




Mitochondrial ROS, a trigger for mitochondrial dysfunction and inflammasome activation and a therapeutic target in liver diseases

Hala Saeed Jaara^{1,2,3}, Sandra Torres^{1,2,3*} 

¹Department of Cell Death and Proliferation, Institute of Biomedical Research of Barcelona (IIBB-CSIC), 08036 Barcelona, Spain

²Liver Unit, Hospital Clinic I Provincial de Barcelona, Instituto de Investigaciones Biomédicas August Pi i Sunyer (IDIBAPS), 08036 Barcelona, Spain

³Center for the Study of Liver and Gastrointestinal Diseases (CIBERehd), Carlos III National Institute of Health, 28029 Madrid, Spain

***Correspondence:** Sandra Torres, Department of Cell Death and Proliferation, Institute of Biomedical Research of Barcelona (IIBB-CSIC), 08036 Barcelona, Spain. sandra.torres@iibb.csic.es

Academic Editor: Ina Bergheim, University of Vienna, Austria

Received: October 11, 2024 **Accepted:** December 1, 2024 **Published:** December 10, 2024

Cite this article: Jaara HS, Torres S. Mitochondrial ROS, a trigger for mitochondrial dysfunction and inflammasome activation and a therapeutic target in liver diseases. *Explor Dig Dis.* 2024;3:474–503. <https://doi.org/10.37349/edd.2024.00062>

Abstract

Mitochondria are essential organelles responsible for intracellular energy production and play crucial roles in cellular metabolism, inflammation, and apoptosis. Reactive oxygen species (ROS) are primarily produced in the mitochondria and endoplasmic reticulum of hepatocytes due to the activity of cytochrome P450 enzymes. Under ideal conditions, cells have specific molecular mechanisms that manage oxidative stress levels, thus ensuring a balance between oxidants and antioxidants. The interplay between ROS-induced mitochondrial dysfunction and the activation of the NLRP3 (nucleotide-binding oligomerization domain-like receptor family, pyrin domain containing 3) inflammasome in the context of liver diseases has been extensively studied. However, the exact mechanisms by which mitochondria promote the activation of the NLRP3 inflammasome and contribute to the onset of liver disease remain unclear. This review aims to elucidate the recently discovered mitochondrial regulation of the NLRP3 inflammasome in liver disorders, including alcohol-related liver disease (ALD), metabolic-associated steatotic liver disease (MASLD), and hepatocellular carcinoma (HCC). Finally, it summarizes various natural and pharmaceutical agents that can mitigate liver damage by modulating the activation of the NLRP3 inflammasome through mitochondrial pathways. This work serves as an important resource for identifying new therapeutic approaches and provides further support for advancing the understanding of liver diseases.

Keywords

ROS, mitochondria, NLRP3 inflammasome, MASLD, ALD, HCC



Introduction

Oxidative stress refers to a condition characterized by an imbalance between the generation of free radicals, known as reactive oxygen species (ROS), and the capacity of the body to counteract their effects through antioxidants. Free radicals are molecules that contain oxygen and are highly reactive due to the presence of unpaired electrons. While they are a natural byproduct of cellular metabolism, especially during processes like energy production in the mitochondria, excessive amounts can lead to cellular damage, with oxidative stress damaging proteins, lipids, and DNA [1]. Mitochondria are a major source of ROS during oxidative phosphorylation (OXPHOS) in the electron transport chain (ETC). Key ROS include superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\bullet OH$) production. Excess ROS can trigger inflammation and activate the NLRP3 (nucleotide-binding oligomerization domain-like receptor family, pyrin domain containing 3) inflammasome, linking mitochondrial dysfunction and increased ROS to immune responses. Disturbances in the balance of ROS production lead to oxidative stress, which is implicated in the progression of liver diseases, where mitochondrial dysfunction and increased ROS are prominent features [2]. Effective regulation of ROS, mitochondrial function, and related pathways is key to maintaining cellular health and preventing liver damage.

This review summarizes the current knowledge on mitochondrial ROS and the interplay with the NLRP3 inflammasome complex in the context of liver diseases.

Mitochondrial ROS sources

ROS are by-products of oxygen metabolism of cell aerobic respiration. They are a family of free radicals, including $O_2^{\bullet-}$, H_2O_2 , $\bullet OH$, and nitric oxide (NO). NO also belongs to the reactive nitrogen species (RNS) category [3].

ROS can come from exogenous sources (such as radiation, anticancer therapy, smoking, consumption of alcohol, and certain drugs) or endogenous sources. Mitochondria are one of the main endogenous sources of ROS, and specifically, it is produced during OXPHOS in the mitochondrial ETC, where ATP synthesis occurs [4]. The ETC is composed of four protein/cytochrome complexes in the mitochondrial inner membrane: complex I (NADH oxidase) which receives electrons from NADH, complex II (Succinate dehydrogenase) which oxidizes succinate to fumarate in the TCA cycle, complex III (Q-cytochrome c oxidoreductase), and complex IV (cytochrome c oxidase). In addition to these complexes, ubiquinone (UQ) and cytochrome c also participate in electron transport. Ultimately, complex V (ATP synthetase) produces ATP using ADP and inorganic phosphate (Pi) as substrates. Complexes I and III of the ETC are the primary sites where $O_2^{\bullet-}$ is formed, and then it is dismutated to H_2O_2 by superoxide dismutase (SOD) and then to water and oxygen by catalase or glutathione peroxidase (Gpx) [5]. ATP synthesis depends on the presence of oxygen (O_2), which facilitates electron transfer reactions through the ETC. Two free radicals ($O_2^{\bullet-}$ and H_2O_2) are formed during the ETC, and they can interact with important biomolecules including DNA, proteins, and membranes [6] (Figure 1).

Besides mitochondria, other intracellular compartments are capable of ROS generation. These include the endoplasmic reticulum (ER), which produces ROS during protein folding [7]; peroxisomes, which generates ROS during the β -oxidation of fatty acids [8]; the plasma membrane, by membrane-bound NADPH oxidases (NOXs) during phagocytosis; in the cytosol, by monooxygenases of cytochrome P450 (CYP) superfamily, or other cytosolic enzymes, such as lipoxygenases and cyclooxygenases; or in the extracellular space, via xanthine oxidase.

Among the functions of mitochondrial ROS in physiological processes, they are important players in metabolic adaptation, differentiation, autophagy activation under starvation, and inflammatory cytokine production under pathogen infection. In terms of adaptive signaling, mitochondrial ROS-driven prolyl hydroxylase enzyme (PHD) inhibition stabilizes hypoxia-inducible factor 1 alpha (HIF1 α) [9–12] and activates hypoxia by activation of HIF1/2 α . Mitochondrial ROS activates cell survival mechanisms as well, such as phosphatase and tensin homolog deleted on chromosome 10 (PTEN), PI3K, protein kinase B (Akt) [13–16], and extracellular signal-regulated kinase (ERK1/2) [17–20]. Interestingly, mitochondrial ROS have

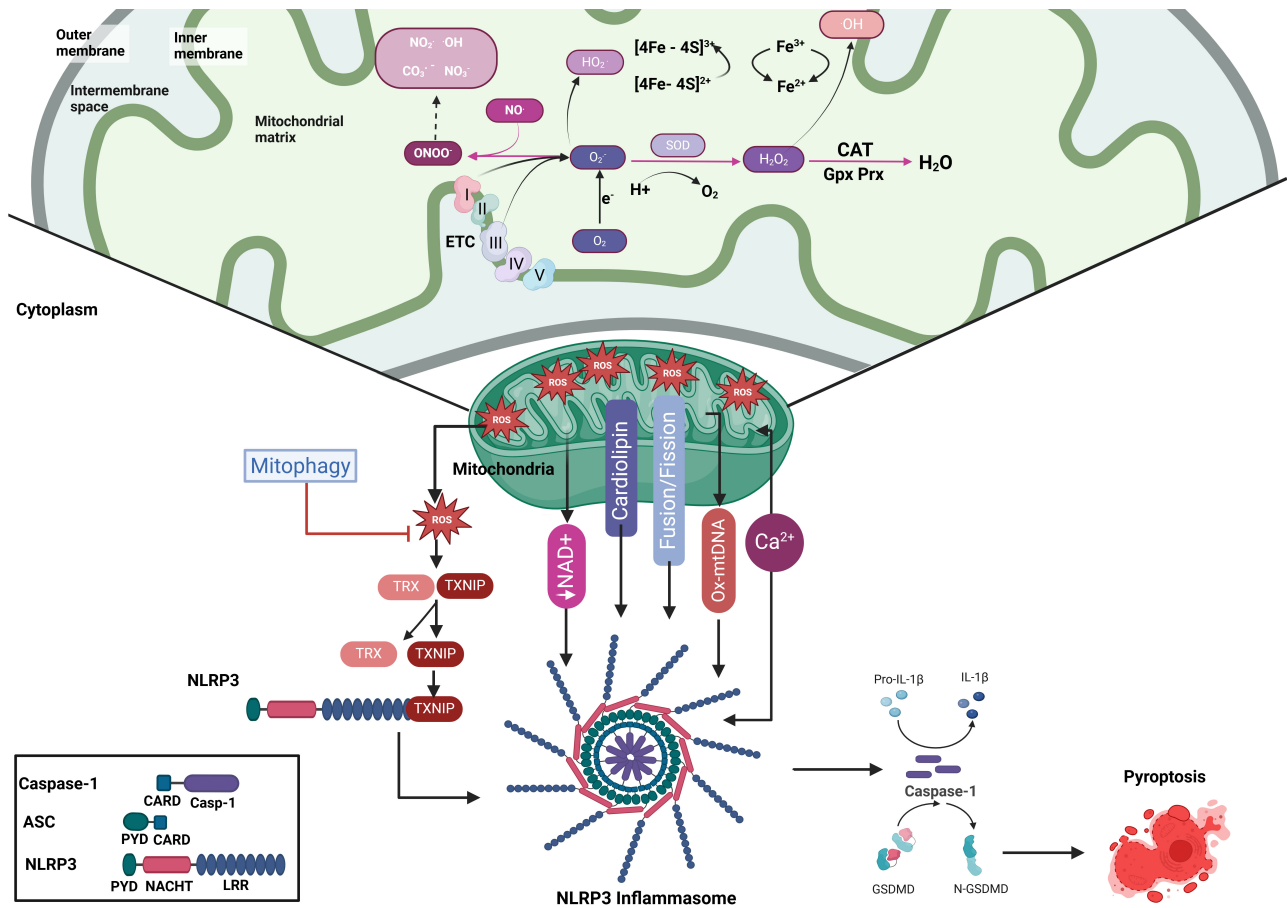


Figure 1. Mitochondrial ROS-induced dysfunction and inflammasome activation leading to pyroptosis. This figure illustrates the role of mitochondrial ROS in promoting mitochondrial dysfunction, inflammasome activation, and eventual pyroptosis. Within the mitochondria, the ETC generates ROS, such as $O_2^{\bullet-}$ and H_2O_2 , which can lead to oxidative damage of mitochondrial components, including proteins and lipids. ROS-mediated damage impairs mitochondrial dynamics, reduces NAD^+ levels, and disrupts fusion and fission balance, contributing to the release of mtDNA. Furthermore, Ca^{2+} dysregulation and cardiolipin translocation amplify inflammasome activation, reinforcing mitochondrial dysfunction and promoting cell death. Mitophagy is key in mitigating ROS-induced damage. Excessive ROS promotes the activation of TXNIP, which binds to the NLRP3 inflammasome complex, composed of NLRP3, ASC, and caspase-1. This activation triggers caspase-1 cleavage, leading to the maturation of pro-inflammatory cytokines, such as IL-1 β , initiating pyroptosis, a form of inflammatory cell death. ASC: apoptosis-associated speck-like protein containing a caspase activation and recruitment domain; Ca^{2+} : calcium; CARD: caspase activation and recruitment domain; CAT: catalase; $CO_3^{\bullet-}$: carbonate radicals; ETC: electron transport chain; Gpx: glutathione peroxidase; GSDMD: gasdermin D; H_2O_2 : hydrogen peroxide; IL-1 β : interleukin-1 β ; LRR: leucine-rich repeat; mtDNA: mitochondrial DNA; NAD^+ : nicotinamide adenine dinucleotide; PYD: pyrin domain; NLRP3: NACHT-, LRR-, and PYD-containing protein 3; NO^{\bullet} : nitric oxide; O_2 : oxygen; $O_2^{\bullet-}$: superoxide anion; $\bullet OH$: hydroxyl radical; $ONOO^-$: peroxynitrite; Ox-mtDNA: oxidized mtDNA; Prx: peroxiredoxin; ROS: reactive oxygen species; SOD: superoxide dismutase; TXNIP: thioredoxin-interacting protein. Created in BioRender. Torres, S. (2024) <https://BioRender.com/b36d741>

also been shown to regulate cellular differentiation and chromatin structure remodeling via the oxidation of architectural chromatin components [21–22].

The equilibrium between ROS production and elimination is critical for maintaining redox homeostasis. Disruption of the redox equilibrium contributes to the pathogenesis of chronic diseases and aging [23, 24]. To counterbalance the excessive ROS production, cells have evolved a complex antioxidant system, which comprises both enzymatic and nonenzymatic mechanisms. The major enzymatic antioxidants are SODs, catalase (CAT) and Gpxs, glutathione-reductase (GR), and superoxide reductases (SOR). Among the most important low molecular weight antioxidants are endogenous, such as glutathione, thioredoxin (Trx), and melatonin, and exogenous antioxidants, such as vitamin C, vitamin E, carotenoids, and flavonoids.

Mitochondrial dysfunction and liver diseases

Mitochondria dysfunction has been shown to contribute to the development and progression of many diseases, including metabolic and neurodegenerative diseases. As mentioned before, mitochondria perform

multiple functions, such as the regulation of cell death signaling, inflammation, ROS production, and mitochondrial DNA (mtDNA) damage, which are factors associated with the progression of liver diseases, such as alcohol-related liver disease (ALD), metabolic-dysfunction associated steatotic liver disease (MASLD), previously known as non-alcoholic fatty liver disease (NAFLD), and hepatocellular carcinoma (HCC) [25, 26].

Mitochondrial dysfunction in ALD

ALD is a condition caused by long-term, excessive alcohol consumption that leads to liver damage [27]. It is a major cause of chronic liver disease worldwide. The worldwide prevalence of alcohol use disorder (AUD) stands at 5.1%, affecting approximately 283 million individuals. The highest rates are observed in the European region, where the prevalence is 14.8% for men and 3.5% for women, followed by the Americas, with rates of 11.5% for men and 5.1% for women [28]. ALD encompasses a spectrum of liver conditions, ranging from simple fatty liver (steatosis) to more severe forms such as alcoholic hepatitis, fibrosis, cirrhosis, and even HCC [29]. The progression and severity of ALD depend on the duration and amount of alcohol intake, as well as individual factors like genetics and underlying health conditions.

Alcohol is metabolized partly in the mitochondria, first in the cytosol by the alcohol dehydrogenase (ADH) to acetaldehyde, and later by aldehyde dehydrogenase 2 (ALDH2) in the mitochondria. A part of ADH, in the cytosol ethanol is metabolized by CYP2E1 in the smooth ER [30]. Because of the alcohol metabolism, there is an increase in oxygen consumption and a decrease in ATP production, causing mitochondrial dysfunction. Alcohol-induced mitochondrial ROS formation [31] damages mtDNA and disrupts the ETC, releasing mtDNA [32, 33]. For example, mice fed with an alcohol diet and treated with manganese SOD (MnSOD), a mitochondrial antioxidant enzyme, were able to prevent mtDNA damage [34]. Furthermore, patients with alcohol-related hepatitis and cirrhosis presented mtDNA deletion and fragmentation [35, 36]. Research indicates that both chronic and acute ethanol exposure reduces intracellular antioxidant levels, particularly within mitochondria, leading to an increased susceptibility to liver damage caused by various apoptotic triggers [37, 38].

