**Copyright Information of the Article Published Online**

|  |  |
| --- | --- |
| **TITLE** | Apoptosis and non-alcoholic fatty liver diseases |
| **AUTHOR(s)** | Tatsuo Kanda, Shunichi Matsuoka, Motomi Yamazaki, Toshikatsu Shibata, Kazushige Nirei, Hiroshi Takahashi, Tomohiro Kaneko, Mariko Fujisawa, Teruhisa Higuchi, Hitomi Nakamura, Naoki Matsumoto, Hiroaki Yamagami, Hiroo Imazu, Masahiro Ogawa, Kazumichi Kuroda and Mitsuhiko Moriyama |
| CITATION | Kanda T, Matsuoka S, Yamazaki M, Shibata T, Nirei K, Takahashi H, Kaneko T, Fujisawa M, Higuchi T, Nakamura H, Matsumoto N, Yamagami H, Ogawa M, Imazu H, Kuroda K, Moriyama M. Apoptosis and non-alcoholic fatty liver diseases. World J Gastroenterol 2018; 24(25): 2661-2672 |
| URL | http://www.wjgnet.com/1007-9327/full/v24/i25/2661.htm |
| DOI | http://dx.doi.org/10.3748/wjg.v24.i25.2661 |
| OPEN ACCESS | This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non-Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/ |
| 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. |
| KEY WORDS | Apoptosis, Autophagy, c-Jun N-terminal kinase, Nonalcoholic fatty liver diseases, and Nonalcoholic steatohepatitis |
| COPYRIGHT  | © The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved. |
| NAME OF JOURNAL | World Journal of Gastroenterology |
| ISSN | 1007-9327 |
| PUBLISHER | Baishideng Publishing Group Inc, 7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA |
| WEBSITE | Http://www.wjgnet.com |

Ms. wjg/20XX REVIEW

Apoptosis and non-alcoholic fatty liver diseases

Tatsuo Kanda, Shunichi Matsuoka, Motomi Yamazaki, Toshikatsu Shibata, Kazushige Nirei, Hiroshi Takahashi, Tomohiro Kaneko, Mariko Fujisawa, Teruhisa Higuchi, Hitomi Nakamura, Naoki Matsumoto, Hiroaki Yamagami, Masahiro Ogawa, Hiroo Imazu, Kazumichi Kuroda, Mitsuhiko Moriyama

Tatsuo Kanda, Shunichi Matsuoka, Motomi Yamazaki, Toshikatsu Shibata, Kazushige Nirei, Hiroshi Takahashi, Tomohiro Kaneko, Mariko Fujisawa, Teruhisa Higuchi, Hitomi Nakamura, Naoki Matsumoto, Hiroaki Yamagami, Masahiro Ogawa, Hiroo Imazu, Kazumichi Kuroda, Mitsuhiko Moriyama, Division of Gastroenterology and Hepatology, Department of Medicine, Nihon University School of Medicine, Itabashi-ku, Tokyo 173-8610, Japan

Author contributions: Kanda T designed research, performed research, and analyzed data; Kanda T, Matsuoka S, Yamazaki M, Shibata T, Nirei K, Takahashi H, Kaneko T, Fujisawa M, Higuchi T, Nakamura H, Matsumoto N, Yamagami H, Ogawa M, Imazu H, Kuroda K, and Moriyama M wrote the paper.

Correspondence to: Tatsuo Kanda, MD, PhD, Associate Professor, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Nihon University School of Medicine, 30-1 Oyaguchi-Kamicho, Itabashi-ku, Tokyo 173-8610, Japan. kanda2t @yahoo.co.jp

Telephone: +81-3-39728111 Fax: +81-3-39568496

Received: April 3, 2018 Revised: June 4, 2018 Accepted: June 22, 2018

Published online: July 7, 2018

**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 NAFLD and NASH. Recently, inhibitors of apoptosis have been developed as drugs for the treatment of NASH and may prevent cirrhosis and HCC. Increased hepatocyte apoptosis may distinguish NASH from 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

Kanda T, Matsuoka S, Yamazaki M, Shibata T, Nirei K, Takahashi H, Kaneko T, Fujisawa M, Higuchi T, Nakamura H, Matsumoto N, Yamagami H, Ogawa M, Imazu H, Kuroda K, Moriyama M. Apoptosis and non-alcoholic fatty liver diseases. *World J Gastroenterol* 2018; 24(25): 2661-2672 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i25/2661.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i25.2661

**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.

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[2].

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[5].

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 (lipoapoptosis), 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 caspase 2 activity and correlated with more severe liver cell apoptosis, inflammation, and fibrosis[18].

It has been reported that Fas, Fas ligand (FasL), and caspase 8 mRNA activation are important contributing factors to NAFLD[19]. Another study showed that children with NASH had significantly higher levels of soluble Fas and soluble FasL than those in the “not NASH” group[20]. Fas apoptosis inhibitory molecule (FAIM), a ubiquitously expressed antiapoptotic protein, functions as a mediator of Akt signaling[21]. Loss of FAIM leads to spontaneous obesity and hepatic steatosis[21].

Hepatic cell apoptosis is associated with miR-34a/Sirtuin 1 (SIRT1)/p53 signaling in NASH[22]. p53 and its transcriptional target, miR34a, have been shown to be involved in the pathogenesis of fatty liver. The p53 inhibitor, pifithrin--p-nitro, was shown to attenuate steatosis, associated oxidative stress, and apoptosis in murine models of NAFLD[23]. The DNA damage checkpoint protein Ataxia telangiectasia mutated pathway plays a role in the response to hepatic fat accumulation and promotes hepatocellular apoptosis and fibrosis in mice models of NAFLD[24]. Massive hepatic progenitor cell expansion, especially in children with NASH, is associated with the degree of liver injury, hepatocyte apoptosis, and cell-cycle arrest[25].

Increased vimentin fragment levels are known to indicate the existence of substantial hepatocellular apoptosis in the progression of NASH[26]. Levels of the augmenter of liver regeneration (ALR) protein were lower in liver tissues from patients with advanced alcoholic liver disease and nonalcoholic steatohepatitis than in liver tissues from controls[27]. Levels of steatosis and apoptosis were reduced in mice with a liver-specific deletion of ALR[27]. Impairment of the formation of a newly discovered ubiquitin ligase complex called linear ubiquitin chain assembly complex, has been shown to result in insufficient NF-B activation and may thus be one of the molecular mechanisms underlying the enhanced apoptotic response of hepatocytes in NASH mouse models[28].

