Szabo G. Fatty acid and endotoxin activate inflammosomes in mouse hepatocytes that release danger signals to stimulate immune cells. *Hepatology* 2011; 54: 133-144 [PMID: 21688066 DOI: 10.1002/hep.24341]

99 Henoa-Mejia J, Elina V, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, Kau AL, Eisenbarth SC, Jureczk MJ, Camporez JP, Shulman GI, Gordon JL, Hoffman HM, Flavell RA. Inflammosome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 2012; 482: 179-185 [PMID: 22297845 DOI: 10.1038/ nature10809]

100 Koutsounas I, Giaginis C, Theocharis S. Farnesoid X Receptor (FXR) from normal to malignant state. *Histol Histopathol* 2012; 27: 835-853 [PMID: 22648540 DOI: 10.14670/HH-27.835]
YJ. Granulocyte colony stimulating factor treatment in non-alcoholic fatty liver disease: beyond marrow cell mobilization. Oncotarget 2017; 8: 97965-97976 [PMID: 29228666 DOI: 10.18632/oncotarget.18967]

Issa D, Wattacheril J, Sanyal AJ. Treatment options for nonalcoholic steatohepatitis - a safety evaluation. Expert Opin Drug Saf 2017; 16: 903-913 [PMID: 28641031 DOI: 10.1080/14740338.2017.1343299]

Huang XL, He Y, Ji LL, Wang KY, Wang YL, Chen DF, Geng Y, OuYang P, Lai WM. Hepatoprotective potential of isoquercitrin against type 2 diabetes-induced hepatic injury in rats. Oncotarget 2017; 8: 101545-101559 [PMID: 29254185 DOI: 10.18632/oncotarget.21074]

Shi T, Yang X, Zhou H, Xi J, Sun J, Ke Y, Zhang J, Shao Y, Jiang X, Pan X, Liu S, Zhuang R. Activated carbon N-acetylcysteine microcapsule protects against nonalcoholic fatty liver disease in young rats via activating telomerase and inhibiting apoptosis. PLoS One 2018; 13: e0189856 [PMID: 29324774 DOI: 10.1371/journal.pone.0189856]

Ou-Yang Q, Xuan CX, Wang X, Luo HQ, Liu JE, Wang LL, Li TT, Chen YP, Liu J. 3-Acetyl-oleanolic acid ameliorates non-alcoholic fatty liver disease in high fat diet-treated rats by activating AMPK-related pathways. Acta Pharmacol Sin 2018; Epub ahead of print [PMID: 29345253 DOI: 10.1038/aps.2017.142]

Huang F, Wang J, Yu F, Tang Y, Ding G, Yang Z, Sun Y. Protective Effect of Meretrix meretrix Oligopeptides on High-Fat-Diet-Induced Non-Alcoholic Fatty Liver Disease in Mice. Mar Drugs 2018; 16: pii: E39 [PMID: 29360762 DOI: 10.3390/md16020039]

Haczeyni F, Wang H, Barn V, Mridha AR, Yeh MM, Haigh WG, Ioannou GN, Choi YJ, McWherter CA, Teoh NC, Farrell GC. The selective peroxisome proliferator-activated receptor-delta agonist seladelpar reverses nonalcoholic steatohepatitis pathology by abrogating lipotoxicity in diabetic obese mice. Hepatol Commun 2017; 1: 663-674 [PMID: 29404484 DOI: 10.1002/hep4.1072]

Hajighasem A, Farzanegi P, Mazaheri Z. Effects of combined therapy with resveratrol, continuous and interval exercises on apoptosis, oxidative stress, and inflammatory biomarkers in the liver of old rats with non-alcoholic fatty liver disease. Arch Physiol Biochem 2018; 1-8 [PMID: 29463133 DOI: 10.1080/13813455.2018.1441872]

Sharma RS, Harrison DJ, Kisielewski D, Cassidy DM, McNeilly AD, Gallagher JR, Walsh SV, Honda T, McCrimmon RJ, Dinkova-Kostova AT, Ashford MLJ, Dillon JF, Hayes JD. Experimental Nonalcoholic Steatohepatitis and Liver Fibrosis Are Ameliorated by Pharmacologic Activation of Nrf2 (NF-E2 p45-Related Factor 2). Cell Mol Gastroenterol Hepatol 2017; 5: 367-398 [PMID: 29552625 DOI: 10.1016/j.jcmgh.2017.11.016]

Nakamura M, Kanda T, Sasaki R, Haga Y, Jiang X, Wu S, Nakamoto S, Yokosuka O. MicroRNA-122 Inhibits the Production of Inflammatory Cytokines by Targeting the PKR Activator PACT in Human Hepatic Stellate Cells. PLoS One 2015; 10: e0144295 [PMID: 26636761 DOI: 10.1371/journal.pone.0144295]

Sumida Y, Yoneda M. Current and future pharmacological therapies for NAFLD/NASH. J Gastroenterol 2018; 53: 362-376 [PMID: 29247356 DOI: 10.1007/s00535-017-1415-1]

Jung Y, Diehl AM. Non-alcoholic steatohepatitis pathogenesis: role of repair in regulating the disease progression. Dig Dis 2010; 28: 225-228 [PMID: 20460916 DOI: 10.1159/000282092]

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