In terms of mitochondrial respiration, experimental models of alcohol intake are shown to be species-dependent. Several studies in rats fed alcohol orally with Lieber–DeCarli liquid diet containing 36% ethanol for 4 weeks showed decreased mitochondrial respiration in isolated liver mitochondria [39–42]. In contrast to these findings, mice fed alcohol orally in a pair-fed liquid diet [5.4% (w/v) ethanol] or by 4 weeks of intragastric alcohol feeding (alcohol accounted for ~38.4% of the total caloric intake) exhibited increased respiration in isolated liver mitochondria [43–45] and a higher complex II relative to complex I-driven OCR [46, 47]. Furthermore, in the liver-humanized mouse models, chimeric mice xenotransplanted with human adult hepatocytes exhibited an increase in mitochondrial respiration after chronic and acute alcohol administration (5%, ethanol; 36% calories) [48].

Since the liver has a well-defined zonation, characterized by the perivenous (PV) and periportal (PP) areas, hypoxia is characteristic of the PV zone, and it is aggravated with alcohol intake that increases hepatic oxygen consumption. Therefore, the morphology and functional activity of mitochondria between PP and PV hepatocytes following alcohol feeding differs. Recent findings showed the presence of the mitochondrial cholesterol transporter, steroidogenic acute regulatory protein 1 (StARD1), predominantly in the PV zone, leading to cholesterol accumulation within the mitochondria that translates into a significant depletion of mitochondrial glutathione (mGSH) defense and increased ROS, lipid peroxidation and liver injury characteristic of ALD [49]. In this line, previous findings demonstrated an increased expression of StARD1 in patients with ALD [50]. Interestingly, the increase in mitochondrial function from alcohol-fed mice was predominantly observed in PV compared with PP hepatocytes. Chronic alcohol-fed rats presented reduced mGSH concentration, as happens in HepG2 treated with acetaldehyde, the primary product of ethanol oxidation [51, 52].

Chronic alcohol exposure also alters the mitochondrial structure and dynamics, producing mitochondrial swelling, cristae formation, and hyper-fragmentation of mitochondria in murine models. Research has demonstrated that exposure to ethanol disturbs the mitochondrial fusion-fission equilibrium,

resulting in rapid changes to their structure in both in vivo and in vitro models. Conversely, a pioneering work described alterations in the structure of hepatic mitochondria from patients with ALD who develop megamitochondria as an adaptive response in alcohol-induced hepatotoxicity [53–55].

Alcohol exposure disrupts mitochondrial calcium homeostasis, leading to increased calcium uptake, which is linked to elevated mitochondrial ROS production [56]. To address dysfunctional mitochondria, cells typically rely on mitophagy for turnover. However, in alcohol-fed mice, mitophagy is impaired through a Parkin-p53 pathway, exacerbating liver injury [57, 58].

Mitochondrial dysfunction in MASLD

In addition to these findings in ALD, mitochondrial dysfunction is considered a hallmark of MASLD. MASLD is characterized by abnormal fat accumulation in the liver, approximately 15% to 20% of MASLD patients develop metabolic dysfunction-associated steatohepatitis (MASH), characterized by the presence of liver damage, inflammation, and fibrosis, and eventually can progress to cirrhosis [59]. The worldwide prevalence of MASLD has increased from 25.3% from 1990 to 2006 to 38.2% between 2016 and 2019, reflecting nearly a 50% increase in its global prevalence over the last thirty years [60]. Hepatic mitochondria are crucial in the development of hepatic steatosis, as their metabolic processes generate acetyl-CoA, which serves as the fundamental component for the synthesis of lipids and cholesterol in the liver. The accumulation of free fatty acids (FFAs) in hepatocytes predisposes the liver to oxidative stress and cellular damage, leading to increased mitochondrial fatty acid import, β -oxidation to generate acetyl-CoA, and increased hepatic de novo lipogenesis [61].

Mitochondrial dysfunction precedes the development of insulin resistance and hepatic steatosis, playing a pivotal role in the progression of MASLD [62]. This dysfunction serves as a link between insulin resistance, steatosis, oxidative stress, and the dysregulation of gluconeogenesis [63, 64]. In the context of hepatic steatosis, there is a disruption in the ETC, leading to a reduction in the activity of mitochondrial complex III. This impairment results in inhibited ATP synthesis and a disturbance in the TCA cycle [65]. Additionally, there is an increase in mtDNA content and mitochondrial density [66], while the production of mitochondrial ROS is elevated. Targeting ROS inhibition is considered a crucial strategy for reducing lipid accumulation in hepatocytes [67].

Animal models of obesity (ob/ob mice) and obese patients with and without MASLD, showed increased hepatic mitochondrial β -oxidation [68, 69]. Studies in rats fed with a high-fat diet (HFD) or choline-deficient diet (CDD) resulted in increased levels of ROS and reduced antioxidant defenses [70–72]. In patients with MASH, increased ROS levels are linked to greater evidence of tissue oxidative damage, such as lipid peroxidation [35, 69, 73], and significantly reduced SOD activity and mGSH levels [73, 74].

Cardiolipin is one of the lipids from the mitochondrial membrane that changes in experimental models of MASH (HFD-fed mice or diabetes mice), but also in MASH patients [75–78]. Cardiolipin exhibits a significant susceptibility to oxidative damage caused by ROS [75]. In addition, cardiolipin is a hallmark of apoptotic cell death and mitochondrial fission [79]. The depletion of cardiolipin from the inner mitochondrial membrane (IMM) results in total disorganization of mitochondrial respiratory complexes, as cardiolipin is essential for the effective coupling of the respiratory chain with ATP synthase [76, 80]. In CDD-fed rats, the peroxidation of cardiolipin results in reduced complex I activity [81].

In addition to cardiolipin, cholesterol trafficking and its accumulation in the mitochondria also play a role in the pathogenesis of MASH [82, 83]. In genetic models, mice lacking niemann-pick type C1 (NPC1), a late endosomal cholesterol trafficking protein, as well as obese ob/ob mice, exhibit mitochondrial cholesterol accumulation, altered mitochondrial membrane fluidity, mGSH depletion, and increased susceptibility to inflammatory cytokines [84, 85]. In this regard, levels of STARD1 are also increased in human MASH patients, which aligns with the reported increase in mitochondrial cholesterol levels and GSH depletion [73, 86, 87].

As occurs in ALD, murine models of MASLD show increased oxidative mtDNA damage with impaired mtDNA repair mechanisms and disrupted ETC [88]. In MASLD patients have an increase in oxidative mitochondrial damage, which leads to defects in the mitochondrial ETC (complexes I, III, IV, and V), OXPHOS, mitochondrial uncoupling, and ATP production [69, 89–91]. HFD-fed mice have reduced complex IV activity and CDD-fed mice have a significant reduction in subunits of complexes I and IV and ATP content [81, 92, 93].

In addition to changes in OXPHOS, mitochondria from MASH patients present alterations in structure, showing megamitochondria [35, 94–97]. Ultrastructural studies have shown that megamitochondria appear swollen, with a loss of cristae, multilamellar membranes, and paracrystalline inclusions [82, 83]. MASH mice models showed megamitochondria formation and liver damage. Recently, a study performed in mice fed with a methionine-choline-deficient (MCD) diet showed that the MCD diet produces megamitochondria in hepatocytes, and the depletion of OPA1, a mitochondrial dynamin-related GTPase that mediates mitochondrial fusion, decreases mitochondrial size in this megamitochondria-associated MASH mouse model [98].

Another critical mitochondrial process involved in the progression into MASH, is mitophagy, the removal of damaged mitochondria through mitochondrial fission, which is impaired in both patients and experimental dietary models [99, 100], such as HFD-fed mice. Recently, it was shown that the formation of megamitochondria in MASH involves arrested mitophagy intermediates [101]. In addition, HFD-fed mice show increased pro-apoptotic proteins and apoptotic cells that correlate to steatosis and inflammation [100]. In the same line, patients with MASH show increased frequency of apoptotic cells as well as increased pro-apoptotic proteins and Fas expression [102].

Mitochondrial dysfunction in liver cancer

HCC is a common form of primary liver cancer and ranks among the foremost causes of cancer-related mortality globally. The prognosis for patients with HCC is poor, with the 5-year relative survival rate being only 18% [103]. In 2018, there were 840,000 cases reported [104]. Asia and Africa have the highest incidence rates worldwide. China has the largest number of cases due to its large population (18.3 per 100,000) and largest population (1.4 billion people), while Mongolia has the highest incidence (93.7 per 100,000) [105]. As a result, the diagnosis and treatment of HCC have become critical focal points for research. Mitochondria are central to HCC progression, affecting energy metabolism, maintaining redox balance, and controlling autophagy and apoptosis.

Aerobic glycolysis is the process by which cancer cells produce more lactate regardless of oxygen availability. Most cancer cells prefer aerobic glycolysis to OXPHOS for glucose metabolism, which is different from normal proliferating cells [106]. The Warburg effect is the name given to this phenomenon [107]. According to Warburg, mitochondrial dysfunction in cancer cells impairs aerobic respiration and increases reliance on glycolytic pathways. The presence of oxygen in healthy cells increases the production of NADH through the TCA cycle, in which NADH acts as an electron donor in the mitochondria, starting the process of electron transfer and ATP synthesis [108]. On the other hand, this electron transfer mechanism is impeded in the anaerobic conditions that are commonly found in tumor cells because oxygen is not present as an electron acceptor [109]. The excessive generation of ROS from this aberrant electron flow within the ETC can lead to oxidative stress, lipid peroxidation, mtDNA damage and mutations, proapoptotic cytokine activation, and a reduction in the effectiveness of OXPHOS. As a result, HCC has fewer copies of mtDNA than normal tissues, which causes mitochondrial dysfunction and tumor growth. Additionally, mtDNA copy number variations and mutations lead to impairments in OXPHOS-mediated ATP production [110]. Nevertheless, many cancer cells have competent OXPHOS activity that can produce ATP, even though the glycolytic phenotype in cancer cells was thought to be caused by defective mitochondrial OXPHOS [111–114]. Moreover, the unfolded protein response (UPR) and worsening of mitochondrial dysfunction can be caused by mtDNA mutation and deletion. Defects in the mitochondrial respiratory system, mtDNA depletion, and mtDNA transcription suppression can therefore promote the development of HCC [115].

Concerning the biology of cancer, aberrant cholesterol metabolism in cancer cells encourages uncontrolled cell division. One molecular factor that specifically coordinates some of these metabolic changes in cancer cells is the accumulation of cholesterol in the mitochondria, which disrupts mitochondrial function [116]. Therefore, the Warburg effect may be partially attributed to mitochondrial cholesterol loading in cancer cells. Paradoxically mitochondrial cholesterol accumulation in cancer cells does not result in mGSH depletion because of compensated overexpression of the SLC25A11 carrier, which counteracts the inhibitory effect of cholesterol, as observed in HCC [117]. Because cholesterol prevents cancer cells from undergoing apoptosis, the adaptive response of HCC cancer cells' mitochondria to show increased mitochondrial cholesterol loading while maintaining unrestricted mGSH is advantageous for tumor growth.

Mitophagy, the deliberate elimination of mitochondria, is probably a contributing component to the drug resistance of cancer cells. Numerous studies that examined the effect of mitophagy deficiency on carcinogenesis have found that inhibiting mitophagy promotes tumor formation [118]. According to recent research, resistant hypoxia-induced HCC cells have reduced expression of the main mitophagy regulatory gene ATPase family AAA domain-containing 3A (*ATAD3A*), and resistance to sorafenib necessitates hyperactivated mitophagy [119]. For patients with HCC, restoring these cells' susceptibility to sorafenib through mitophagy inhibition may provide a novel therapeutic strategy.

Inflammasome complex

Immunity is the body's sophisticated defense network, designed to identify and neutralize harmful invaders like bacteria, viruses, and damaged cells. At its core, the immune system is divided into two main branches: innate and adaptive immunity [120]. The innate immune system acts as the first responder, rapidly detecting common threats through pattern recognition and initiating an immediate response. In contrast, the adaptive immune system provides a more tailored defense, learning to recognize and remember specific pathogens for future protection [121]. This intricate balance between rapid action and long-term memory keeps the body resilient against external infections and internal disruptions.

Inflammasomes are critical components of the cytoplasmic architecture of immune cells, including macrophages and dendritic cells. These complexes play a pivotal role in the innate immune system by recognizing and responding to cellular stress and danger signals [122]. Central to this process are pattern recognition receptors (PRRs), which mediate the recognition of conserved molecular signatures, known as pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). PRRs are categorized based on their location; membrane-bound PRRs include toll-like receptors (TLRs) and C-type lectin receptors (CLRs), and cytoplasmic PRRs include NACHT-like receptors (NLRs) and RIG-I-like receptors (RLRs) [123].

The NLRs, a type of intracellular PRR, share a conserved structure consisting of three key domains. At the C-terminus, the leucine-rich repeat (LRR) domain is responsible for ligand recognition and autoregulation. This domain is linked to a central NACHT domain, which mediates the assembly of the inflammasome, which facilitates nucleotide binding and oligomerization, essential for receptor activation [124]. Phylogenetic analyses of the NACHT domain allowed the classification of NLRs into five distinct subfamilies based on their N-terminal effector domain. These subfamilies include NLRs (nucleotide-binding oligomerization domain-like receptors caspase activation and recruitment domain-containing proteins), which possess a caspase activation and recruitment domain (CARD); NLRPs, which comprise a pyrin domain (PYD); and NLRPA, NLRPB, NLRPX1, which lack an effector domain. The specific effector domain determines the functional properties and immune response mechanisms of each NLR subfamily [125].

The NLRP proteins, also known as NALPs, are critical components of the innate immune system. The NLRP subfamily is composed of 14 proteins [123, 126]. Among them, NLRP3 was first found in the human brain specifically in microglia, and has been the most extensively studied for its role in responding to different stimuli of TLR, pathogens, proinflammatory cytokines, and intracellular components [127]. The

NLRP3 inflammasome is the most extensively characterized member of the NLR family and has been the subject of considerable research in the context of liver disease. Within the liver, NLRP3 is primarily expressed in macrophages and monocytes, while hepatocytes and stellate cells demonstrate comparatively lower levels of NLRP3 expression [128]. The N-terminal PYD of NLRP3 is responsible for initiating immune responses by facilitating the formation of inflammasomes [129].

The NLRP3 inflammasome plays a critical role in orchestrating inflammatory responses. This multiprotein complex is involved in detecting a broad array of PAMPs and DAMPs, leading to the activation of inflammatory cascades and pyroptotic cell death. It is typically composed of a cytosolic NLR sensor, the adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD), and the effector enzyme pro-caspase-1 [127].

The cytosolic sensor NLR, specifically from the NLRP3, detects danger signals (PAMPs and DAMPs) and recruits downstream signaling molecules. Its PYD domain is important for protein-protein interactions, particularly with the adaptor protein ASC, which helps to form the inflammasome complex [127]. Pro-caspase-1 and NLRP3 are connected by ASC, which consists of two domains: CARD, which interacts with pro-caspase-1, and PYD, which binds to NLRP3. Upon the activation of NLRP3 as an inflammasome sensor molecule, ASC oligomerizes to form a large cytoplasmic structure known as the “ASC speck”, which acts as a bridge that connects NLRP3 to caspase-1 through CARD-CARD, PYD-PYD interactions for pro-caspase-1 recruitment and activation. Caspase-1 is a cysteine effector protease synthesized from its inactive precursor (pro-caspase-1). Upon recruitment to the inflammasome complex by ASC, pro-caspase-1 undergoes autocatalytic cleavage to become active [130].

Inflammasomes are activated upon receiving the initial signal, converting caspase-1 to its cleaved form. This activated caspase-1 is responsible for processing pro-inflammatory cytokines, such as pro-IL-1 β and pro-IL-18, into their mature forms (IL-1 β and IL-18) [123]. Additionally, it activates the cytoplasmic protein gasdermin D (GSDMD), which facilitates pore formation in the plasma membrane. The formation of these pores by GSDMD can lead to pyroptosis, a type of cell death characterized by DNA fragmentation and increased plasma membrane permeability, allowing for the release of IL-1 β and IL-18 into the extracellular space [131] (Figure 1).