IgM-free apoptosis inhibitor of macrophage serum levels appear to be a sensitive diagnostic marker for NASH-HCC[29]. Activation of apoptosis signal-regulating kinase 1 (ASK1) in hepatocytes is a key step in the progression of nonalcoholic steatohepatitis[30]. Additionally, tumor necrosis factor alpha-induced protein 3 directly interacts with and deubiquitinates ASK1 in hepatocytes[30].

Thus, the activation of caspases and other molecules that are involved in apoptosis are frequently observed in the livers of NASH patients and may be related to 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-B activation even though anti-apoptotic B-cell lymphoma 2 (Bcl-2) is strongly expressed[31,32]. 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 be play an important role in apoptosis in NAFLD[19], 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[34].

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)[35]. 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 lipoapoptosis.

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 lipoapoptosis 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[38]. 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 L02 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.

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--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.

APOPTOSIS, 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 dysplayed 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-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, chemoattraction 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 (TNFRDKO mice) with an MCD-diet for 8 weeks attenuated liver steatosis and fibrosis and also suppressed hepatic induction of TNF-, 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- and IL6 in rats with NASH[58]. COX-2 is highly expressed in NASH.

Lipoapoptotic supernatants stimulated monocyte migration to a similar magnitude as monocyte che­moattractant protein, CCL2 (MCP-1)[59]. 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 chemotactic and inflammatory cytokines (CCL2 and CXCL2, and IL1, 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 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[62].

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 (TGF) 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].

TGF signaling activates Smad- and TGF-activated kinase 1-dependent signaling and plays a role in regulating cell survival, proliferation, fibrosis, and tumorigenesis. In hepatocytes, TGF 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-B 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 1 (HIF-1) 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-1 increases the level of CHOP. These finding indicate that hepatocyte lipotoxicity is associated with decreased HIF-1 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-B through a pathway involving the expression of IL1 receptor associated kinase 2. This activation results in the direct secretion of the cytokines IL8 and TNF 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 3 (LC3)-II, a hallmark of autophagic flux, was founded 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 PKC 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 sequestrosome (SQSTM)1/p62-dependent autophagy activates JNK, upregulates expression of Bim and PUMA, and contributes to hepatocyte apoptosis induced by saturated FFAs[85]. Parkin-mediated mitophagy may mitigate hepatocyte apoptosis, improve mitochondrial quality, and suppress steatosis (lipid accumulation) in animal models of alcoholic fatty liver disease[86]. In rats treated with ethanol-enhanced hepatic mitophagy was associated with Parkin mitochondrial translocation, which was triggered by oxidative mitochondrial DNA damage[86]. Rubicon is overexpressed and plays a pathogenic role in NAFLD by accelerating hepatocellular lipoapoptosis and lipid accumulation and inhibiting autophagy[87]. Sirtuin 3 (SIRT3) is a nicotinamide adenine dinucleotide-dependent deacetylase that is primarily located inside the mitochondria[88]. SIRT3 negatively regulates autophagy, thereby enhancing the susceptibility of hepatocytes to SFA-induced cytotoxicity[88].

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[89]. 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[90].

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[91]. 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[92].

Intrahepatic expression of dipeptidyl peptidase-4 (DPP4) and circulating DPP4 (cDPP4) levels and its enzymatic activity are all increased in NAFLD[93]. Circulating DPP4 activity correlates with measures of hepatocyte apoptosis and fibrosis in NAFLD in patients with type 2 diabetes mellitus and/or obesity[93]. Senescence marker protein-30 is involved in both glucose metabolism disorder and NAFLD[94].

TRAIL receptor signaling was also found to be involved in the pathogenesis of NASH in mice with a genetic deletion of the TRAIL receptor[95]. Furthermore, patients with NASH had significantly reduced plasma TRAIL concentrations compared to controls, patients with simple steatosis, or obese individuals[96]. 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[96].

LIPID METABOLISM AND APOPTOSIS

The serine/threonine kinases, glycogen synthase kinase GSK-3 and GSK-3, can participate in pro-apoptotic signaling during FFA-induced lipoapoptosis[35]. More specifically, saturated fatty acids strongly induce hepatocyte apoptosis[97]. Saturated fatty acids up-regulate the inflammasome in hepatocytes and lead to sensitization to LPS-induced inflammasome activation and inflammatory injury[98,99]. Saturated fatty acids also induce hepatocyte apoptosis and the activation of caspase 8, which triggers the release of dangerous molecules[98].

Resistance to lipoapoptosis is, in part, due to an autocrine hedgehog signaling pathway[14]. 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[100]. 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[101]. Palmitate-induced lipoapoptosis is dependent on calcium-stimulated mitochondrial activation, which induces oxidative stress and hepatic cell lipotoxicity[102].

Free cholesterol accumulates in NASH patients but not in simple steatosis. Mitochondrial free cholesterol deposition causes hepatocyte apoptosis and necrosis by activating JNK1[103]. High-mobility-group-box 1 and toll-like receptor 4 are both involved in this activation mechanism[103]. Cholesterol markedly promoted the apoptosis of steatosis HepG2 cells *in vitro*, likely through the up-regulation of expression of Bax and caspase 3[104]. Palmitate activation by fatty acid transport protein 4 triggers hepatocellular apoptosis via altered phospholipid composition and steatosis by acylation into complex lipids[105]. These complex lipids are involved in the development of NAFLD[105]. E2F transcription factors are known regulators of the cell cycle, proliferation, apoptosis, and differentiation. E2F1 regulates lipid synthesis and glycolysis and thus contributes to the development of NAFLD[106].

Androgen-dependent proapoptotic polycystic ovarian syndrome (PCOS) may directly contribute to NAFLD progression in PCOS patients[107]. 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 , a key miR-21 target, was decreased[108]. 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[108].