Inflammasomes can be broadly categorized into canonical and non-canonical inflammasomes based on their activation mechanisms and downstream signaling pathways [132]. The canonical inflammasome involves a sensor protein, an adaptor protein (ASC), and caspase-1, with a well-defined priming and activation pathway. The sensor protein is upregulated through signaling pathways like those involving TLRs and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B). The non-canonical inflammasome includes components like NLRC4 and Pypin. These are characterized by the direct activation of caspase-11 (or caspase-4/5 in humans), without the need for the ASC adaptor. The sensor protein in this case can sense bacterial flagellin or other specific stimuli. The NLRP3, NLRP1, and absent in melanoma 2 (AIM2) inflammasomes fall into the canonical category, with a well-characterized pathway involving priming and subsequent activation leading to inflammation and pyroptosis [123].

Inflammasome activation

The activation of the NLRP3 inflammasome is a highly regulated, multi-step process that incorporates various cellular stress signals, resulting in the formation of the inflammasome complex and the commencement of an inflammatory response. In response to these triggers, a downstream inflammatory pathway is activated to eliminate microbial infection and repair damaged tissues [133]. This pathway requires two steps: the first step, priming, consists of a transcriptional process that positively regulates NLRP3 and proinflammatory mediators, preparing the cell for the second step, which is the activation of the inflammasome [134].

The priming phase of NLRP3 inflammasome activation, also known as signal 1, is a critical step that prepares immune cells, such as macrophages, for subsequent inflammasome assembly. This phase is initiated by PAMPs, such as bacterial lipopolysaccharide (LPS), or DAMPs released by damaged cells. These

signals activate PRRs such as TLRs or NLRs, leading to the activation of the transcription factor NF- κ B. NF- κ B drives the transcriptional upregulation of NLRP3, pro-IL-1 β , and pro-IL-18, the inactive precursors of inflammatory cytokines [133]. Importantly, these components are not constitutively expressed at high levels, so priming ensures their availability in preparation for inflammasome assembly. This step can also involve post-translational modifications like NLRP3 phosphorylation, which further prepares NLRP3 for activation.

While the first stage involves cell priming, a second phase in the inflammasome activation process takes place leading to full activation and inflammasome formation once an NLRP3 activator is recognized [134]. The activation of the NLRP3 inflammasome is triggered by a range of stimuli that induce common intracellular signals, including ionic flux (K^+ , Cl^- , Ca^{2+}), lysosomal damage, and ROS production by damaged mitochondria. These processes collectively lead to the activation of NLRP3, which is crucial for initiating the inflammatory response [133].

The release of K^+ and Ca^{2+} mobilization is prompted by stimuli such as ATP binding to P2X7 receptors, nigericin (a K^+ ionophore), and certain microbial agents [129]. K^+ efflux from the cytoplasm to the extracellular media occurs through specific ion channels or transporters, resulting in a decrease in intracellular K^+ concentration that is critical for inflammasome activation, as lower K^+ levels facilitate the assembly and functional activation of the inflammasome complex by mediating IL-1 maturation of macrophages and monocytes, thereby driving the inflammatory response. On the other hand, Ca^{2+} mobilization occurs through voltage-dependent anion channels (VDAC) at the mitochondria-associated membranes (MAMs). This mobilization of Ca^{2+} is achieved by opening the plasma membrane channels or releasing intracellular Ca^{2+} from the ER. The efflux of K^+ , which acts as a counter ion, helps the entry of Ca^{2+} through the plasma membrane channels and promotes the release of Ca^{2+} stores linked to the ER. Therefore, the release of K^+ and Ca^{2+} mobilization are linked to achieving the same goal.

In addition to ion flux, lysosomal disruption acts as a signal for NLRP3 activation. Lysosomes are essential intracellular organelles responsible for degrading biomolecules and maintaining cellular homeostasis by breaking down cellular waste, damaged organelles, and pathogens. Under pathological conditions, such as the phagocytosis of crystals, lysosomal membrane integrity can be compromised, leading to lysosomal membrane permeabilization (LMP). This process results in the release of lysosomal contents, including cathepsins, into the cytoplasm, which serve as critical signals for activating the NLRP3 inflammasome. This activation relies on potassium efflux, suggesting that molecules released during lysosomal damage may interact with the cellular membrane to trigger this process [130].

Mitochondrial dysfunction, often producing ROS, is another key signal for NLRP3 inflammasome activation. ROS can directly or indirectly activate NLRP3 by disrupting mitochondrial integrity. Mitochondrial damage, caused by stressors such as ROS or toxins, can lead to the release of mtDNA into the cytosol. This release is particularly concerning when the mtDNA is oxidized (Ox-mtDNA), as its proximity to the ETC makes it highly susceptible to oxidative damage. The presence of mtDNA outside the mitochondria is recognized as a danger signal by the immune system, triggering the activation of the NLRP3 inflammasome [135]. Additionally, recent studies indicate that priming triggers the synthesis of mtDNA via interferon regulatory factor 1 (IRF1) activation, which is required for full NLRP3 activation [136].

As mentioned before, cardiolipin is a phospholipid crucial for mitochondrial function and highly sensitive to ROS-induced oxidative damage [75, 76]. Moreover, Ca^{2+} influx promotes the translocation of cardiolipin to the outer membrane during mitochondrial stress, especially at MAMs, where cardiolipin's movement facilitates NLRP3 inflammasome assembly by influencing mitochondrial membrane integrity and signaling [137, 138] (Figure 1). Thus, cardiolipin creates a favorable environment for inflammasome formation and amplifies NLRP3 activation.

Interplay between mitochondrial ROS and inflammasome complex in liver disease

Elevated ROS levels play a significant role in the pathogenesis of various liver diseases. The liver is particularly vulnerable to oxidative stress because of its central role in detoxification, metabolism, and exposure to environmental toxins. ROS-driven damage occurs through various mechanisms, such as lipid peroxidation, in which ROS attack cell membranes; DNA damage, leading to mutations; mitochondrial dysfunction, which impairs energy production and amplifies ROS generation; inflammation, by activating signaling pathways that promote inflammatory responses; and fibrosis, where ROS activate stellate cells, contributing to scar tissue formation and liver damage [139]. These processes collectively drive the progression of liver diseases, from initial injury to more advanced stages such as cirrhosis and cancer.

ALD

Acetaldehyde, the primary byproduct of ethanol metabolism, has pro-inflammatory and fibrogenic properties that significantly contribute to the development of steatohepatitis and fibrosis in ALD [140]. The progression of ALD involves the activation of a pro-inflammatory cascade that occurs both in the liver and systemically. In the liver, this inflammatory response is initiated by PAMPs from the gut and DAMPs released from injured hepatocytes, which subsequently activate the inflammasome [141]. When alcohol is consumed, it passes into the intestines, where it disrupts the intestinal barrier—a protective layer that blocks harmful substances. This disruption weakens the tight junctions between the epithelial cells, compromising the barrier's integrity [142]. As a result, LPS, molecules found on the surface of certain bacteria, begin to pass into the bloodstream. The translocation of LPS triggers an immune response, as these bacterial components, recognized as DAMPs, are detected by TLRs. This activation initiates inflammatory signals in the liver [143, 144] (Figure 2).

Meanwhile, ethanol is metabolized in hepatocytes by ADH and the enzyme CYP2E1. During this process, CYP2E1 utilizes oxygen to convert ethanol into acetaldehyde, which generates free radicals and ROS as byproducts. The excessive production of ROS, along with inducible nitric oxide synthase (iNOS), induces ER stress and activates inflammatory pathways through the TLR4/MyD88/NF- κ B signaling axis, which significantly enhances NLRP3 inflammasome activation [145, 146]. Studies employing the Lieber-DeCarli model demonstrated that mice expressing human CYP2E1 exhibited increased susceptibility to the progression of ALD, displaying more severe liver damage, oxidative stress, inflammation, and mild fibrosis compared to wild-type mice [147].

The development of ALD is driven by various factors, including an imbalance in mitochondrial homeostasis. CYP2E1, an enzyme highly active in hepatocytes, has a strong catalytic capacity for ethanol, leading to excessive lipid accumulation, increased ROS production, mitochondrial dysfunction, and eventually hepatocyte pyroptosis [148]. Moreover, ROS plays a significant role in regulating inflammatory pathways, making them key contributors to the inflammation and progression of ALD [149].

Numerous studies have demonstrated a notable correlation between ALD and increased concentrations of uric acid and ATP. These elevated levels can trigger the activation of the NLRP3 inflammasome within Kupffer cells (KCs), the macrophages that reside in the liver, leading to the activation of caspase-1 [150] (Figure 2). Within these immune cells, NLRP3 activation is associated with intracellular mechanisms involving ROS and spleen tyrosine kinase (SYK) [151]. This inflammatory response is closely linked to chronic alcohol consumption, as ethanol and its metabolites are major contributors to hepatocyte necrosis. When hepatocytes become necrotic, they release ATP, which binds to the P2X7 receptor on KCs, causing K⁺ efflux and Ca²⁺ influx. This cascade of events further promotes NLRP3 inflammasome activation, highlighting the intricate relationship between necrosis and inflammation in the context of ALD [152]. Furthermore, alcohol consumption results in the release of extracellular ATP and uric acid from injured liver cells, while simultaneously enhancing the transport of gut-derived LPS to the liver a phenomenon attributed to increased gut permeability, which is partially facilitated by IL-18 [153]. This intricate relationship between the gut and liver, exacerbated by alcohol intake, highlights how alcohol promotes an

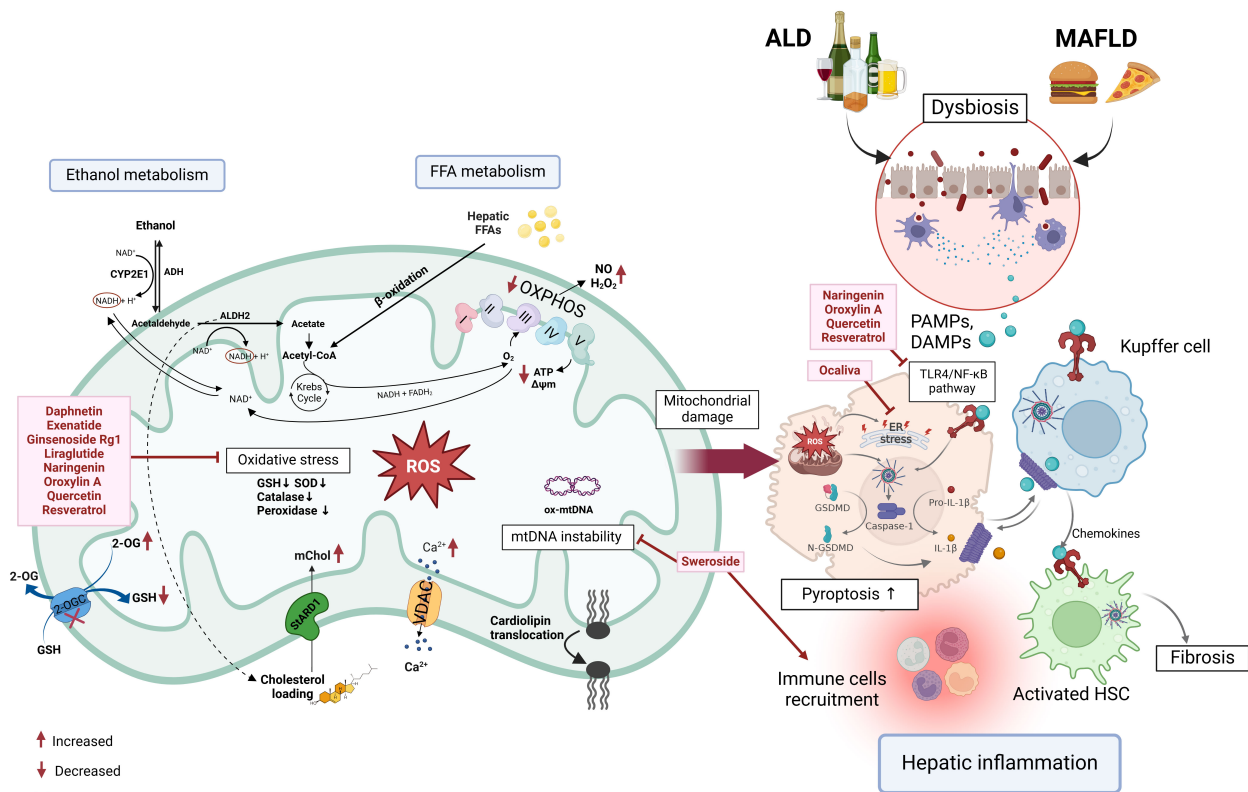


Figure 2. Targeting mitochondrial ROS-induced inflammasome in ALD and MASLD. Ethanol intake, along with diets high in FFA and cholesterol, can compromise the integrity of the intestinal barrier. This disruption allows PAMPs and DAMPs, such as FFA or microbial byproducts, to traverse the impaired tight junctions, thereby stimulating the activation of the NLRP3 inflammasome in hepatic cells. This process initiates the activation of caspase-1, which facilitates the conversion of pro-IL-1β and GSDMD into their active forms. Furthermore, the metabolism of ethanol and FFA in hepatocytes elevates ROS levels within the mitochondria, which subsequently triggers inflammasome activation. The intake of ethanol and fatty diets enhances the transport of cholesterol into mitochondria via the StARD1 protein. Cholesterol increases the membrane rigidity altering the membrane proteins and the antioxidants (GSH) transport into mitochondria to reduce ROS. Ethanol intake and fatty diets increase the cholesterol in mitochondria through StARD1 protein. PAMPs and DAMPs activate KCs and HSCs, leading to the recruitment of immune cells and the onset of hepatic inflammation, which may progress to advanced stages of ALD. CYP2E1: cytochrome P450 2E1; ADH: alcohol dehydrogenase; ALDH₂: aldehyde dehydrogenase 2; 2-OG: 2-oxoglutarate; GSH: glutathione; NAD⁺/NADH: nicotinamide adenine dinucleotide; SOD: superoxide dismutase; mChol: mitochondrial cholesterol; StARD1: steroidal acute regulatory protein 1; FFA: free fatty acid; FADH₂: flavin adenine dinucleotide; VDAC: voltage-dependent anion channel; ROS: reactive oxygen species; NO: nitric oxide; O₂: oxygen; H₂O₂: hydrogen peroxide; ALD: alcohol-related liver disease; MASLD: metabolic-dysfunction associated steatotic liver disease; PAMPs: pathogen-associated molecular patterns; DAMPs: damage-associated molecular patterns; TLR4: toll-like receptor 4; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; ER: endoplasmic reticulum; IL-1β: interleukin-1β; HSC: hepatic stellate cell; GSDMD: gasdermin D; KCs: Kupffer cells; mtDNA: mitochondrial DNA; Ox-mtDNA: oxidized mtDNA; OXPHOS: oxidative phosphorylation. Created in BioRender. Torres, S. (2024) <https://BioRender.com/g11w167>

environment that fosters liver inflammation and damage. Such conditions can elevate levels of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and IL-6, in both clinical patients and experimental models of alcohol exposure. This cascade subsequently triggers the activation of the NLRP3 inflammasome in liver cells, leading to increased production of inflammatory cytokines and further aggravating liver inflammation and injury [147].

Pyroptosis can be triggered by either canonical or noncanonical inflammasomes, primarily through activating proinflammatory caspases, specifically caspase-1 and caspase-11, via the cleavage of GSDMD [128]. This inflammatory response leads to pyroptosis in hepatocytes. Khanova et al. [154] utilized a hybrid-feeding model that combined a Western diet with intragastric ethanol administration to induce alcoholic steatohepatitis (ASH). Their findings indicated an upregulation of caspase-1 and increased processing of GSDMD, underscoring the significant role of pyroptosis in the pathogenesis of alcoholic hepatitis [154].