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- 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 disease. *Liver Int* 2017; **37** Suppl 1: 81-84 [PMID: 28052624 DOI: 10.1111/liv.13299]

3 **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]

4 **Povero D**, Feldstein AE. Novel Molecular Mechanisms in the Development of Non-Alcoholic Steatohepatitis. *Diabetes Metab J* 2016; **40**: 1-11 [PMID: 26912150 DOI: 10.4093/dmj.2016.40.1.1]

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

6 **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]

7 **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]

8 **Feldstein AE**, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokeratin-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]

9 **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 overweight and obese paediatric population. *Pediatr Obes* 2018; **13**: 342-347 [PMID: 28730729 DOI: 10.1111/ijpo.12228]

10 **Kucukoglu O**, Guldiken N, Chen Y, Usachov V, El-Heliebi A, Haybaeck J, Denk H, Trautwein C, Strnad P. High-fat diet triggers Mallory-Denk body formation through misfolding and crosslinking of excess keratin 8. *Hepatology* 2014; **60**: 169-178 [PMID: 24519272 DOI: 10.1002/hep.27068]

11 **Witek RP**, Stone WC, Karaca FG, Syn WK, Pereira TA, Agboola 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. *Hepatology* 2009; **50**: 1421-1430 [PMID: 19676126 DOI: 10.1002/hep.23167]

12 **Anstee QM**, Concas D, Kudo H, Levene A, Pollard J, Charlton P, Thomas HC, Thursz MR, Goldin RD. Impact of pan-caspase inhibition in animal models of established steatosis and non-alcoholic steatohepatitis. *J Hepatol* 2010; **53**: 542-550 [PMID: 20557969 DOI: 10.1016/j.jhep.2010.03.016]

13 **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]

14 **Kakisaka K**, Cazanave SC, Werneburg NW, Razumilava N, Mertens JC, Bronk SF, Gores GJ. A hedgehog survival pathway in ‘undead’ lipotoxic hepatocytes. *J Hepatol* 2012; **57**: 844-851 [PMID: 22641094 DOI: 10.1016/j.jhep.2012.05.011]

15 **Hatting M**, Zhao G, Schumacher F, Sellge G, Al Masaoudi M, Gaβler N, Boekschoten M, Müller M, Liedtke C, Cubero FJ, Trautwein C. Hepatocyte caspase-8 is an essential modulator of steatohepatitis in rodents. *Hepatology* 2013; **57**: 2189-2201 [PMID: 23339067 DOI: 10.1002/hep.26271]

16 **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 lipoapoptosis. *J Biol Chem* 2013; **288**: 14463-14475 [PMID: 23553630 DOI: 10.1074/jbc.M112.437210]

17 **Machado MV**, Michelotti GA, Pereira Tde A, Boursier J, Kruger L, Swiderska-Syn M, Karaca G, Xie G, Guy CD, Bohinc B, Lindblom KR, Johnson E, Kornbluth S, Diehl AM. Reduced lipoapoptosis, 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-2014-307362]

18 **Machado MV**, Kruger L, Jewell ML, Michelotti GA, Pereira Tde A, Xie G, Moylan CA, Diehl AM. Vitamin B5 and N-Acetylcysteine in Nonalcoholic Steatohepatitis: A Preclinical Study in a Dietary Mouse Model. *Dig Dis Sci* 2016; **61**: 137-148 [PMID: 26403427 DOI: 10.1007/s10620-015-3871-x]

19 **Li CP**, Li JH, He SY, Li P, Zhong XL. Roles of Fas/Fasl, Bcl-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]

20 **Alkhouri N**, Alisi A, Okwu V, Matloob A, Ferrari F, Crudele A, De Vito R, Lopez R, Feldstein AE, Nobili V. Circulating Soluble Fas and Fas Ligand Levels Are Elevated in Children with Nonalcoholic Steatohepatitis. *Dig Dis Sci* 2015; **60**: 2353-2359 [PMID: 25764498 DOI: 10.1007/s10620-015-3614-z]

21 **Huo J**, Ma Y, Liu JJ, Ho YS, Liu S, Soh LY, Chen S, Xu S, Han W, Hong A, Lim SC, Lam KP. Loss of Fas apoptosis inhibitory molecule leads to spontaneous obesity and hepatosteatosis. *Cell Death Dis* 2016; **7**: e2091 [PMID: 26866272 DOI: 10.1038/cddis.2016.12]

22 **Castro RE**, Ferreira DM, Afonso MB, Borralho PM, Machado MV, Cortez-Pinto H, Rodrigues CM. miR-34a/SIRT1/p53 is suppressed by ursodeoxycholic acid in the rat liver and activated by disease severity in human non-alcoholic fatty liver disease. *J Hepatol* 2013; **58**: 119-125 [PMID: 22902550 DOI: 10.1016/j.jhep.2012.08.008]

23 **Derdak Z**, Villegas KA, Harb R, Wu AM, Sousa A, Wands JR. Inhibition of p53 attenuates steatosis and liver injury in a mouse model of non-alcoholic fatty liver disease. *J Hepatol* 2013; **58**: 785-791 [PMID: 23211317 DOI: 10.1016/j.jhep.2012.11.042]

24 **Daugherity EK**, Balmus G, Al Saei A, Moore ES, Abi Abdallah D, Rogers AB, Weiss RS, Maurer KJ. The DNA damage checkpoint protein ATM promotes hepatocellular apoptosis and fibrosis in a mouse model of non-alcoholic fatty liver disease. *Cell Cycle* 2012; **11**: 1918-1928 [PMID: 22544329 DOI: 10.4161/cc.20259]

25 **Nobili V**, Carpino G, Alisi A, Franchitto A, Alpini G, De Vito R, Onori P, Alvaro D, Gaudio E. Hepatic progenitor cells activation, fibrosis, and adipokines production in pediatric nonalcoholic fatty liver disease. *Hepatology* 2012; **56**: 2142-2153 [PMID: 22467277 DOI: 10.1002/hep.25742]

26 **Lee SJ**, Yoo JD, Choi SY, Kwon OS. The expression and secretion of vimentin in the progression of non-alcoholic steatohepatitis. *BMB Rep* 2014; **47**: 457-462 [PMID: 24325816]

27 **Gandhi CR**, Chaillet JR, Nalesnik MA, Kumar S, Dangi A, Demetris AJ, Ferrell R, Wu T, Divanovic S, Stankeiwicz T, Shaffer B, Stolz DB, Harvey SA, Wang J, Starzl TE. Liver-specific deletion of augmenter of liver regeneration accelerates development of steatohepatitis and hepatocellular carcinoma in mice. *Gastroenterology* 2015; **148**: 379-391.e4 [PMID: 25448926 DOI: 10.1053/j.gastro.2014.10.008]