In the context of ALD, studies have demonstrated a link between pyroptosis in hepatocytes and the activation of the NLRP3 inflammasome. Recent experimental models of ALD have shown a notable decrease

in the expression of microRNA 148a (miR-148a) in hepatocytes. This miRNA is regulated by FoxO1, a key metabolic regulator and a tumor suppressor in the liver. This reduction promotes the overexpression of Trx-interacting protein (TXNIP), further activating the NLRP3 inflammasome and leading to pyroptosis [155] (Figure 1).

MASLD

A key characteristic of MASLD is chronic liver inflammation, which is closely associated to both mitochondrial dysfunction and activation of the NLRP3 inflammasome [156]. Understanding these interconnected processes is essential for unraveling the progression of MASLD and identifying potential therapeutic interventions.

The accumulation of FFAs and triglycerides within liver cells compromises mitochondrial integrity, leading to overproduction of ROS, primarily generated during mitochondrial β -oxidation of fatty acids [157], that consequently leads to inflammasome activation, cellular injury, and eventually fibrosis, hallmarks of MASH [2] (Figure 2). Furthermore, mitochondrial dysfunction initiates ER stress by activating the UPR, a protective mechanism that becomes maladaptive under prolonged stress [158]. This ER stress promotes *de novo* lipogenesis, worsening hepatic steatosis and accelerating the transition from simple fatty liver to the more inflammatory and fibrotic stages of MASH [159]. The tight relationship between mitochondrial health, oxidative stress, and lipid metabolism underscores mitochondria as a key driver in the pathogenesis of MASLD [160].

The NLRP3 inflammasome is another critical player in the inflammatory response associated with MASLD. Elevated levels of NLRP3 inflammasome components, such as caspase-1, have been detected in MASLD patients, and their presence is strongly correlated with the severity of liver damage [128]. In particular, therapies that modulate cholesterol metabolism, restore mitochondrial integrity, or inhibit receptors, such as the P2X7 receptor, which is a specific NLRP3 activator triggered by potassium efflux, may potentially delay or even reverse MASH progression [161]. Experimental models in mice demonstrate that deletion of the NLRP3 inflammasome reduces hepatic inflammation and fibrosis, highlighting its central role in disease progression. Pharmacological NLRP3 inhibitors like MCC950 have shown promising results in blocking NLRP3 activation, mitigating inflammation, and protecting against liver injury [162]. The intricate link between mitochondrial dysfunction and NLRP3 inflammasome activation represents a crucial pathway in MASLD progression. DAMPs, such as saturated FFAs, further enhance this activation in the early stages of MASH, making the liver more vulnerable to subsequent inflammatory insults, including LPS exposure [150]. Cholesterol overload adds another layer of complexity to this process. Excessive cholesterol, particularly when it accumulates within mitochondria, disrupts mitochondrial membrane integrity and amplifies oxidative stress [163]. In addition, cholesterol crystals can activate the NLRP3 inflammasome within KCs, accelerating the inflammatory process and contributing to fibrosis [164]. Studies have shown that reducing mitochondrial cholesterol or using cholesterol-lowering agents, like statins, can alleviate steatohepatitis in experimental models, further emphasizing the interplay between cholesterol metabolism, mitochondrial function, and NLRP3 activation [165]. However, further research is needed to assess their long-term effects on steatosis and cholesterol deposition.

In mouse models of MASLD induced by an HFD, mitochondrial damage emerges as a critical factor. This damage results in the release of mtDNA into the cytoplasm, which activates inflammasomes and worsens liver inflammation. Notably, mtDNA promotes activation of the NLRP3 and other inflammasome complexes, like AIM2 inflammasome, in liver cells, which, in turn, triggers pyroptosis—a highly inflammatory form of cell death—further aggravating liver pathology [166]. The AIM2 inflammasome, like NLRP3, responds to mitochondrial dysfunction by initiating the formation of inflammasomes, which leads to the maturation and release of pro-inflammatory cytokines, including IL-1 β and IL-18 [167]. These cytokines amplify inflammation through pyroptosis, thereby advancing the disease state in MASLD [167].

Mitochondrial dysfunction and NLRP3 inflammasome activation are tightly interconnected processes that drive the progression of MASLD from simple steatosis to MASH [150]. These pathways contribute to

inflammation, oxidative stress, and fibrosis—key aspects of the disease. Understanding these mechanisms not only enhances our knowledge of MASLD pathogenesis but also points to potential therapeutic targets. Future research focusing on therapies that improve mitochondrial health or inhibit inflammasome activation holds significant promise for more effective management of MASLD and its complications in clinical practice.

Liver cancer

Chronic liver diseases, characterized by sustained inflammation—including those with NLRP3 inflammasome activation—drive the progression from liver damage to HCC. While hepatotropic viruses evade NLRP3 to maintain chronic infections with minimal immune activation, the established HCC environment exhibits active NLRP3 signaling, which paradoxically promotes tumor growth [168]. In HCC, NLRP3 activation drives a pro-inflammatory and pro-tumorigenic environment, supporting cancer cell survival, immune evasion, and angiogenesis [169].

The NLRP3 inflammasome plays a pivotal role in shaping the HCC tumor microenvironment (TME), which is heavily influenced by chronic liver inflammation. The HCC TME consists of diverse cell populations—cancer-associated fibroblasts (CAFs), myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), and regulatory T cells (Tregs)—all of which contribute to immune evasion and tumor progression [169, 170]. Inflammatory cytokines released upon NLRP3 activation can recruit immune cells to the tumor site, where they might support tumor growth and metastasis by promoting an immunosuppressive TME. Elevated levels of IL-1 β , for example, have been shown to promote the recruitment of MDSCs, which contribute to chemotherapy resistance.

Recent research has investigated how NLRP3 expression in HCC cells influences NK cell-mediated cancer surveillance, reducing their number and functionality. Findings indicate that knocking out NLRP3 in HCC cells enhances NK cell cytotoxic effects. In line with this data, in xenograft mouse models, NLRP3 knockout in HCC cells was associated with slower tumor progression and metastasis, as well as an increased susceptibility of the tumor to NK cell attacks. Altogether, this suggests that targeting NLRP3 in HCC may boost NK cell immunosurveillance, offering a promising strategy for NK cell-based immunotherapies in liver cancer [171]. In addition to this, NLRP3 activation impacts metabolic pathways within the TME. For instance, it promotes fatty acid oxidation (FAO) in TAMs, fostering an immunosuppressive M2 macrophage phenotype conducive to tumor progression [172]. Furthermore, ROS generated through FAO activate the NLRP3/IL-1 β signaling pathway, which is crucial for shaping immune responses within the TME [173]. Further, recent studies indicate that receptor-interacting protein kinase 3 (RIPK3) influences fatty acid metabolism and HCC progression through NLRP3/caspase-1 signaling in TAMs.

On the other hand, other studies suggest that NLRP3 activation has anti-tumor potential in HCC, notably through inducing pyroptosis, which can suppress tumor progression by stimulating the recruitment of NK cells to the tumor site. In this line, *in vitro* studies indicate that NLRP3 inflammasome activation may inhibit tumor progression by reducing cell proliferation and migration. Specifically, in Bel-7402 and SMMC-7721 HCC cell lines, NLRP3 activation suppresses DNA replication, slows cell proliferation, and induces G1 cell cycle arrest. Combined LPS and ATP treatment significantly reduces the migration of these HCC cells without inducing apoptosis, suggesting that NLRP3 activation may inhibit tumor growth by limiting cancer cell proliferation and migration [173]. Similarly, research suggests that inflammasomes may initially act to minimize tumor growth in response to lactate buildup in the TME. However, tumor cells often adapt by increasing their production of TGF- β , a cytokine that promotes further immunosuppressive mechanisms, thereby counteracting this restriction [174].

Lastly, MiR-223-3p has been shown to regulate HCC by binding to the 3'-untranslated region of NLRP3, thereby suppressing its expression [175, 176]. This suppression of the NLRP3 inflammasome induces apoptosis and inhibits the proliferation of HCC cells [177]. However, this observation contrasts with previous studies suggesting that NLRP3 inflammasome inhibition promotes apoptosis in HCC cells [178].

In summary, the NLRP3 inflammasome plays a complex and dualistic role in HCC progression, acting as both a tumor suppressor and promoter depending on the context [179–181]. In early stages, NLRP3 activation may enhance anti-tumor immunity and tumor cell death, whereas, in established HCC, it can facilitate immune evasion and tumor growth through inflammatory pathways involving NF- κ B and ATP. Targeting NLRP3 or its downstream pathways thus presents a promising therapeutic approach to modulate the TME in favor of anti-tumor responses. Context-dependent effects of NLRP3 require tailored therapeutic strategies that account for factors such as HCC stage, underlying liver disease (e.g., viral hepatitis, NAFLD), and the patient's immune status [178]. Further research is essential to clarify NLRP3's mechanisms across diverse cell types and disease stages, which could ultimately inform the development of treatments that enhance immune surveillance while minimizing tumor-promoting inflammation in HCC.

Mitochondria and inflammasome as a therapeutic goal in liver disease

Since mitochondria are associated with the regulation of NLRP3 activation, this has led to the development of new methods and drugs that target mitochondria for the treatment of inflammation-associated human pathologies.

Numerous drug candidates have been discovered through chemical synthesis or the analysis of natural products and their derivatives. Several of these drug candidates have demonstrated potential efficacy in treating diseases in both in vitro and in vivo models, and a few have advanced to the clinical trial stage.

From a mechanistic perspective, numerous steps involved in NLRP3 inflammasome activation and signaling present viable targets for pharmacological intervention in NLRP3 inflammasome-associated liver diseases (Figure 2).

Liraglutide, an analog of glucagon-like peptide-1 (GLP-1), has recently been recognized as an effective first-line therapy for type 2 diabetes mellitus (T2DM) and has demonstrated the ability to reduce hepatic steatosis. In a specific mouse model, liraglutide was shown to improve MASLD experimental models and to alleviate hepatitis by inhibiting the activation of the hepatic NLRP3 inflammasome [182]. Additionally, recent findings suggested that liraglutide could mitigate MASLD by regulating the activation of the NLRP3 inflammasome and pyroptosis, thereby influencing mitophagy [183].

Clinical evidence also supports the notion that liraglutide can enhance liver function and promote histological improvement in MASLD patients [184, 185]. These findings suggest that liraglutide may be a viable option for early intervention in patients with T2DM and MASLD as a preventive measure.

Exenatide is classified as a GLP-1 receptor agonist that promotes insulin secretion and is utilized in the management of T2DM. Research has demonstrated that exenatide effectively reduces the severity of MASLD in both in vivo and in vitro settings. Exenatide treatment has demonstrated the ability to enhance the flux of the mitochondrial tricarboxylic acid cycle, while also significantly reducing insulin resistance, steatosis, and lipotoxicity in hepatocytes [186]. Additionally, exenatide mitigates oxidative stress-induced damage and regulates NLRP3 inflammasome activation by modulating mitophagy and autophagy pathways in the liver, providing protective effects in murine models of MASLD and diabetes [186]. In clinical trials, exenatide has been shown to mitigate the pathological symptoms of MASLD in patients with T2DM, promoting weight reduction, partially suppressing inflammation, and hindering the advancement of steatosis [187–189]. Therefore, exenatide may be integrated into personalized treatment strategies for MASLD.

Ocaliva (obeticholic acid) functions as a ligand for farnesoid X receptor (FXR), which is essential for maintaining liver homeostasis and is crucial for cellular and mitochondrial functionality [190]. The expression levels of hepatic FXR are inversely correlated with the activation of the NLRP3 inflammasome in MASLD [191], which can be attributed to hepatitis B virus infection or liver failure in patients, as well as liver injury in murine models [192–195]. FXR has been shown to inhibit the activation of the NLRP3 inflammasome induced by ER stress in hepatocytes, while also downregulating the expression of NLRP3 and TXNIP through the PERK–CHOP signaling pathway [196]. Ocaliva has indeed been utilized in various clinical trials for the treatment of MASLD, showing a significant reduction in fibrosis and an improvement in pathological symptoms among patients with MASH, indicating a potential clinical benefit [197].

In this regard, numerous natural products have been recognized for their ability to influence NLRP3 inflammasome activation, either directly or indirectly, by enhancing mitochondrial function. Among them, ginsenoside Rg1, recognized as the predominant component in ginseng saponin [99], demonstrates protective properties in both in vivo and in vitro experimental models against hepatic steatosis, inflammation, and fibrosis associated with various liver diseases [198–200]. The hepatoprotective mechanisms of Rg1 include the action of antioxidants, the reduction of ROS release, the inhibition of apoptosis-related proteins and inflammatory cytokines, and the suppression of NLRP3 inflammasome activation [201]. More specifically, Rg1 facilitates the blockade of steatosis in hepatocytes, inhibits fibrosis in hepatic stellate cells, and promotes mitophagy in KCs, thereby further inhibiting NLRP3 inflammasome activation [202–205].

Another natural product that protects against liver disease is the polyphenolic plant pigment quercetin. Numerous studies have indicated that quercetin plays a positive role in MASLD, ALD, and T2DM experimental models by modulating the expression of lipid metabolism genes and influencing inflammatory mediators' release [206, 207]. Recent findings in alcohol-induced acute liver injury in rats showed that quercetin might protect against liver damage by upregulating the expression of *HO-1*, an Nrf2-dependent gene, thereby inhibiting NLRP3 inflammasome activation and inflammatory cytokine secretion [208]. In mice fed with an MCD diet, quercetin ameliorates liver pathology by facilitating mitophagy through the activation of AMPK [209]. This indicates that enhancing mitophagy through the upregulation of AMPK could serve as a potential therapeutic approach for combating MASLD.

Sweroside, a key ingredient in traditional Chinese medicine for the treatment of hepatitis, demonstrates a notable protective effect against liver injury and fibrosis induced by chemicals [210, 211]. In experimental models of MASLD, sweroside has been shown to enhance the infiltration of hepatic immune cells while also regulating lipid metabolism, liver fibrosis, and inflammatory responses. The mechanism of action for sweroside involves the inhibition of de novo synthesis of mtDNA in the liver, which plays a role in reducing the activation of the hepatic NLRP3 inflammasome and lowering levels of hepatic IL-1 β and caspase-1 [212, 213].

Oroxylin A and daphnetin are additional natural compounds recognized for their hepatoprotective properties [214–216]. Both substances demonstrate the ability to mitigate oxidative stress and mitochondrial dysfunction, thereby preventing the activation of the NLRP3 inflammasome, albeit through distinct mechanisms. For instance, in models of ALD, oroxylin A has been found to inhibit the activation of pyroptosis mediated by mitochondrial ROS via the peroxisome proliferator-activated receptor gamma coactivator 1 α (PGC-1 α)/Mfn2 pathway [217]. Conversely, daphnetin exhibits protective effects in models of drug-induced liver injury by inhibiting the activation of the ROS-TXNIP/NLRP3 inflammasome pathway [218].

Resveratrol, a natural polyphenol with phytoalexin properties, exhibits beneficial effects on lipid accumulation and lipid profiles, enhances insulin sensitivity, and possesses significant anti-inflammatory and antioxidant characteristics, positioning it as a potential hepatoprotective agent for the treatment of MASLD. The administration of resveratrol in a rodent model of HFD-induced obesity has been shown to alleviate hepatic metaflammation and fatty liver, which is associated with changes in NLRP3 inflammasomes and a reduction in pro-inflammatory marker levels [219]. Resveratrol also improves the condition of ALD by reducing the expression of HIF1 α and decreasing the production of mitochondrial ROS [220]. However, a meta-analysis involving 302 patients with MASLD across seven randomized clinical trials concluded that the current evidence is inadequate to confirm the effectiveness of resveratrol in managing and treating MASLD [221]. Further investigation in this field is warranted.

Naringenin is a flavonoid predominantly found in high levels within citrus fruits. It has demonstrated protective effects against liver damage, attributed to its anti-inflammatory, anti-fibrotic, antioxidant, and anticancer characteristics [222]. Studies involving naringenin administration have revealed its beneficial impact on the management of MASLD by affecting lipid metabolism, which encompasses the regulation of lipid and cholesterol oxidation, accumulation, and synthesis. Additionally, naringenin decreases the

expression of NLRP3 and IL-1 β , while also inhibiting the advancement of MASLD by targeting the NLRP3/NF- κ B signaling pathway in hepatocytes and KCs [223]. As a result, naringenin is considered a promising candidate for the treatment of liver diseases.

Conclusions

NLRP3 inflammasome activation is acknowledged as a critical contributor to the initiation and advancement of numerous liver diseases. Mitochondria, which are among the most abundant organelles in the liver, are essential in modulating NLRP3 inflammasome activation in the context of liver injury. This modulation takes place through various mechanisms, such as enhancing the release of DAMPs, promoting the recruitment of NLRP3 to mitochondria, inducing mitochondrial calcium overload, increasing mitochondrial ROS production, and the inhibitory effects of mitophagy. The overproduction of ROS and disruptions in OXPHOS play a crucial role in mitochondrial dysfunction, along with oxidative damage that affects both mitochondrial respiratory chain complexes and mtDNA. This damage partially obstructs electron flow, leading to an increased production of mitochondrial ROS, which in turn creates a harmful cycle of escalating damage. Understanding the role of mitochondria in NLRP3 inflammasome activation during liver injury provides valuable insights for developing novel therapeutic strategies targeting NLRP3 inflammasome-related conditions. Both pharmacological derivatives and natural compounds have shown the potential to alleviate liver damage by influencing NLRP3 inflammasome activation through mitochondrial pathways. Studies conducted on human and animal models highlight the promise of new antioxidant strategies to mitigate mitochondrial ROS production, providing a promising approach for the prevention and treatment of liver diseases.

Abbreviations

•OH: hydroxyl radical

ADH: alcohol dehydrogenase

AIM2: absent in melanoma 2

ALD: alcohol-related liver disease

ASC: apoptosis-associated speck-like protein containing a caspase activation and recruitment domain

CARD: caspase activation and recruitment domain

CDD: choline-deficient diet

CYP: cytochrome P450

DAMPs: damage-associated molecular patterns

ER: endoplasmic reticulum

ETC: electron transport chain

FAO: fatty acid oxidation

FFAs: free fatty acids

FXR: farnesoid X receptor

GLP-1: glucagon-like peptide-1

Gpx: glutathione peroxidase

GSDMD: gasdermin D

H₂O₂: hydrogen peroxide

HCC: hepatocellular carcinoma

HFD: high-fat diet

HIF1 α : hypoxia-inducible factor 1 alpha

KCs: Kupffer cells
LPS: lipopolysaccharide
MAM: mitochondria-associated membranes
MASH: metabolic dysfunction-associated steatohepatitis
MASLD: metabolic-dysfunction associated steatotic liver disease
MCD: methionine-choline-deficient
MDSCs: myeloid-derived suppressor cells
mGSH: mitochondrial glutathione
mtDNA: mitochondrial DNA
NAFLD: non-alcoholic fatty liver disease
NF- κ B: nuclear factor kappa-light-chain-enhancer of activated B cells
NLRCs: nucleotide-binding oligomerization domain-like receptors caspase activation and recruitment domain-containing proteins
NLRP3: nucleotide-binding oligomerization domain-like receptor family, pyrin domain containing 3
NLRs: NACHT-like receptors
NO: nitric oxide
 $O_2^{\bullet -}$: superoxide anion
OXPHOS: oxidative phosphorylation
PAMPs: pathogen-associated molecular patterns
PP: periportal
PRRs: pattern recognition receptors
PV: perivenous
PYD: pyrin domain
ROS: reactive oxygen species
SOD: superoxide dismutase
StARD1: steroidogenic acute regulatory protein 1
T2DM: type 2 diabetes mellitus
TAMs: tumor-associated macrophages
TLRs: toll-like receptor
TME: tumor microenvironment
Trx: thioredoxin
TXNIP: thioredoxin-interacting protein
UPR: unfolded protein response

Declarations

Author contributions

HSJ: Conceptualization, Investigation, Writing—original draft, Writing—review & editing. ST: Validation, Writing—review & editing, Supervision. Both authors read and approved the submitted version.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

Not applicable.

Funding

We acknowledge the support from the grants [PID2022-140169OB-C22] from Plan Nacional de I+D funded by the Agencia Estatal de Investigación (AEI) and from Beatriu de Pinós Post-doctoral grant [2021 BP00187]; as well as from the CIBER, the intramural project grant for junior researchers [CIBEREHD 2023] and the support from AGAUR of the Generalitat de Catalunya [SGR-2021-00491]. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright

© The Author(s) 2024.

References

1. Hernansanz-Agustín P, Enríquez JA. Generation of Reactive Oxygen Species by Mitochondria. *Antioxidants (Basel)*. 2021;10:415. [DOI] [PubMed] [PMC]
2. Allameh A, Niayesh-Mehr R, Aliarab A, Sebastiani G, Pantopoulos K. Oxidative Stress in Liver Pathophysiology and Disease. *Antioxidants (Basel)*. 2023;12:1653. [DOI] [PubMed] [PMC]
3. Mandal M, Sarkar M, Khan A, Biswas M, Masi A, Rakwal R, et al. Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) in Plants– Maintenance of Structural Individuality and Functional Blend. *Adv Redox Res*. 2022;5:100039. [DOI]
4. Conde de la Rosa L, Goicoechea L, Torres S, Garcia-Ruiz C, Fernandez-Checa JC. Role of Oxidative Stress in Liver Disorders. *Livers*. 2022;2:283–314. [DOI]
5. Jomova K, Raptova R, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, et al. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. *Arch Toxicol*. 2023;97: 2499–574. [DOI] [PubMed] [PMC]
6. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. *Clin Interv Aging*. 2018;13:757–72. [DOI] [PubMed] [PMC]
7. Cao J, Wang C, Hao N, Fujiwara T, Wu T. Endoplasmic Reticulum Stress and Reactive Oxygen Species in Plants. *Antioxidants (Basel)*. 2022;11:1240. [DOI] [PubMed] [PMC]
8. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. *Indian J Clin Biochem*. 2015;30:11–26. [DOI] [PubMed] [PMC]
9. Lartigue L, Kushnareva Y, Seong Y, Lin H, Faustin B, Newmeyer DD. Caspase-independent mitochondrial cell death results from loss of respiration, not cytotoxic protein release. *Mol Biol Cell*. 2009;20:4871–84. [DOI] [PubMed] [PMC]

10. Lin X, David CA, Donnelly JB, Michaelides M, Chandel NS, Huang X, et al. A chemical genomics screen highlights the essential role of mitochondria in HIF-1 regulation. *Proc Natl Acad Sci U S A*. 2008;105:174–9. [DOI] [PubMed] [PMC]
11. Chandel NS, McClintock DS, Feliciano CE, Wood TM, Melendez JA, Rodriguez AM, et al. Reactive Oxygen Species Generated at Mitochondrial Complex III Stabilize Hypoxia-inducible Factor-1 α during Hypoxia: a mechanism of O₂ sensing. *J Biol Chem*. 2000;275:25130–8. [DOI] [PubMed]
12. Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, Schumacker PT. Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. *Proc Natl Acad Sci U S A*. 1998;95:11715–20. [DOI] [PubMed] [PMC]
13. Kennedy SG, Kandel ES, Cross TK, Hay N. Akt/Protein kinase B inhibits cell death by preventing the release of cytochrome c from mitochondria. *Mol Cell Biol*. 1999;19:5800–10. [DOI] [PubMed] [PMC]
14. Gottlob K, Majewski N, Kennedy S, Kandel E, Robey RB, Hay N. Inhibition of early apoptotic events by Akt/PKB is dependent on the first committed step of glycolysis and mitochondrial hexokinase. *Genes Dev*. 2001;15:1406–18. [DOI] [PubMed] [PMC]
15. Datta SR, Ranger AM, Lin MZ, Sturgill JF, Ma Y, Cowan CW, et al. Survival factor-mediated BAD phosphorylation raises the mitochondrial threshold for apoptosis. *Dev Cell*. 2002;3:631–43. [DOI] [PubMed]
16. Yamaguchi H, Wang HG. The protein kinase PKB/Akt regulates cell survival and apoptosis by inhibiting Bax conformational change. *Oncogene*. 2001;20:7779–86. [DOI] [PubMed]
17. Balmanno K, Cook SJ. Tumour cell survival signalling by the ERK1/2 pathway. *Cell Death Differ*. 2009;16:368–77. [DOI] [PubMed]
18. Cassano S, Agnese S, D'Amato V, Papale M, Garbi C, Castagnola P, et al. Reactive oxygen species, Ki-Ras, and mitochondrial superoxide dismutase cooperate in nerve growth factor-induced differentiation of PC12 cells. *J Biol Chem*. 2010;285:24141–53. [DOI] [PubMed] [PMC]
19. Park MA, Zhang G, Mitchell C, Rahmani M, Hamed H, Hagan MP, et al. Mitogen-Activated Protein Kinase Kinase 1/2 Inhibitors and 17-Allylamino-17-Demethoxygeldanamycin Synergize to Kill Human Gastrointestinal Tumor Cells *in Vitro* via Suppression of c-FLIP-s Levels and Activation of CD95. *Mol Cancer Ther*. 2008;7:2633–48. [DOI]
20. Bhagatte Y, Lodwick D, Storey N. Mitochondrial ROS production and subsequent ERK phosphorylation are necessary for temperature preconditioning of isolated ventricular myocytes. *Cell Death Dis*. 2012;3:e345. [DOI] [PubMed] [PMC]
21. Desai R, East DA, Hardy L, Faccenda D, Rigon M, Crosby J, et al. Mitochondria form contact sites with the nucleus to couple prosurvival retrograde response. *Sci Adv*. 2020;6:eabc9955. [DOI] [PubMed] [PMC]
22. Tormos KV, Anso E, Hamanaka RB, Eisenbart J, Joseph J, Kalyanaraman B, et al. Mitochondrial complex III ROS regulate adipocyte differentiation. *Cell Metab*. 2011;14:537–44. [DOI] [PubMed] [PMC]
23. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007;39:44–84. [DOI] [PubMed]
24. HARMAN D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol*. 1956;11:298–300. [DOI] [PubMed]
25. Nunnari J, Suomalainen A. Mitochondria: in sickness and in health. *Cell*. 2012;148:1145–59. [DOI] [PubMed] [PMC]
26. Middleton P, Vergis N. Mitochondrial dysfunction and liver disease: role, relevance, and potential for therapeutic modulation. *Therap Adv Gastroenterol*. 2021;14:17562848211031394. [DOI] [PubMed] [PMC]
27. Osna NA, Jr TMD, Kharbanda KK. Alcoholic Liver Disease: Pathogenesis and Current Management. *Alcohol Res*. 2017;38:147–61. [PubMed] [PMC]

28. Devarbhavi H, Asrani SK, Arab JP, Nartey YA, Pose E, Kamath PS. Global burden of liver disease: 2023 update. *J Hepatol.* 2023;79:516–37. [DOI] [PubMed]
29. Mackowiak B, Fu Y, Maccioni L, Gao B. Alcohol-associated liver disease. *J Clin Invest.* 2024;134:e176345. [DOI] [PubMed] [PMC]
30. Jung F, Sánchez V, Brandt A, Berghheim I. Alcohol-related liver disease: also a question of what you drink? *Explor Dig Dis.* 2023;2:118–32. [DOI] [PubMed] [PMC]
31. Wu D, Cederbaum AI. Oxidative stress and alcoholic liver disease. *Semin Liver Dis.* 2009;29:141–54. [DOI] [PubMed]
32. Mansouri A, Gaou I, Kerguenec CD, Amsellem S, Haouzi D, Berson A, et al. An alcoholic binge causes massive degradation of hepatic mitochondrial DNA in mice. *Gastroenterology.* 1999;117:181–90. [DOI] [PubMed]
33. Mansouri A, Fromenty B, Berson A, Robin MA, Grimbert S, Beaugrand M, et al. Multiple hepatic mitochondrial DNA deletions suggest premature oxidative aging in alcoholic patients. *J Hepatol.* 1997;27:96–102. [DOI] [PubMed]
34. Larosche I, Lettéron P, Berson A, Fromenty B, Huang T, Moreau R, et al. Hepatic mitochondrial DNA depletion after an alcohol binge in mice: probable role of peroxynitrite and modulation by manganese superoxide dismutase. *J Pharmacol Exp Ther.* 2010;332:886–97. [DOI] [PubMed]
35. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology.* 2001;120:1183–92. [DOI] [PubMed]
36. Caldwell SH, Swerdlow RH, Khan EM, Iezzoni JC, Hespeneheide EE, Parks JK, et al. Mitochondrial abnormalities in non-alcoholic steatohepatitis. *J Hepatol.* 1999;31:430–4. [DOI] [PubMed]
37. Samuvel DJ, Li L, Krishnasamy Y, Goos M, Takemoto K, Woster PM, et al. Mitochondrial depolarization after acute ethanol treatment drives mitophagy in living mice. *Autophagy.* 2022;18:2671–85. [DOI] [PubMed] [PMC]
38. Adachi M, Ishii H. Role of mitochondria in alcoholic liver injury. *Free Radic Biol Med.* 2002;32:487–91. [DOI] [PubMed]
39. Chacko BK, Srivastava A, Johnson MS, Benavides GA, Chang MJ, Ye Y, et al. Mitochondria-targeted ubiquinone (MitoQ) decreases ethanol-dependent micro and macro hepatosteatosis. *Hepatology.* 2011;54:153–63. [DOI] [PubMed] [PMC]
40. Spach PI, Cunningham CC. Control of state 3 respiration in liver mitochondria from rats subjected to chronic ethanol consumption. *Biochim Biophys Acta.* 1987;894:460–7. [DOI] [PubMed]
41. Cederbaum AI, Lieber CS, Rubin E. Effects of chronic ethanol treatment on mitochondrial functions damage to coupling site I. *Arch Biochem Biophys.* 1974;165:560–9. [DOI] [PubMed]
42. Bernstein JD, Penniall R. Effects of chronic ethanol treatment upon rat liver mitochondria. *Biochem Pharmacol.* 1978;27:2337–42. [DOI] [PubMed]
43. Venkatraman A, Shiva S, Wigley A, Ulasova E, Chhieng D, Bailey SM, et al. The role of iNOS in alcohol-dependent hepatotoxicity and mitochondrial dysfunction in mice. *Hepatology.* 2004;40:565–73. [DOI] [PubMed]
44. Zhang X, Tachibana S, Wang H, Hisada M, Williams GM, Gao B, et al. Interleukin-6 is an important mediator for mitochondrial DNA repair after alcoholic liver injury in mice. *Hepatology.* 2010;52:2137–47. [DOI] [PubMed] [PMC]
45. Han D, Ybanez MD, Johnson HS, McDonald JN, Mesrobian L, Sancheti H, et al. Dynamic adaptation of liver mitochondria to chronic alcohol feeding in mice: biogenesis, remodeling, and functional alterations. *J Biol Chem.* 2012;287:42165–79. [DOI] [PubMed] [PMC]
46. Goikoetxea-Usandizaga N, Bravo M, Egiá-Mendikute L, Abecia L, Serrano-Maciá M, Urduñigo RG, et al. The outcome of boosting mitochondrial activity in alcohol-associated liver disease is organ-dependent. *Hepatology.* 2023;78:878–95. [DOI] [PubMed] [PMC]