28 **Matsunaga Y**, Nakatsu Y, Fukushima T, Okubo H, Iwashita M, Sakoda H, Fujishiro M, Yamamotoya T, Kushiyama A, Takahashi S, Tsuchiya Y, Kamata H, Tokunaga F, Iwai K, Asano T. LUBAC Formation Is Impaired in the Livers of Mice with MCD-Dependent Nonalcoholic Steatohepatitis. *Mediators Inflamm* 2015; **2015**: 125380 [PMID: 26170532 DOI: 10.1155/2015/125380]

29 **Koyama N**, Yamazaki T, Kanetsuki Y, Hirota J, Asai T, Mitsumoto Y, Mizuno M, Shima T, Kanbara Y, Arai S, Miyazaki T, Okanoue T. Activation of apoptosis inhibitor of macrophage is a sensitive diagnostic marker for NASH-associated hepatocellular carcinoma. *J Gastroenterol* 2018; **53**: 770-779 [PMID: 29086016 DOI: 10.1007/s00535-017-1398-y]

30 **Zhang P**, Wang PX, Zhao LP, Zhang X, Ji YX, Zhang XJ, Fang C, Lu YX, Yang X, Gao MM, Zhang Y, Tian S, Zhu XY, Gong J, Ma XL, Li F, Wang Z, Huang Z, She ZG, Li H. The deubiquitinating enzyme TNFAIP3 mediates inactivation of hepatic ASK1 and ameliorates nonalcoholic steatohepatitis. *Nat Med* 2018; **24**: 84-94 [PMID: 29227477 DOI: 10.1038/nm.4453]

31 **Ramalho RM**, Cortez-Pinto H, Castro RE, Solá S, Costa A, Moura MC, Camilo ME, Rodrigues CM. Apoptosis and Bcl-2 expression in the livers of patients with steatohepatitis. *Eur J Gastroenterol Hepatol* 2006; **18**: 21-29 [PMID: 16357615]

32 **Ribeiro PS**, Cortez-Pinto H, Solá S, Castro RE, Ramalho RM, Baptista A, Moura MC, Camilo ME, Rodrigues CM. Hepatocyte apoptosis, expression of death receptors, and activation of NF-kappaB in the liver of nonalcoholic and alcoholic steatohepatitis patients. *Am J Gastroenterol* 2004; **99**: 1708-1717 [PMID: 15330907]

33 **Malhi H**, Bronk SF, Werneburg NW, Gores GJ. Free fatty acids induce JNK-dependent hepatocyte lipoapoptosis. *J Biol Chem* 2006; **281**: 12093-12101 [PMID: 16505490]

34 **El Bassat H**, Ziada 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]

35 **Ibrahim SH**, Akazawa Y, Cazanave SC, Bronk SF, Elmi NA, Werneburg NW, Billadeau DD, Gores GJ. Glycogen synthase kinase-3 (GSK-3) inhibition attenuates hepatocyte lipoapoptosis. *J Hepatol* 2011; **54**: 765-772 [PMID: 21147505 DOI: 10.1016/j.jhep.2010.09.039]

36 **Cazanave SC**, Mott JL, Elmi NA, Bronk SF, Masuoka HC, Charlton MR, Gores GJ. A role for miR-296 in the regulation of lipoapoptosis by targeting PUMA. *J Lipid Res* 2011; **52**: 1517-1525 [PMID: 21633093 DOI: 10.1194/jlr.M014654]

37 **Kuo TF**, Tatsukawa H, Matsuura T, Nagatsuma K, Hirose S, Kojima S. Free fatty acids induce transglutaminase 2-dependent apoptosis in hepatocytes via ER stress-stimulated PERK pathways. *J Cell Physiol* 2012; **227**: 1130-1137 [PMID: 21567402 DOI: 10.1002/jcp.22833]

38 **Wang HN**, Chen HD, Chen KY, Xiao JF, He K, Xiang GA, Xie X. Highly expressed MT-ND3 positively associated with histological severity of hepatic steatosis. *APMIS* 2014; **122**: 443-451 [PMID: 24020820 DOI: 10.1111/apm.12166]

39 **Kroy DC**, Schumacher F, Ramadori P, Hatting M, Bergheim I, Gassler N, Boekschoten MV, Müller M, Streetz KL, Trautwein C. Hepatocyte specific deletion of c-Met leads to the development of severe non-alcoholic steatohepatitis in mice. *J Hepatol* 2014; **61**: 883-890 [PMID: 24845607 DOI: 10.1016/j.jhep.2014.05.019]

40 **Yin X**, Zheng F, Pan Q, Zhang S, Yu D, Xu Z, Li H. Glucose fluctuation increased hepatocyte apoptosis under lipotoxicity and the involvement of mitochondrial permeability transition opening. *J Mol Endocrinol* 2015; **55**: 169-181 [PMID: 26464382 DOI: 10.1530/JME-15-0101]

41 **Eguchi A**, De Mollerat Du Jeu X, Johnson CD, Nektaria A, Feldstein AE. Liver Bid suppression for treatment of fibrosis associated with non-alcoholic steatohepatitis. *J Hepatol* 2016; **64**: 699-707 [PMID: 26555271 DOI: 10.1016/j.jhep.2015.11.002]

42 **Jin X**, Liu J, Chen YP, Xiang Z, Ding JX, Li YM. Effect of miR-146 targeted HDMCP up-regulation in the pathogenesis of nonalcoholic steatohepatitis. *PLoS One* 2017; **12**: e0174218 [PMID: 28346483 DOI: 10.1371/journal.pone.0174218]

43 **Zhang W**, Kudo H, Kawai K, Fujisaka S, Usui I, Sugiyama T, Tsukada K, Chen N, Takahara T. Tumor necrosis factor-alpha accelerates apoptosis of steatotic hepatocytes from a murine model of non-alcoholic fatty liver disease. *Biochem Biophys Res Commun* 2010; **391**: 1731-1736 [PMID: 20043871 DOI: 10.1016/j.bbrc.2009.12.144]

44 **Ibrahim SH**, Gores GJ, Hirsova P, Kirby M, Miles L, Jaeschke A, Kohli R. Mixed lineage kinase 3 deficient mice are protected against the high fat high carbohydrate diet-induced steatohepatitis. *Liver Int* 2014; **34**: 427-437 [PMID: 24256559 DOI: 10.1111/liv.12353]

45 **Win S**, Than TA, Le BH, García-Ruiz C, Fernandez-Checa JC, Kaplowitz N. Sab (Sh3bp5) dependence of JNK mediated inhibition of mitochondrial respiration in palmitic acid induced hepatocyte lipotoxicity. *J Hepatol* 2015; **62**: 1367-1374 [PMID: 25666017 DOI: 10.1016/j.jhep.2015.01.032]

46 **Zhang QY**, Zhao LP, Tian XX, Yan CH, Li Y, Liu YX, Wang PX, Zhang XJ, Han YL. The novel intracellular protein CREG inhibits hepatic steatosis, obesity, and insulin resistance. *Hepatology* 2017; **66**: 834-854 [PMID: 28508477 DOI: 10.1002/hep.29257]

47 **Rensen SS**, Slaats Y, Driessen A, Peutz-Kootstra CJ, Nijhuis J, Steffensen R, Greve JW, Buurman WA. Activation of the complement system in human nonalcoholic fatty liver disease. *Hepatology* 2009; **50**: 1809-1817 [PMID: 19821522 DOI: 10.1002/hep.23228]

48 **Kahraman A**, Schlattjan M, Kocabayoglu P, Yildiz-Meziletoglu S, Schlensak M, Fingas CD, Wedemeyer I, Marquitan G, Gieseler RK, Baba HA, Gerken G, Canbay A. Major histocompatibility complex class I-related chains A and B (MIC A/B): a novel role in nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 92-102 [PMID: 19998387 DOI: 10.1002/hep.23253]

49 **Gomez-Santos L**, Luka Z, Wagner C, Fernandez-Alvarez S, Lu SC, Mato JM, Martinez-Chantar ML, Beraza N. Inhibition of natural killer cells protects the liver against acute injury in the absence of glycine N-methyltransferase. *Hepatology* 2012; **56**: 747-759 [PMID: 22392635 DOI: 10.1002/hep.25694]

50 **Wu R**, Nakatsu G, Zhang X, Yu J. Pathophysiological 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]

51 **Kazankov K**, Barrera F, Møller HJ, Rosso C, Bugianesi E, David E, Ibrahim Kamal Jouness R, Esmaili S, Eslam M, McLeod D, Bibby BM, Vilstrup H, George J, Grønbaek H. The macrophage activation marker sCD163 is associated with morphological disease stages in patients with non-alcoholic fatty liver disease. *Liver Int* 2016; **36**: 1549-1557 [PMID: 27102725 DOI: 10.1111/liv.13150]

52 **Locatelli I**, Sutti S, Vacchiano M, Bozzola C, Albano E. NF-κB1 deficiency stimulates the progression of non-alcoholic steatohepatitis (NASH) in mice by promoting NKT-cell-mediated responses. *Clin Sci* (Lond) 2013; **124**: 279-287 [PMID: 22970906 DOI: 10.1042/CS20120289]

53 **Bieghs V**, Wouters K, van Gorp PJ, Gijbels MJ, de Winther MP, Binder CJ, Lütjohann D, Febbraio M, Moore KJ, van Bilsen M, Hofker MH, Shiri-Sverdlov R. Role of scavenger receptor A and CD36 in diet-induced nonalcoholic steatohepatitis in hyperlipidemic mice. *Gastroenterology* 2010; **138**: 2477-2486, 2486.e1-2486.e3 [PMID: 20206177 DOI: 10.1053/j.gastro.2010.02.051]

54 **Wobser H**, Dorn C, Weiss TS, Amann T, Bollheimer C, Büttner R, Schölmerich J, Hellerbrand C. Lipid accumulation in hepatocytes induces fibrogenic activation of hepatic stellate cells. *Cell Res* 2009; **19**: 996-1005 [PMID: 19546889 DOI: 10.1038/cr.2009.73]

55 **Pulli B**, Ali M, Iwamoto Y, Zeller MW, Schob S, Linnoila JJ, Chen JW. Myeloperoxidase-Hepatocyte-Stellate Cell Cross Talk Promotes Hepatocyte Injury and Fibrosis in Experimental Nonalcoholic Steatohepatitis. *Antioxid Redox Signal* 2015; **23**: 1255-1269 [PMID: 26058518 DOI: 10.1089/ars.2014.6108]

56 **Tomita K**, Tamiya G, Ando S, Ohsumi K, Chiyo T, Mizutani A, Kitamura N, Toda K, Kaneko T, Horie Y, Han JY, Kato S, Shimoda M, Oike Y, Tomizawa M, Makino S, Ohkura T, Saito H, Kumagai N, Nagata H, Ishii H, Hibi T. Tumour necrosis factor alpha signalling through activation of Kupffer cells plays an essential role in liver fibrosis of non-alcoholic steatohepatitis in mice. *Gut* 2006; **55**: 415-424 [PMID: 16174657]

57 **Aparicio-Vergara M**, Hommelberg PP, Schreurs M, Gruben N, Stienstra R, Shiri-Sverdlov R, Kloosterhuis NJ, de Bruin A, van de Sluis B, Koonen DP, Hofker MH. Tumor necrosis factor receptor 1 gain-of-function mutation aggravates nonalcoholic fatty liver disease but does not cause insulin resistance in a murine model. *Hepatology* 2013; **57**: 566-576 [PMID: 22941955 DOI: 10.1002/hep.26046]

58 **Cheng Q**, Li N, Chen M, Zheng J, Qian Z, Wang X, Huang C, Xu S, Shi G. Cyclooxygenase-2 promotes hepatocellular apoptosis by interacting with TNF-α and IL-6 in the pathogenesis of nonalcoholic steatohepatitis in rats. *Dig Dis Sci* 2013; **58**: 2895-2902 [PMID: 23975340 DOI: 10.1007/s10620-013-2823-6]

59 **Xiao F**, Waldrop SL, Bronk SF, Gores GJ, Davis LS, Kilic G. Lipoapoptosis induced by saturated free fatty acids stimulates monocyte migration: a novel role for Pannexin1 in liver cells. *Purinergic Signal* 2015; **11**: 347-359 [PMID: 26054298 DOI: 10.1007/s11302-015-9456-5]

60 **Henkel J**, Coleman CD, Schraplau A, Johrens K, Weber D, Castro JP, Hugo M, Schulz TJ, Krämer S, Schürmann A, Püschel GP. Induction of steatohepatitis (NASH) with insulin resistance in wildtype B6 mice by a western-type diet containing soybean oil and cholesterol. *Mol Med* 2017; **23**: Epub ahead of print [PMID: 28332698 DOI: 10.2119/molmed.2016.00203]