47. Han D, Johnson HS, Rao MP, Martin G, Sancheti H, Silkwood KH, et al. Mitochondrial remodeling in the liver following chronic alcohol feeding to rats. *Free Radic Biol Med.* 2017;102:100–10. [DOI] [PubMed] [PMC]
48. Insausti N, Solsona-Vilarrasa E, Fucho R, Torres S, Alarcón-Vila C, Nuñez S, et al. Chronic Alcohol Feeding Increases Mitochondrial Respiration in Frg Mice with Humanized Liver. *Hepatology.* 2018;68:799A.
49. Fucho R, Solsona-Vilarrasa E, Torres S, Nuñez S, Insausti-Urkiá N, Edo A, et al. Zonal expression of StARD1 and oxidative stress in alcoholic-related liver disease. *J Lipid Res.* 2023;64:100413. [DOI] [PubMed] [PMC]
50. Fernandez A, Matias N, Fucho R, Ribas V, Montfort CV, Nuño N, et al. ASMase is required for chronic alcohol induced hepatic endoplasmic reticulum stress and mitochondrial cholesterol loading. *J Hepatol.* 2013;59:805–13. [DOI] [PubMed] [PMC]
51. García-Ruiz C, Morales A, Ballesta A, Rodés J, Kaplowitz N, Fernández-Checa JC. Effect of chronic ethanol feeding on glutathione and functional integrity of mitochondria in periportal and perivenous rat hepatocytes. *J Clin Invest.* 1994;94:193–201. [DOI] [PubMed] [PMC]
52. Lluís JM, Colell A, García-Ruiz C, Kaplowitz N, Fernández-Checa JC. Acetaldehyde impairs mitochondrial glutathione transport in HepG2 cells through endoplasmic reticulum stress. *Gastroenterology.* 2003;124:708–24. [DOI] [PubMed]
53. Palma E, Ma X, Riva A, Iansante V, Dhawan A, Wang S, et al. Dynamin-1-Like Protein Inhibition Drives Megamitochondria Formation as an Adaptive Response in Alcohol-Induced Hepatotoxicity. *Am J Pathol.* 2019;189:580–9. [DOI] [PubMed] [PMC]
54. Yan M, Zhu P, Liu H, Zhang H, Liu L. Ethanol induced mitochondria injury and permeability transition pore opening: role of mitochondria in alcoholic liver disease. *World J Gastroenterol.* 2007;13:2352–6. [DOI] [PubMed] [PMC]
55. Zhong Z, Ramshesh VK, Rehman H, Liu Q, Theruvath TP, Krishnasamy Y, et al. Acute ethanol causes hepatic mitochondrial depolarization in mice: role of ethanol metabolism. *PLoS One.* 2014;9:e91308. [DOI] [PubMed] [PMC]
56. Wang G, Mémin E, Murali I, Gaspers LD. The effect of chronic alcohol consumption on mitochondrial calcium handling in hepatocytes. *Biochem J.* 2016;473:3903–21. [DOI] [PubMed] [PMC]
57. Williams JA, Ni H, Ding Y, Ding W. Parkin regulates mitophagy and mitochondrial function to protect against alcohol-induced liver injury and steatosis in mice. *Am J Physiol Gastrointest Liver Physiol.* 2015;309:G324–40. [DOI] [PubMed] [PMC]
58. Zhou H, Zhu P, Wang J, Toan S, Ren J. DNA-PKcs promotes alcohol-related liver disease by activating Drp1-related mitochondrial fission and repressing FUNDC1-required mitophagy. *Signal Transduct Target Ther.* 2019;4:56. [DOI] [PubMed] [PMC]
59. Ribas V. Role of Cholesterol Homeostasis in MASH-Driven Hepatocellular Carcinoma: Not Just a Neutral Fat. *Explor Dig Dis.* 2024;3:203–25. [DOI]
60. Younossi ZM, Golabi P, Paik JM, Henry A, Dongen CV, Henry L. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. *Hepatology.* 2023;77:1335–47. [DOI] [PubMed] [PMC]
61. Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. *Nat Med.* 2018;24:908–22. [DOI] [PubMed] [PMC]
62. Rector RS, Thyfault JP, Uptergrove GM, Morris EM, Naples SP, Borengasser SJ, et al. Mitochondrial dysfunction precedes insulin resistance and hepatic steatosis and contributes to the natural history of non-alcoholic fatty liver disease in an obese rodent model. *J Hepatol.* 2010;52:727–36. [DOI] [PubMed] [PMC]
63. Muoio DM, Newgard CB. Molecular and metabolic mechanisms of insulin resistance and β -cell failure in type 2 diabetes. *Nat Rev Mol Cell Biol.* 2008;9:193–205. [DOI] [PubMed]

64. Savage DB, Petersen KF, Shulman GI. Disordered lipid metabolism and the pathogenesis of insulin resistance. *Physiol Rev.* 2007;87:507–20. [DOI] [PubMed] [PMC]
65. Lee K, Haddad A, Osme A, Kim C, Borzou A, Ilchenko S, et al. Hepatic Mitochondrial Defects in a Nonalcoholic Fatty Liver Disease Mouse Model Are Associated with Increased Degradation of Oxidative Phosphorylation Subunits. *Mol Cell Proteomics.* 2018;17:2371–86. [DOI] [PubMed] [PMC]
66. Kamfar S, Alavian SM, Houshmand M, Yadegarazari R, Zarei BS, Khalaj A, et al. Liver Mitochondrial DNA Copy Number and Deletion Levels May Contribute to Nonalcoholic Fatty Liver Disease Susceptibility. *Hepat Mon.* 2016;16:e40774. [DOI] [PubMed] [PMC]
67. Arroyave-Ospina JC, Wu Z, Geng Y, Moshage H. Role of Oxidative Stress in the Pathogenesis of Non-Alcoholic Fatty Liver Disease: Implications for Prevention and Therapy. *Antioxidants (Basel).* 2021; 10:174. [DOI] [PubMed] [PMC]
68. Brady LJ, Brady PS, Romsos DR, Hoppel CL. Elevated hepatic mitochondrial and peroxisomal oxidative capacities in fed and starved adult obese (*ob/ob*) mice. *Biochem J.* 1985;231:439–44. [DOI] [PubMed] [PMC]
69. Koliaki C, Szendroedi J, Kaul K, Jelenik T, Nowotny P, Jankowiak F, et al. Adaptation of hepatic mitochondrial function in humans with non-alcoholic fatty liver is lost in steatohepatitis. *Cell Metab.* 2015;21:739–46. [DOI] [PubMed]
70. Santos JC, de Araújo OR, Valentim IB, de Andrade KQ, Moura FA, Smaniotto S, et al. Choline and Cystine Deficient Diets in Animal Models with Hepatocellular Injury: Evaluation of Oxidative Stress and Expression of RAGE, TNF- α , and IL-1 β . *Oxid Med Cell Longev.* 2015;2015:121925. [DOI] [PubMed] [PMC]
71. Satapati S, Kucejova B, Duarte JAG, Fletcher JA, Reynolds L, Sunny NE, et al. Mitochondrial metabolism mediates oxidative stress and inflammation in fatty liver. *J Clin Invest.* 2015;125: 4447–62. [DOI] [PubMed] [PMC]
72. Lu Y, Li Y, Sun Y, Ma S, Zhang K, Tang X, et al. Differences in energy metabolism and mitochondrial redox status account for the differences in propensity for developing obesity in rats fed on high-fat diet. *Food Sci Nutr.* 2021;9:1603–13. [DOI] [PubMed] [PMC]
73. Serviddio G, Bellanti F, Tamborra R, Rollo T, Capitanio N, Romano AD, et al. Uncoupling protein-2 (UCP2) induces mitochondrial proton leak and increases susceptibility of non-alcoholic steatohepatitis (NASH) liver to ischaemia-reperfusion injury. *Gut.* 2008;57:957–65. [DOI] [PubMed]
74. Videla LA, Rodrigo R, Orellana M, Fernandez V, Tapia G, Quiñones L, et al. Oxidative stress-related parameters in the liver of non-alcoholic fatty liver disease patients. *Clin Sci (Lond).* 2004;106:261–8. [DOI] [PubMed]
75. Win S, Than TA, Kaplowitz N, Wong N, Arya A, Win Z. et al.. et al. The Central Role of Mitochondrial Metabolism in Hepatic Steatosis. *Explor Dig Dis.* 2024;3:42–68. [DOI]
76. Ferreira FM, Seica R, Oliveira PJ, Coxito PM, Moreno AJ, Palmeira CM, et al. Diabetes induces metabolic adaptations in rat liver mitochondria: role of coenzyme Q and cardiolipin contents. *Biochim Biophys Acta.* 2003;1639:113–20. [DOI] [PubMed]
77. Sagar S, Faizan MI, Chaudhary N, Singh V, Singh P, Gheware A, et al. Obesity impairs cardiolipin-dependent mitophagy and therapeutic intercellular mitochondrial transfer ability of mesenchymal stem cells. *Cell Death Dis.* 2023;14:324. [DOI] [PubMed] [PMC]
78. Li J, Romestaing C, Han X, Li Y, Hao X, Wu Y, et al. Cardiolipin remodeling by ALCAT1 links oxidative stress and mitochondrial dysfunction to obesity. *Cell Metab.* 2010;12:154–65. [DOI] [PubMed] [PMC]
79. Fàbrega L, Fernández-Checa JC, Rosa LC. de la; Garcia-Ruiz C. Impact of Mitochondrial Lipid Alterations on Liver Disease Mechanisms and Progression. *Explor Dig Dis.* 2024;3:382–413. [DOI]
80. Tu C, Xiong H, Hu Y, Wang W, Mei G, Wang H, et al. Cardiolipin Synthase 1 Ameliorates NASH Through Activating Transcription Factor 3 Transcriptional Inactivation. *Hepatology.* 2020;72: 1949–67. [DOI] [PubMed]

81. Petrosillo G, Portincasa P, Grattagliano I, Casanova G, Matera M, Ruggiero FM, et al. Mitochondrial dysfunction in rat with nonalcoholic fatty liver: Involvement of complex I, reactive oxygen species and cardiolipin. *Biochim Biophys Acta*. 2007;1767:1260–7. [DOI] [PubMed]
82. Ribas V, Rosa LCdl, Robles D, Núñez S, Segalés P, Insausti-Urkia N, et al. Dietary and Genetic Cholesterol Loading Rather Than Steatosis Promotes Liver Tumorigenesis and NASH-Driven HCC. *Cancers (Basel)*. 2021;13:4091. [DOI] [PubMed] [PMC]
83. Ioannou GN. The Role of Cholesterol in the Pathogenesis of NASH. *Trends Endocrinol Metab*. 2016;27:84–95. [DOI] [PubMed]
84. Marí M, Caballero F, Colell A, Morales A, Caballeria J, Fernandez A, et al. Mitochondrial free cholesterol loading sensitizes to TNF- and Fas-mediated steatohepatitis. *Cell Metab*. 2006;4:185–98. [DOI] [PubMed]
85. Caballero F, Fernández A, Matías N, Martínez L, Fucho R, Elena M, et al. Specific contribution of methionine and choline in nutritional nonalcoholic steatohepatitis: impact on mitochondrial S-adenosyl-L-methionine and glutathione. *J Biol Chem*. 2010;285:18528–36. [DOI] [PubMed] [PMC]
86. Caballero F, Fernández A, Lacy AMD, Fernández-Checa JC, Caballería J, García-Ruiz C. Enhanced free cholesterol, SREBP-2 and StAR expression in human NASH. *J Hepatol*. 2009;50:789–96. [DOI] [PubMed]
87. Anuka E, Gal M, Stocco DM, Orly J. Expression and roles of steroidogenic acute regulatory (StAR) protein in 'non-classical', extra-adrenal and extra-gonadal cells and tissues. *Mol Cell Endocrinol*. 2013;371:47–61. [DOI] [PubMed]
88. Gao D, Wei C, Chen L, Huang J, Yang S, Diehl AM. Oxidative DNA damage and DNA repair enzyme expression are inversely related in murine models of fatty liver disease. *Am J Physiol Gastrointest Liver Physiol*. 2004;287:G1070–7. [DOI] [PubMed]
89. Pérez-Carreras M, Hoyo PD, Martín MA, Rubio JC, Martín A, Castellano G, et al. Defective hepatic mitochondrial respiratory chain in patients with nonalcoholic steatohepatitis. *Hepatology*. 2003;38:999–1007. [DOI] [PubMed]
90. Sookoian S, Flichman D, Scian R, Rohr C, Dopazo H, Gianotti TF, et al. Mitochondrial genome architecture in non-alcoholic fatty liver disease. *J Pathol*. 2016;240:437–49. [DOI] [PubMed]
91. Cortez-Pinto H, Chatham J, Chacko VP, Arnold C, Rashid A, Diehl AM. Alterations in liver ATP homeostasis in human nonalcoholic steatohepatitis: a pilot study. *JAMA*. 1999;282:1659–64. [DOI] [PubMed]
92. Aharoni-Simon M, Hann-Obercyger M, Pen S, Madar Z, Tirosh O. Fatty liver is associated with impaired activity of PPAR γ -coactivator 1 α (PGC1 α) and mitochondrial biogenesis in mice. *Lab Invest*. 2011;91:1018–28. [DOI] [PubMed]
93. Hensley K, Kotake Y, Sang H, Pye QN, Wallis GL, Kolker LM, et al. Dietary choline restriction causes complex I dysfunction and increased H₂O₂ generation in liver mitochondria. *Carcinogenesis*. 2000;21:983–9. [DOI] [PubMed]
94. Le TH, Caldwell SH, Redick JA, Sheppard BL, Davis CA, Arseneau KO, et al. The zonal distribution of megamitochondria with crystalline inclusions in nonalcoholic steatohepatitis. *Hepatology*. 2004;39:1423–9. [DOI] [PubMed]
95. Noureddin M, Yates KP, Vaughn IA, Neuschwander-Tetri BA, Sanyal AJ, McCullough A, et al. Clinical and histological determinants of nonalcoholic steatohepatitis and advanced fibrosis in elderly patients. *Hepatology*. 2013;58:1644–54. [DOI] [PubMed] [PMC]
96. Takahashi Y, Fukusato T. Histopathology of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol*. 2014;20:15539–48. [DOI] [PubMed] [PMC]
97. Kleiner DE. Histopathology, grading and staging of nonalcoholic fatty liver disease. *Minerva Gastroenterol Dietol*. 2018;64:28–38. [DOI] [PubMed]