61 **Hirsova P**, Ibrahim SH, Krishnan A, Verma VK, Bronk SF, Werneburg NW, Charlton MR, Shah VH, Malhi H, Gores GJ. Lipid-Induced Signaling Causes Release of Inflammatory Extracellular Vesicles From Hepatocytes. *Gastroenterology* 2016; **150**: 956-967 [PMID: 26764184 DOI: 10.1053/j.gastro.2015.12.037]

62 **Xu Z**, Zhang X, Lau J, Yu J. C-X-C motif chemokine 10 in non-alcoholic steatohepatitis: role as a pro-inflammatory factor and clinical implication. *Expert Rev Mol Med* 2016; **18**: e16 [PMID: 27669973]

63 **Wang YG**, Fang WL, Wei J, Wang T, Wang N, Ma JL, Shi M. The involvement of NLRX1 and NLRP3 in the development of nonalcoholic steatohepatitis in mice. *J Chin Med Assoc* 2013; **76**: 686-692 [PMID: 24084392 DOI: 10.1016/j.jcma.2013.08.010]

64 **Gautheron J**, Vucur M, Reisinger F, Cardenas DV, Roderburg C, Koppe C, Kreggenwinkel K, Schneider AT, Bartneck M, Neumann UP, Canbay A, Reeves HL, Luedde M, Tacke F, Trautwein C, Heikenwalder M, Luedde T. A positive feedback loop between RIP3 and JNK controls non-alcoholic steatohepatitis. *EMBO Mol Med* 2014; **6**: 1062-1074 [PMID: 24963148 DOI: 10.15252/emmm.201403856]

65 **Roychowdhury S**, McCullough RL, Sanz-Garcia C, Saikia P, Alkhouri N, Matloob A, Pollard KA, McMullen MR, Croniger CM, Nagy LE. Receptor interacting protein 3 protects mice from high-fat diet-induced liver injury. *Hepatology* 2016; **64**: 1518-1533 [PMID: 27301788 DOI: 10.1002/hep.28676]

66 **Ikura Y**, Ohsawa M, Suekane T, Fukushima H, Itabe H, Jomura H, Nishiguchi S, Inoue T, Naruko T, Ehara S, Kawada N, Arakawa T, Ueda M. Localization of oxidized phosphatidylcholine in nonalcoholic fatty liver disease: impact on disease progression. *Hepatology* 2006; **43**: 506-514 [PMID: 16496325]

67 **Tomita K**, Teratani T, Suzuki T, Oshikawa T, Yokoyama H, Shimamura K, Nishiyama K, Mataki N, Irie R, Minamino T, Okada Y, Kurihara C, Ebinuma H, Saito H, Shimizu I, Yoshida Y, Hokari R, Sugiyama K, Hatsuse K, Yamamoto J, Kanai T, Miura S, Hibi T. p53/p66Shc-mediated signaling contributes to the progression of non-alcoholic steatohepatitis in humans and mice. *J Hepatol* 2012; **57**: 837-843 [PMID: 22641095 DOI: 10.1016/j.jhep.2012.05.013]

68 **Maliken BD**, Nelson JE, Klintworth HM, Beauchamp M, Yeh MM, Kowdley KV. Hepatic reticuloendothelial system cell iron deposition is associated with increased apoptosis in nonalcoholic fatty liver disease. *Hepatology* 2013; **57**: 1806-1813 [PMID: 23325576 DOI: 10.1002/hep.26238]

69 **Yang L**, Roh YS, Song J, Zhang B, Liu C, Loomba R, Seki E. Transforming growth factor beta signaling in hepatocytes participates in steatohepatitis through regulation of cell death and lipid metabolism in mice. *Hepatology* 2014; **59**: 483-495 [PMID: 23996730 DOI: 10.1002/hep.26698]

70 **Liang S**, Kisseleva T, Brenner DA. The Role of NADPH Oxidases (NOXs) in Liver Fibrosis and the Activation of Myofibroblasts. *Front Physiol* 2016; **7**: 17 [PMID: 26869935 DOI: 10.3389/fphys.2016.00017]

71 **Park S**, Kim JW, Yun H, Choi SJ, Lee SH, Choi KC, Lim CW, Lee K, Kim B. Mainstream cigarette smoke accelerates the progression of nonalcoholic steatohepatitis by modulating Kupffer cell-mediated hepatocellular apoptosis in adolescent mice. *Toxicol Lett* 2016; **256**: 53-63 [PMID: 27180087 DOI: 10.1016/j.toxlet.2016.05.012]

72 **Mann JP**, Raponi M, Nobili V. Clinical implications of understanding the association between oxidative stress and pediatric NAFLD. *Expert Rev Gastroenterol Hepatol* 2017; **11**: 371-382 [PMID: 28162008 DOI: 10.1080/17474124.2017.1291340]

73 **Kuo TF**, Tatsukawa H, Kojima S. New insights into the functions and localization of nuclear transglutaminase 2. *FEBS J* 2011; **278**: 4756-4767 [PMID: 22051117 DOI: 10.1111/j.1742-4658.2011.08409.x]

74 **Toriguchi K**, Hatano E, Tanabe K, Takemoto K, Nakamura K, Koyama Y, Seo S, Taura K, Uemoto S. Attenuation of steatohepatitis, fibrosis, and carcinogenesis in mice fed a methionine-choline deficient diet by CCAAT/enhancer-binding protein homologous protein deficiency. *J Gastroenterol Hepatol* 2014; **29**: 1109-1118 [PMID: 24329600 DOI: 10.1111/jgh.12481]

75 **Yoo W**, Noh KH, Ahn JH, Yu JH, Seo JA, Kim SG, Choi KM, Baik SH, Choi DS, Kim TW, Kim HJ, Kim NH. HIF-1α expression as a protective strategy of HepG2 cells against fatty acid-induced toxicity. *J Cell Biochem* 2014; **115**: 1147-1158 [PMID: 24402912 DOI: 10.1002/jcb.24757]

76 **Miyamoto Y**, Mauer AS, Kumar S, Mott JL, Malhi H. Mmu-miR-615-3p regulates lipoapoptosis by inhibiting C/EBP homologous protein. *PLoS One* 2014; **9**: e109637 [PMID: 25314137 DOI: 10.1371/journal.pone.0109637]

77 **Willy JA**, Young SK, Stevens JL, Masuoka HC, Wek RC. CHOP links endoplasmic reticulum stress to NF-κB activation in the pathogenesis of nonalcoholic steatohepatitis. *Mol Biol Cell* 2015; **26**: 2190-2204 [PMID: 25904325 DOI: 10.1091/mbc.E15-01-0036]

78 **Ao N**, Yang J, Wang X, Du J. Glucagon-like peptide-1 preserves non-alcoholic fatty liver disease through inhibition of the endoplasmic reticulum stress-associated pathway. *Hepatol Res* 2016; **46**: 343-353 [PMID: 26147696 DOI: 10.1111/hepr.12551]

79 **Liu X**, Henkel AS, LeCuyer BE, Schipma MJ, Anderson KA, Green RM. Hepatocyte X-box binding protein 1 deficiency increases liver injury in mice fed a high-fat/sugar diet. *Am J Physiol Gastrointest Liver Physiol* 2015; **309**: G965-G974 [PMID: 26472223 DOI: 10.1152/ajpgi.00132.2015]

80 **Mota M**, Banini BA, Cazanave SC, Sanyal AJ. Molecular mechanisms of lipotoxicity and glucotoxicity in nonalcoholic fatty liver disease. *Metabolism* 2016; **65**: 1049-1061 [PMID: 26997538 DOI: 10.1016/j.metabol.2016.02.014]

81 **Tu QQ**, Zheng RY, Li J, Hu L, Chang YX, Li L, Li MH, Wang RY, Huang DD, Wu MC, Hu HP, Chen L, Wang HY. Palmitic acid induces autophagy in hepatocytes via JNK2 activation. *Acta Pharmacol Sin* 2014; **35**: 504-512 [PMID: 24608675 DOI: 10.1038/aps.2013.170]

82 **Cai N**, Zhao X, Jing Y, Sun K, Jiao S, Chen X, Yang H, Zhou Y, Wei L. Autophagy protects against palmitate-induced apoptosis in hepatocytes. *Cell Biosci* 2014; **4**: 28 [PMID: 24904743 DOI: 10.1186/2045-3701-4-28]

83 **Xie F**, Jia L, Lin M, Shi Y, Yin J, Liu Y, Chen D, Meng Q. ASPP2 attenuates triglycerides to protect against hepatocyte injury by reducing autophagy in a cell and mouse model of non-alcoholic fatty liver disease. *J Cell Mol Med* 2015; **19**: 155-164 [PMID: 25256142 DOI: 10.1111/jcmm.12364]

84 **Lou J**, Wang Y, Wang X, Jiang Y. Uncoupling protein 2 regulates palmitic acid-induced hepatoma cell autophagy. *Biomed Res Int* 2014; **2014**: 810401 [PMID: 25512910 DOI: 10.1155/2014/810401]

85 **Cazanave SC**, Sanyal AJ. KEAP the balance between life and death. *Mol Cell Oncol* 2014; **2**: e968065 [PMID: 27308418 DOI: 10.4161/23723548.2014.968065]

86 **Eid N**, Ito Y, Otsuki Y. Triggering of Parkin Mitochondrial Translocation in Mitophagy: Implications for Liver Diseases. *Front Pharmacol* 2016; **7**: 100 [PMID: 27199746 DOI: 10.3389/fphar.2016.00100]

87 **Tanaka S**, Hikita H, Tatsumi T, Sakamori R, Nozaki Y, Sakane S, Shiode Y, Nakabori T, Saito Y, Hiramatsu N, Tabata K, Kawabata T, Hamasaki M, Eguchi H, Nagano H, Yoshimori T, Takehara T. Rubicon inhibits autophagy and accelerates hepatocyte apoptosis 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**, Dou 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, Miquilena-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, 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, Pierpont B, Caprio 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**: 2993-3000 [PMID: 23626003 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, Veillard AS, Shackel NA, Brooks B, Bu Y, Cavanagh E, Raleigh J, McLennan SV, McCaughan GW, Keane FM, Zekry A, Gorrell MD, Twigg SM. Circulating dipeptidyl peptidase-4 activity correlates with measures of hepatocyte apoptosis and fibrosis in non-alcoholic fatty liver disease in type 2 diabetes mellitus and obesity: A dual cohort cross-sectional study. *J Diabetes* 2015; **7**: 809-819 [PMID: 25350950 DOI: 10.1111/1753-0407.12237]

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/ggi.12722]

95 **Idrissova L**, Malhi H, Werneburg NW, LeBrasseur NK, Bronk SF, Fingas C, Tchkonia T, Pirtskhalava T, White TA, Stout MB, Hirsova P, Krishnan A, Liedtke C, Trautwein C, Finnberg N, El-Deiry WS, Kirkland JL, Gores GJ. TRAIL receptor deletion in mice suppresses the inflammation of nutrient excess. *J Hepatol* 2015; **62**: 1156-1163 [PMID: 25445398 DOI: 10.1016/j.jhep.2014.11.033]

96 **Cartland 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 deletion in mice. *Sci Rep* 2017; **7**: 1898 [PMID: 28507343 DOI: 10.1038/s41598-017-01721-4]

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

98 **Csak T**, Ganz M, Pespisa J, Kodys K, Dolganiuc A, Szabo G. Fatty acid and endotoxin activate inflammasomes in mouse hepatocytes that release danger signals to stimulate immune cells. *Hepatology* 2011; **54**: 133-144 [PMID: 21488066 DOI: 10.1002/hep.24341]

99 **Henao-Mejia J**, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaiss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez JP, Shulman GI, Gordon JI, Hoffman HM, Flavell RA. Inflammasome-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]

101 **Akazawa Y**, Guicciardi ME, Cazanave SC, Bronk SF, Werneburg NW, Kakisaka K, Nakao K, Gores GJ. Degradation of cIAPs contributes to hepatocyte lipoapoptosis. *Am J Physiol Gastrointest Liver Physiol* 2013; **305**: G611-G619 [PMID: 24008361 DOI: 10.1152/ajpgi.00111.2013]

102 **Egnatchik RA**, Leamy AK, Jacobson DA, Shiota M, Young JD. ER calcium release promotes mitochondrial dysfunction and hepatic cell lipotoxicity in response to palmitate overload. *Mol Metab* 2014; **3**: 544-553 [PMID: 25061559 DOI: 10.1016/j.molmet.2014.05.004]