98. Yamada T, Murata D, Kleiner DE, Anders R, Rosenberg AZ, Kaplan J, et al. Prevention and regression of megamitochondria and steatosis by blocking mitochondrial fusion in the liver. *iScience*. 2022;25:103996. [DOI] [PubMed] [PMC]
99. Undamatla R, Fagunloye OG, Chen J, Edmunds LR, Murali A, Mills A, et al. Reduced mitophagy is an early feature of NAFLD and liver-specific PARKIN knockout hastens the onset of steatosis, inflammation and fibrosis. *Sci Rep*. 2023;13:7575. [DOI] [PubMed] [PMC]
100. Zhang N, Liu X, Xie L, Shen X, Wu J. Impaired mitophagy triggers NLRP3 inflammasome activation during the progression from nonalcoholic fatty liver to nonalcoholic steatohepatitis. *Lab Invest*. 2019;99:749–63. [DOI] [PubMed]
101. Yamada T, Murata D, Adachi Y, Itoh K, Kameoka S, Igarashi A, et al. Mitochondrial Stasis Reveals p62-Mediated Ubiquitination in Parkin-Independent Mitophagy and Mitigates Nonalcoholic Fatty Liver Disease. *Cell Metab*. 2018;28:588–604.e5. [DOI] [PubMed] [PMC]
102. Feldstein AE, Canbay A, Angulo P, Taniai M, Burgart LJ, Lindor KD, et al. Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. *Gastroenterology*. 2003;125:437–43. [DOI] [PubMed]
103. Bian J, Zhang D, Wang Y, Qin H, Yang W, Cui R, et al. Mitochondrial Quality Control in Hepatocellular Carcinoma. *Front Oncol*. 2021;11:713721. [DOI] [PubMed] [PMC]
104. Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, et al. Hepatocellular carcinoma. *Nat Rev Dis Primers*. 2021;7:6. [DOI] [PubMed]
105. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68:394–424. [DOI] [PubMed]
106. Feng J, Li J, Wu L, Yu Q, Ji J, Wu J, et al. Emerging roles and the regulation of aerobic glycolysis in hepatocellular carcinoma. *J Exp Clin Cancer Res*. 2020;39:126. [DOI] [PubMed] [PMC]
107. WEINHOUSE S. On respiratory impairment in cancer cells. *Science*. 1956;124:267–9. [DOI] [PubMed]
108. Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*. 2009;324:1029–33. [DOI] [PubMed] [PMC]
109. Liberti MV, Locasale JW. The Warburg Effect: How Does it Benefit Cancer Cells? *Trends Biochem Sci*. 2016;41:211–8. [DOI] [PubMed] [PMC]
110. Lee H, Wei Y. Mitochondrial DNA instability and metabolic shift in human cancers. *Int J Mol Sci*. 2009;10:674–701. [DOI] [PubMed] [PMC]
111. Alam MM, Lal S, FitzGerald KE, Zhang L. A holistic view of cancer bioenergetics: mitochondrial function and respiration play fundamental roles in the development and progression of diverse tumors. *Clin Transl Med*. 2016;5:3. [DOI] [PubMed] [PMC]
112. Liu G, Luo Q, Li H, Liu Q, Ju Y, Song G. Increased Oxidative Phosphorylation Is Required for Stemness Maintenance in Liver Cancer Stem Cells from Hepatocellular Carcinoma Cell Line HCCLM3 Cells. *Int J Mol Sci*. 2020;21:5276. [DOI] [PubMed] [PMC]
113. DeWaal D, Nogueira V, Terry AR, Patra KC, Jeon S, Guzman G, et al. Hexokinase-2 depletion inhibits glycolysis and induces oxidative phosphorylation in hepatocellular carcinoma and sensitizes to metformin. *Nat Commun*. 2018;9:446. [DOI] [PubMed] [PMC]
114. Tan JL, Li F, Yeo JZ, Yong KJ, Bassal MA, Ng GH, et al. New High-Throughput Screening Identifies Compounds That Reduce Viability Specifically in Liver Cancer Cells That Express High Levels of SALL4 by Inhibiting Oxidative Phosphorylation. *Gastroenterology*. 2019;157:1615–29.e17. [DOI] [PubMed] [PMC]
115. Patel A, Oshi M, Yan L, Matsuyama R, Endo I, Takabe K. The Unfolded Protein Response Is Associated with Cancer Proliferation and Worse Survival in Hepatocellular Carcinoma. *Cancers (Basel)*. 2021;13:4443. [DOI] [PubMed] [PMC]

116. Rosa LCdl, Garcia-Ruiz C, Vallejo C, Baulies A, Nuñez S, Monte MJ, et al. STARD1 promotes NASH-driven HCC by sustaining the generation of bile acids through the alternative mitochondrial pathway. *J Hepatol.* 2021;74:1429–41. [DOI] [PubMed] [PMC]
117. Baulies A, Montero J, Matías N, Insausti N, Terrones O, Basañez G, et al. The 2-oxoglutarate carrier promotes liver cancer by sustaining mitochondrial GSH despite cholesterol loading. *Redox Biol.* 2018;14:164–77. [DOI] [PubMed] [PMC]
118. Dong Y, Zhang X. Targeting cellular mitophagy as a strategy for human cancers. *Front Cell Dev Biol.* 2024;12:1431968. [DOI] [PubMed] [PMC]
119. Wu H, Wang T, Liu Y, Li X, Xu S, Wu C, et al. Mitophagy Promotes Sorafenib Resistance through Hypoxia-Inducible ATAD3A Dependent Axis. *J Exp Clin Cancer Res.* 2020;39:274. [DOI]
120. Sun L, Wang X, Saredy J, Yuan Z, Yang X, Wang H. Innate-adaptive immunity interplay and redox regulation in immune response. *Redox Biol.* 2020;37:101759. [DOI] [PubMed] [PMC]
121. Chi H, Pepper M, Thomas PG. Principles and therapeutic applications of adaptive immunity. *Cell.* 2024;187:2052–78. [DOI] [PubMed] [PMC]
122. Dai Y, Zhou J, Shi C. Inflammasome: structure, biological functions, and therapeutic targets. *MedComm (2020).* 2023;4:e391. [DOI] [PubMed] [PMC]
123. Xu Z, Kombe AJK, Deng S, Zhang H, Wu S, Ruan J, et al. NLRP inflammasomes in health and disease. *Mol Biomed.* 2024;5:14. [DOI] [PubMed] [PMC]
124. Shen Y, Qian L, Luo H, Li X, Ruan Y, Fan R, et al. The Significance of NLRP Inflammasome in Neuropsychiatric Disorders. *Brain Sci.* 2022;12:1057. [DOI] [PubMed] [PMC]
125. Chen L, Cao S, Lin Z, He S, Zuo J. NOD-like receptors in autoimmune diseases. *Acta Pharmacol Sin.* 2021;42:1742–56. [DOI] [PubMed] [PMC]
126. Martinon F, Mayor A, Tschopp J. The inflammasomes: guardians of the body. *Annu Rev Immunol.* 2009;27:229–65. [DOI] [PubMed]
127. Fu J, Wu H. Structural Mechanisms of NLRP3 Inflammasome Assembly and Activation. *Annu Rev Immunol.* 2023;41:301–16. [DOI] [PubMed] [PMC]
128. Ribeiro MDC, Szabo G. Role of the Inflammasome in Liver Disease. *Annu Rev Pathol.* 2022;17:345–65. [DOI]
129. Nomura J, So A, Tamura M, Busso N. Intracellular ATP Decrease Mediates NLRP3 Inflammasome Activation upon Nigericin and Crystal Stimulation. *J Immunol.* 2015;195:5718–24. [DOI] [PubMed]
130. Mangan MSJ, Olhava EJ, Roush WR, Seidel HM, Glick GD, Latz E. Targeting the NLRP3 inflammasome in inflammatory diseases. *Nat Rev Drug Discov.* 2018;17:588–606. [DOI] [PubMed]
131. Mamun AA, Akter A, Hossain S, Sarker T, Safa SA, Mustafa QG, et al. Role of NLRP3 inflammasome in liver disease. *J Dig Dis.* 2020;21:430–6. [DOI] [PubMed]
132. Platnich JM, Muruve DA. NOD-like receptors and inflammasomes: A review of their canonical and non-canonical signaling pathways. *Arch Biochem Biophys.* 2019;670:4–14. [DOI] [PubMed]
133. Kelley N, Jeltema D, Duan Y, He Y. The NLRP3 Inflammasome: An Overview of Mechanisms of Activation and Regulation. *Int J Mol Sci.* 2019;20:3328. [DOI] [PubMed] [PMC]
134. Blevins HM, Xu Y, Biby S, Zhang S. The NLRP3 Inflammasome Pathway: A Review of Mechanisms and Inhibitors for the Treatment of Inflammatory Diseases. *Front Aging Neurosci.* 2022;14:879021. [DOI] [PubMed] [PMC]
135. Xian H, Watari K, Sanchez-Lopez E, Offenberger J, Onyuru J, Sampath H, et al. Oxidized DNA fragments exit mitochondria via mPTP- and VDAC-dependent channels to activate NLRP3 inflammasome and interferon signaling. *Immunity.* 2022;55:1370–85.e8. [DOI] [PubMed] [PMC]
136. Zhong Z, Liang S, Sanchez-Lopez E, He F, Shalapour S, Lin X, et al. New mitochondrial DNA synthesis enables NLRP3 inflammasome activation. *Nature.* 2018;560:198–203. [DOI] [PubMed] [PMC]
137. Iyer SS, He Q, Janczy JR, Elliott EI, Zhong Z, Olivier AK, et al. Mitochondrial cardiolipin is required for Nlrp3 inflammasome activation. *Immunity.* 2013;39:311–23. [DOI] [PubMed] [PMC]

138. Gros Lambert M, Py BF. Spotlight on the NLRP3 inflammasome pathway. *J Inflamm Res.* 2018;11:359–74. [DOI] [PubMed] [PMC]
139. Koyama Y, Brenner DA. Liver inflammation and fibrosis. *J Clin Invest.* 2017;127:55–64. [DOI] [PubMed] [PMC]
140. Hyun J, Han J, Lee C, Yoon M, Jung Y. Pathophysiological Aspects of Alcohol Metabolism in the Liver. *Int J Mol Sci.* 2021;22:5717. [DOI] [PubMed] [PMC]
141. Taru V, Szabo G, Mehal W, Reiberger T. Inflammasomes in chronic liver disease: Hepatic injury, fibrosis progression and systemic inflammation. *J Hepatol.* 2024;81:895–910. [DOI] [PubMed]
142. Gupta H, Suk KT, Kim DJ. Gut Microbiota at the Intersection of Alcohol, Brain, and the Liver. *J Clin Med.* 2021;10:541. [DOI] [PubMed] [PMC]
143. DeSantis DA, Ko C, Liu Y, Liu X, Hise AG, Nunez G, et al. Alcohol-induced liver injury is modulated by Nlrp3 and Nlrc4 inflammasomes in mice. *Mediators Inflamm.* 2013;2013:751374. [DOI] [PubMed] [PMC]
144. Soares J, Pimentel-Nunes P, Roncon-Albuquerque R, Leite-Moreira A. The role of lipopolysaccharide/toll-like receptor 4 signaling in chronic liver diseases. *Hepatol Int.* 2010;4:659–72. [DOI] [PubMed] [PMC]
145. Hoyt LR, Randall MJ, Ather JL, DePuccio DP, Landry CC, Qian X, et al. Mitochondrial ROS induced by chronic ethanol exposure promote hyper-activation of the NLRP3 inflammasome. *Redox Biol.* 2017;12:883–96. [DOI] [PubMed] [PMC]
146. Brahadeeswaran S, Dasgupta T, Manickam V, Saraswathi V, Tamizhselvi R. NLRP3: a new therapeutic target in alcoholic liver disease. *Front Immunol.* 2023;14:1215333. [DOI] [PubMed] [PMC]
147. Lu Y, Wu D, Wang X, Ward SC, Cederbaum AI. Chronic alcohol-induced liver injury and oxidant stress are decreased in cytochrome P4502E1 knockout mice and restored in humanized cytochrome P4502E1 knock-in mice. *Free Radic Biol Med.* 2010;49:1406–16. [DOI] [PubMed] [PMC]
148. LeFort KR, Rungratanawanich W, Song B. Contributing roles of mitochondrial dysfunction and hepatocyte apoptosis in liver diseases through oxidative stress, post-translational modifications, inflammation, and intestinal barrier dysfunction. *Cell Mol Life Sci.* 2024;81:34. [DOI] [PubMed] [PMC]
149. Park J, Rustamov N, Roh Y. The Roles of NFR2-Regulated Oxidative Stress and Mitochondrial Quality Control in Chronic Liver Diseases. *Antioxidants (Basel).* 2023;12:1928. [DOI] [PubMed] [PMC]
150. Torres S, Segalés P, García-Ruiz C, Fernández-Checa JC. Mitochondria and the NLRP3 Inflammasome in Alcoholic and Nonalcoholic Steatohepatitis. *Cells.* 2022;11:1475. [DOI] [PubMed] [PMC]
151. Lin Y, Huang D, Wang J, Lin Y, Hsieh S, Huang K, et al. Syk is involved in NLRP3 inflammasome-mediated caspase-1 activation through adaptor ASC phosphorylation and enhanced oligomerization. *J Leukoc Biol.* 2015;97:825–35. [DOI] [PubMed]
152. Sayaf K, Battistella S, Russo FP. NLRP3 Inflammasome in Acute and Chronic Liver Diseases. *Int J Mol Sci.* 2024;25:4537. [DOI] [PubMed] [PMC]
153. Wang H, Mehal W, Nagy LE, Rotman Y. Immunological mechanisms and therapeutic targets of fatty liver diseases. *Cell Mol Immunol.* 2021;18:73–91. [DOI] [PubMed] [PMC]
154. Khanova E, Wu R, Wang W, Yan R, Chen Y, French SW, et al. Pyroptosis by caspase11/4-gasdermin-D pathway in alcoholic hepatitis in mice and patients. *Hepatology.* 2018;67:1737–53. [DOI] [PubMed] [PMC]
155. Heo MJ, Kim TH, You JS, Blaya D, Sancho-Bru P, Kim SG. Alcohol dysregulates miR-148a in hepatocytes through FoxO1, facilitating pyroptosis via TXNIP overexpression. *Gut.* 2019;68:708–20. [DOI] [PubMed] [PMC]
156. Dong T, Li J, Liu Y, Zhou S, Wei X, Hua H, et al. Roles of immune dysregulation in MASLD. *Biomed Pharmacother.* 2024;170:116069. [DOI] [PubMed]
157. Mihajlovic M, Vinken M. Mitochondria as the Target of Hepatotoxicity and Drug-Induced Liver Injury: Molecular Mechanisms and Detection Methods. *Int J Mol Sci.* 2022;23:3315. [DOI] [PubMed] [PMC]

158. Ajoalabady A, Liu S, Klionsky DJ, Lip GYH, Tuomilehto J, Kavalakatt S, et al. ER stress in obesity pathogenesis and management. *Trends Pharmacol Sci.* 2022;43:97–109. [DOI] [PubMed] [PMC]
159. Kim JY, Garcia-Carbonell R, Yamachika S, Zhao P, Dhar D, Loomba R, et al. ER Stress Drives Lipogenesis and Steatohepatitis via Caspase-2 Activation of S1P. *Cell.* 2018;175:133–45.e15. [DOI] [PubMed] [PMC]
160. García-Ruiz C, Fernández-Checa JC. Mitochondrial Oxidative Stress and Antioxidants Balance in Fatty Liver Disease. *HepatoL Commun.* 2018;2:1425–39. [DOI] [PubMed] [PMC]
161. Rossato M, Vincenzo AD, Pagano C, Hadi HE, Vettor R. The P2X7 Receptor and NLRP3 Axis in Non-Alcoholic Fatty Liver Disease: A Brief Review. *Cells.* 2020;9:1047. [DOI] [PubMed] [PMC]
162. Mridha AR, Wree A, Robertson AAB, Yeh MM, Johnson CD, Rooyen DMV, et al. NLRP3 inflammasome blockade reduces liver inflammation and fibrosis in experimental NASH in mice. *J Hepatol.* 2017;66:1037–46. [DOI] [PubMed] [PMC]
163. Goicoechea L, Rosa LCdl, Torres S, García-Ruiz C, Fernández-Checa JC. Mitochondrial cholesterol: Metabolism and impact on redox biology and disease. *Redox Biol.* 2023;61:102643. [DOI] [PubMed] [PMC]
164. Yu L, Hong W, Lu S, Li Y, Guan Y, Weng X, et al. The NLRP3 Inflammasome in Non-Alcoholic Fatty Liver Disease and Steatohepatitis: Therapeutic Targets and Treatment. *Front Pharmacol.* 2022;13:780496. [DOI] [PubMed] [PMC]
165. Zhang S, Ren X, Zhang B, Lan T, Liu B. A Systematic Review of Statins for the Treatment of Nonalcoholic Steatohepatitis: Safety, Efficacy, and Mechanism of Action. *Molecules.* 2024;29:1859. [DOI] [PubMed] [PMC]
166. Xu L, Zhou J, Che J, Wang H, Yang W, Zhou W, et al. Mitochondrial DNA enables AIM2 inflammasome activation and hepatocyte pyroptosis in nonalcoholic fatty liver disease. *Am J Physiol Gastrointest Liver Physiol.* 2021;320:G1034–44. [DOI] [PubMed]
167. Lugrin J, Martinon F. The AIM2 inflammasome: Sensor of pathogens and cellular perturbations. *Immunol Rev.* 2018;281:99–114. [DOI] [PubMed]
168. Ramos-Tovar E, Muriel P. NLRP3 inflammasome in hepatic diseases: A pharmacological target. *Biochem Pharmacol.* 2023;217:115861. [DOI] [PubMed]
169. Papadakos SP, Dedes N, Kouroumalis E, Theocharis S. The Role of the NLRP3 Inflammasome in HCC Carcinogenesis and Treatment: Harnessing Innate Immunity. *Cancers (Basel).* 2022;14:3150. [DOI] [PubMed] [PMC]
170. Arvanitakis K, Koletsa T, Mitroulis I, Germanidis G. Tumor-Associated Macrophages in Hepatocellular Carcinoma Pathogenesis, Prognosis and Therapy. *Cancers (Basel).* 2022;14:226. [DOI] [PubMed] [PMC]
171. Lee HH, Kim D, Jung J, Kang H, Cho H. NLRP3 Deficiency in Hepatocellular Carcinoma Enhances Surveillance of NK-92 through a Modulation of MICA/B. *Int J Mol Sci.* 2021;22:9285. [DOI] [PubMed] [PMC]
172. Shi G, Tang N, Qiu J, Zhang D, Huang F, Cheng Y, et al. Irisin stimulates cell proliferation and invasion by targeting the PI3K/AKT pathway in human hepatocellular carcinoma. *Biochem Biophys Res Commun.* 2017;493:585–91. [DOI] [PubMed]
173. Zhang J, Zhang Q, Lou Y, Fu Q, Chen Q, Wei T, et al. Hypoxia-inducible factor-1 α /interleukin-1 β signaling enhances hepatoma epithelial-mesenchymal transition through macrophages in a hypoxic-inflammatory microenvironment. *Hepatology.* 2018;67:1872–89. [DOI] [PubMed]
174. Tu C, Hu Y, Zhou P, Guo X, Gu C, Zhang Y, et al. Lactate and TGF- β antagonistically regulate inflammasome activation in the tumor microenvironment. *J Cell Physiol.* 2021;236:4528–37. [DOI] [PubMed]
175. Deng B, Hu Y, Sheng X, Zeng H, Huo Y. miR-223-3p reduces high glucose and high fat-induced endothelial cell injury in diabetic mice by regulating NLRP3 expression. *Exp Ther Med.* 2020;20:1514–20. [DOI] [PubMed] [PMC]

176. Wang X, He Y, Mackowiak B, Gao B. MicroRNAs as regulators, biomarkers and therapeutic targets in liver diseases. *Gut*. 2021;70:784–95. [DOI] [PubMed]
177. Wan L, Yuan X, Liu M, Xue B. miRNA-223-3p regulates NLRP3 to promote apoptosis and inhibit proliferation of hep3B cells. *Exp Ther Med*. 2018;15:2429–35. [DOI] [PubMed] [PMC]
178. Zhao H, Zhang Y, Zhang Y, Chen C, Liu H, Yang Y, et al. The role of NLRP3 inflammasome in hepatocellular carcinoma. *Front Pharmacol*. 2023;14:1150325. [DOI] [PubMed] [PMC]
179. Sharma BR, Kanneganti T. NLRP3 inflammasome in cancer and metabolic diseases. *Nat Immunol*. 2021;22:550–9. [DOI] [PubMed] [PMC]
180. Hamarsheh S, Zeiser R. NLRP3 Inflammasome Activation in Cancer: A Double-Edged Sword. *Front Immunol*. 2020;11:1444. [DOI] [PubMed] [PMC]
181. Moossavi M, Parsamanesh N, Bahrami A, Atkin SL, Sahebkar A. Role of the NLRP3 inflammasome in cancer. *Mol Cancer*. 2018;17:158. [DOI] [PubMed] [PMC]
182. Zhu W, Feng P, He K, Li S, Gong J. Liraglutide protects non-alcoholic fatty liver disease via inhibiting NLRP3 inflammasome activation in a mouse model induced by high-fat diet. *Biochem Biophys Res Commun*. 2018;505:523–9. [DOI] [PubMed]
183. Yu X, Hao M, Liu Y, Ma X, Lin W, Xu Q, et al. Liraglutide ameliorates non-alcoholic steatohepatitis by inhibiting NLRP3 inflammasome and pyroptosis activation via mitophagy. *Eur J Pharmacol*. 2019;864:172715. [DOI] [PubMed]
184. Armstrong MJ, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet*. 2016;387:679–90. [DOI] [PubMed]
185. Feng W, Bi Y, Li P, Yin T, Gao C, Shen S, et al. Effects of liraglutide, metformin and gliclazide on body composition in patients with both type 2 diabetes and non-alcoholic fatty liver disease: A randomized trial. *J Diabetes Investig*. 2019;10:399–407. [DOI] [PubMed] [PMC]
186. Shao N, Yu X, Ma X, Lin W, Hao M, Kuang H. Exenatide Delays the Progression of Nonalcoholic Fatty Liver Disease in C57BL/6 Mice, Which May Involve Inhibition of the NLRP3 Inflammasome through the Mitophagy Pathway. *Gastroenterol Res Pract*. 2018;2018:1864307. [DOI] [PubMed] [PMC]
187. Liu L, Yan H, Xia M, Zhao L, Lv M, Zhao N, et al. Efficacy of exenatide and insulin glargine on nonalcoholic fatty liver disease in patients with type 2 diabetes. *Diabetes Metab Res Rev*. 2020;36:e3292. [DOI] [PubMed]
188. Fan H, Pan Q, Xu Y, Yang X. Exenatide improves type 2 diabetes concomitant with non-alcoholic fatty liver disease. *Arq Bras Endocrinol Metabol*. 2013;57:702–8. [DOI] [PubMed]
189. Ghazanfar H, Kandhi SD, Nawaz I, Javed N, Abraham MC, Farag M, et al. Role of Glucagon-Like Peptide-1 Receptor Agonists in the Management of Non-Alcoholic Steatohepatitis: A Clinical Review Article. *Cureus*. 2021;13:e15141. [DOI] [PubMed] [PMC]
190. Papazyan R, Liu X, Liu J, Dong B, Plummer EM, 2nd RDL, et al. FXR activation by obeticholic acid or nonsteroidal agonists induces a human-like lipoprotein cholesterol change in mice with humanized chimeric liver. *J Lipid Res*. 2018;59:982–93. [DOI] [PubMed] [PMC]
191. Huang S, Wu Y, Zhao Z, Wu B, Sun K, Wang H, et al. A new mechanism of obeticholic acid on NASH treatment by inhibiting NLRP3 inflammasome activation in macrophage. *Metabolism*. 2021;120:154797. [DOI] [PubMed]
192. Wang Y, Chen W, Wang M, Yu D, Forman BM, Huang W. Farnesoid X receptor antagonizes nuclear factor κ B in hepatic inflammatory response. *Hepatology*. 2008;48:1632–43. [DOI] [PubMed] [PMC]
193. Wang H, Ge C, Zhou J, Guo Y, Cui S, Huang N, et al. Noncanonical farnesoid X receptor signaling inhibits apoptosis and impedes liver fibrosis. *EBioMedicine*. 2018;37:322–33. [DOI] [PubMed] [PMC]
194. Yan T, Yan N, Wang H, Yagai T, Luo Y, Takahashi S, et al. FXR-Deoxycholic Acid-TNF- α Axis Modulates Acetaminophen-Induced Hepatotoxicity. *Toxicol Sci*. 2021;181:273–84. [DOI] [PubMed] [PMC]

195. Hao H, Cao L, Jiang C, Che Y, Zhang S, Takahashi S, et al. Farnesoid X Receptor Regulation of the NLRP3 Inflammasome Underlies Cholestasis-Associated Sepsis. *Cell Metab.* 2017;25:856–67.e5. [DOI] [PubMed] [PMC]
196. Han CY, Rho HS, Kim A, Kim TH, Jang K, Jun DW, et al. FXR Inhibits Endoplasmic Reticulum Stress-Induced NLRP3 Inflammasome in Hepatocytes and Ameliorates Liver Injury. *Cell Rep.* 2018;24:2985–99. [DOI] [PubMed]
197. Hoofnagle JH. FXR Agonists as Therapy for Liver Disease. *Hepatology.* 2020;72:1–3. [DOI] [PubMed]
198. Gao Y, Chu S, Zhang Z, Chen N. Hepatoprotective effects of ginsenoside Rg1 - A review. *J Ethnopharmacol.* 2017;206:178–83. [DOI] [PubMed]
199. Cao L, Zou Y, Zhu J, Fan X, Li J. Ginsenoside Rg1 attenuates concanavalin A-induced hepatitis in mice through inhibition of cytokine secretion and lymphocyte infiltration. *Mol Cell Biochem.* 2013;380:203–10. [DOI] [PubMed]
200. Xin Y, Wei J, Chunhua M, Danhong Y, Jianguo Z, Zongqi C, et al. Protective effects of Ginsenoside Rg1 against carbon tetrachloride-induced liver injury in mice through suppression of inflammation. *Phytomedicine.* 2016;23:583–8. [DOI] [PubMed]
201. Lin J, Huang H, Yang S, Duan J, Qu S, Yuan B, et al. The effect of Ginsenoside Rg1 in hepatic ischemia reperfusion (I/R) injury ameliorates ischemia-reperfusion-induced liver injury by inhibiting apoptosis. *Biomed Pharmacother.* 2020;129:110398. [DOI] [PubMed]
202. Li Y, Zhang D, Li L, Han Y, Dong X, Yang L, et al. Ginsenoside Rg1 ameliorates aging-induced liver fibrosis by inhibiting the NOX4/NLRP3 inflammasome in SAMP8 mice. *Mol Med Rep.* 2021;24:801. [DOI] [PubMed] [PMC]
203. Zhao J, He B, Zhang S, Huang W, Li X. Ginsenoside Rg1 alleviates acute liver injury through the induction of autophagy and suppressing NF- κ B/NLRP3 inflammasome signaling pathway. *Int J Med Sci.* 2021;18:1382–9. [DOI] [PubMed] [PMC]
204. Zhang Q, Hu J, Mao A, Liu M, Guan S, Lu J. Ginsenoside Rb1 alleviated concanavalin A-induced hepatocyte pyroptosis by activating mitophagy. *Food Funct.* 2023;14:3793–803. [DOI] [PubMed]
205. Wang F, Park J, Ma Y, Ma H, Lee Y, Lee G, et al. Ginseng Saponin Enriched in Rh1 and Rg2 Ameliorates Nonalcoholic Fatty Liver Disease by Inhibiting Inflammasome Activation. *Nutrients.* 2021;13:856. [DOI] [PubMed] [PMC]
206. Simón J, Casado-Andrés M, Goikoetxea-Usandizaga N, Serrano-Maciá M, Martínez-Chantar ML. Nutraceutical Properties of Polyphenols against Liver Diseases. *Nutrients.* 2020;12:3517. [DOI] [PubMed] [PMC]
207. Vergani L, Vecchione G, Baldini F, Grasselli E, Voci A, Portincasa P, et al. Polyphenolic extract attenuates fatty acid-induced steatosis and oxidative stress in hepatic and endothelial cells. *Eur J Nutr.* 2018;57:1793–805. [DOI] [PubMed]
208. Zhang L, Xu L, Tang F, Liu D, Zhao X, Zhang J, et al. New perspectives on the therapeutic potential of quercetin in non-communicable diseases: Targeting Nrf2 to counteract oxidative stress and inflammation. *J Pharm Anal.* 2024;14:100930. [DOI] [PubMed] [PMC]
209. Cao P, Wang Y, Zhang C, Sullivan MA, Chen W, Jing X, et al. Quercetin ameliorates nonalcoholic fatty liver disease (NAFLD) via the promotion of AMPK-mediated hepatic mitophagy. *J Nutr Biochem.* 2023;120:109414. [DOI] [PubMed]
210. Yang Q, Yang F, Gong J, Tang X, Wang G, Wang Z, et al. Sweroside ameliorates α -naphthylisothiocyanate-induced cholestatic liver injury in mice by regulating bile acids and suppressing pro-inflammatory responses. *Acta Pharmacol Sin.* 2016;37:1218–28. [DOI] [PubMed] [PMC]
211. Ding Y, Chen Y, Hu K, Yang Q, Li Y, Huang M. Sweroside alleviates hepatic steatosis in part by activating AMPK/mTOR-mediated autophagy in mice. *J Cell Biochem.* 2023;124:1012–22. [DOI] [PubMed]

212. Yang G, Jang JH, Kim SW, Han S, Ma K, Jang J, et al. Sweroside Prevents Non-Alcoholic Steatohepatitis by Suppressing Activation of the NLRP3 Inflammasome. *Int J Mol Sci.* 2020;21:2790. [DOI] [PubMed] [PMC]
213. Yang Q, Shu F, Gong J, Ding P, Cheng R, Li J, et al. Sweroside ameliorates NAFLD in high-fat diet induced obese mice through the regulation of lipid metabolism and inflammatory response. *J Ethnopharmacol.* 2020;255:112556. [DOI] [PubMed]
214. Li T, Yang G, Hao Q, Zhang X, Zhang X. Daphnetin Ameliorates the Expansion of Chemically Induced Hepatocellular Carcinoma via Reduction of Inflammation and Oxidative Stress. *J Oleo Sci.* 2022;71: 575–85. [DOI] [PubMed]
215. Zhu R, Zeng G, Chen Y, Zhang Q, Liu B, Liu J, et al. Oroxylin A accelerates liver regeneration in CCl₄-induced acute liver injury mice. *PLoS One.* 2013;8:e71612. [DOI] [PubMed] [PMC]
216. Sajeev A, Hegde M, Daimary UD, Kumar A, Girisa S, Sethi G, et al. Modulation of diverse oncogenic signaling pathways by oroxylin A: An important strategy for both cancer prevention and treatment. *Phytomedicine.* 2022;105:154369. [DOI] [PubMed]
217. Kai J, Yang X, Wang Z, Wang F, Jia Y, Wang S, et al. Oroxylin a promotes PGC-1 α /Mfn2 signaling to attenuate hepatocyte pyroptosis via blocking mitochondrial ROS in alcoholic liver disease. *Free Radic Biol Med.* 2020;153:89–102. [DOI] [PubMed]
218. Lv H, Zhu C, Wei W, Lv X, Yu Q, Deng X, et al. Enhanced Keap1-Nrf2/Trx-1 axis by daphnetin protects against oxidative stress-driven hepatotoxicity via inhibiting ASK1/JNK and Txnip/NLRP3 inflammasome activation. *Phytomedicine.* 2020;71:153241. [DOI] [PubMed]
219. Yang SJ, Lim Y. Resveratrol ameliorates hepatic metaflammation and inhibits NLRP3 inflammasome activation. *Metabolism.* 2014;63:693–701. [DOI] [PubMed]
220. Ma Z, Zhang Y, Li Q, Xu M, Bai J, Wu S. Resveratrol improves alcoholic fatty liver disease by downregulating HIF-1 α expression and mitochondrial ROS production. *PLoS One.* 2017;12: e0183426. [DOI] [PubMed] [PMC]
221. Jakubczyk K, Skonieczna-Żydecka K, Kałduńska J, Stachowska E, Gutowska I, Janda K. Effects of Resveratrol Supplementation in Patients with Non-Alcoholic Fatty Liver Disease—A Meta-Analysis. *Nutrients.* 2020;12:2435. [DOI] [PubMed] [PMC]
222. Bhia M, Motallebi M, Abadi B, Zarepour A, Pereira-Silva M, Saremnejad F, et al. Naringenin Nano-Delivery Systems and Their Therapeutic Applications. *Pharmaceutics.* 2021;13:291. [DOI] [PubMed] [PMC]
223. Wang Q, Ou Y, Hu G, Wen C, Yue S, Chen C, et al. Naringenin attenuates non-alcoholic fatty liver disease by down-regulating the NLRP3/NF- κ B pathway in mice. *Br J Pharmacol.* 2020;177:1806–21. [DOI] [PubMed] [PMC]