103 **Gan LT**, Van Rooyen DM, Koina ME, McCuskey RS, Teoh NC, Farrell GC. Hepatocyte free cholesterol lipotoxicity results from JNK1-mediated mitochondrial injury and is HMGB1 and TLR4-dependent. *J Hepatol* 2014; **61**: 1376-1384 [PMID: 25064435 DOI: 10.1016/j.jhep.2014.07.024]

104 **Zhu C**, Xie P, Zhao F, Zhang L, An W, Zhan Y. Mechanism of the promotion of steatotic HepG2 cell apoptosis by cholesterol. *Int J Clin Exp Pathol* 2014; **7**: 6807-6813 [PMID: 25400762]

105 **Seeßle J**, Liebisch G, Schmitz G, Stremmel W, Chamulitrat W. Palmitate activation by fatty acid transport protein 4 as a model system for hepatocellular apoptosis and steatosis. *Biochim Biophys Acta* 2015; **1851**: 549-565 [PMID: 25603556 DOI: 10.1016/j.bbalip.2015.01.004]

106 **Denechaud PD**, Lopez-Mejia IC, Giralt A, Lai Q, Blanchet E, Delacuisine B, Nicolay BN, Dyson NJ, Bonner C, Pattou F, Annicotte JS, Fajas L. E2F1 mediates sustained lipogenesis and contributes to hepatic steatosis. *J Clin Invest* 2016; **126**: 137-150 [PMID: 26619117 DOI: 10.1172/JCI81542]

107 **Baranova A**, Tran TP, Afendy A, Wang L, Shamsaddini A, Mehta R, Chandhoke V, Birerdinc A, Younossi ZM. Molecular signature of adipose tissue in patients with both non-alcoholic fatty liver disease (NAFLD) and polycystic ovarian syndrome (PCOS). *J Transl Med* 2013; **11**: 133 [PMID: 23721173 DOI: 10.1186/1479-5876-11-133]

108 **Rodrigues PM**, Afonso MB, Simão AL, Carvalho CC, Trindade A, Duarte A, Borralho PM, Machado MV, Cortez-Pinto H, Rodrigues CM, Castro RE. miR-21 ablation and obeticholic acid ameliorate nonalcoholic steatohepatitis in mice. Cell Death Dis 2017; 8: e2748 [PMID: 28406477 DOI: 10.1038/cddis.2017.172]

109 **Kirsch R**, Clarkson V, Verdonk RC, Marais AD, Shephard EG, Ryffel B, de la M Hall P. Rodent nutritional model of steatohepatitis: effects of endotoxin (lipopolysaccharide) and tumor necrosis factor alpha deficiency. *J Gastroenterol Hepatol* 2006; **21**: 174-182 [PMID: 16706830]

110 **Kudo H**, Takahara T, Yata Y, Kawai K, Zhang W, Sugiyama T. Lipopolysaccharide triggered TNF-alpha-induced hepatocyte apoptosis in a murine non-alcoholic steatohepatitis model. *J Hepatol* 2009; **51**: 168-175 [PMID: 19446916 DOI: 10.1016/j.jhep.2009.02.032]

111 **Hirsova P**, Ibrahim SH, Gores GJ, Malhi H. Lipotoxic lethal and sublethal stress signaling in hepatocytes: relevance to NASH pathogenesis. *J Lipid Res* 2016; **57**: 1758-1770 [PMID: 27049024]

112 **Dechandt CRP**, Zuccolotto-Dos-Reis FH, Teodoro BG, Fernandes AMAP, Eberlin MN, Kettelhut IC, Curti C, Alberici LC. Triacsin C reduces lipid droplet formation and induces mitochondrial biogenesis in primary rat hepatocytes. *J Bioenerg Biomembr* 2017; **49**: 399-411 [PMID: 28918598 DOI: 10.1007/s10863-017-9725-9]

113 **Kim SH**, Kim G, Han DH, Lee M, Kim I, Kim B, Kim KH, Song YM, Yoo JE, Wang HJ, Bae SH, Lee YH, Lee BW, Kang ES, Cha BS, Lee MS. Ezetimibe ameliorates steatohepatitis via AMP activated protein kinase-TFEB-mediated activation of autophagy and NLRP3 inflammasome inhibition. *Autophagy* 2017; **13**: 1767-1781 [PMID: 28933629 DOI: 10.1080/15548627.2017.1356977]

114 **Zhang J**, Zhang H, Deng X, Zhang N, Liu B, Xin S, Li G, Xu K. Baicalin attenuates non-alcoholic steatohepatitis by suppressing key regulators of lipid metabolism, inflammation and fibrosis in mice. *Life Sci* 2018; **192**: 46-54 [PMID: 29158052 DOI: 10.1016/j.lfs.2017.11.027]

115 **Nam HH**, Jun DW, Jang K, Saeed WK, Lee JS, Kang HT, Chae 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]

116 **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]

117 **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]

118 **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]

119 **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]

120 **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]

121 **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]

122 **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]

123 **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]

124 **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]

125 **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]

126 **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]

Figure Legends



**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.

Footnotes

Manuscript source: Invited manuscript

**Specialty type:** Gastroenterology and hepatology

**Country of origin:** Japan

**Peer-review report classification**

Grade A (Excellent): 0

Grade B (Very good): B, B

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0

Conflict-of-interest statement: Kanda T received research grants from AbbVie, MSD, Chugai Pharma, and Sysmex. These companies played no role in this study. Other authors declare no conflict of interests related to this publication.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Peer-review started: April 3, 2018

First decision: May 29, 2018

Article in press: June 22, 2018

**P- Reviewer**: Namisaki T, Shimizu Y, Takahashi T **S- Editor**:Wang JL **L- Editor**: A **E- Editor**:Huang Y

**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-B | NF-B anti-inflammation signaling pathways | [114] |
| Granulocyte colony stimulating factor (G-CSF) | PI3K/Akt | Activation of PI3K and Akt pathway | [115] |
| Elafibranor (GFT505) | PPAR/ | Agonist of PPAR/ receptors | [116] |
| Isoquercitrin | Dipeptidyl peptidase-IV(DPP-IV)  | Activation of glucagonlike 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-B | NF-B anti-inflammation signaling pathways | [120] |
| Seladelpar (MBX-8025) | Proliferator-activated receptor- delta (PPAR-) | Selective PPAR- agonist | [121] |
| Resveratrol | Sirt1 | Antioxidant | [122] |
| TBE-31 | NF-E2 p45-related factor 2 (Nrf2) | Regulation of intracellular redox homeostasis | [124] |