Life Sciences 236 (2019) 116464

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Review article

Mitochondrial DNA in liver inflammation and oxidative stress

Xufei Zhang[a,c], Xiuwen Wu[b,c,*], Qiongyuan Hu[b,c], Jie Wu[b,c], Gefei Wang[b,c], Zhiwu Hong[b,c], Jianan Ren[a,c,**], Lab for Trauma and Surgical Infections[c]

[a] Research Institute of General Surgery, Jinling Hospital, Nanjing Medical University, Nanjing 210002, PR China
[b] Research Institute of General Surgery, Jinling Hospital, Medical School of Nanjing University, Nanjing 210002, PR China
[c] Lab for Trauma and Surgical Infections, Jinling Hospital, Nanjing 210002, PR China

ARTICLE INFO

Keywords:
Liver injury
mtDNA
STING
Oxidative stress
HBV
Hepatocellular carcinoma

ABSTRACT

The function of liver is highly dependent on mitochondria producing ATP for biosynthetic and detoxifying properties. Accumulating evidence indicates that most hepatic disorders are characterized by profound mitochondrial dysfunction. Mitochondrial dysfunction not only exhibits mitochondrial DNA (mtDNA) damage and depletion, but also releases mtDNA. mtDNA is a closed circular molecule encoding 13 of the polypeptides of the oxidative phosphorylation system. Extensive mtDNA lesions could exacerbate mitochondrial oxidative stress and subsequently cause damage to hepatocytes. When mtDNA leaves the confines of mitochondria to the cytosolic and extracellular environment, it can act as damage-associated molecular patterns (DAMPs) to trigger the inflammatory response through the Toll-like receptor 9, inflammasomes, and stimulator of interferon genes (STING) pathways and further exacerbate hepatocellular damage and even remote organs injury. In addition, mtDNA also plays a vital role in hepatitis B virus (HBV)-related liver injury and hepatocellular carcinoma (HCC). In this review, we describe mtDNA alterations during liver injury, focusing on the mechanisms of mtDNA-mediated liver inflammation and oxidative stress injury.

1. Introduction

The liver is an organ with high energy requirements, playing a pivotal role in multiple synthesis and secretions of endogenous compounds. Hepatic diseases are closely associated with mitochondrial dysfunction [1]. Mounting studies suggest that mitochondrial dysfunction is a critical factor in the initiation and progression of some diseases [2]. Mitochondria are intracellular double-membrane-bound organelles responsible for energy production, protein synthesis, calcium homeostasis, and cell death [2,3]. Mitochondrial dysfunction not only exhibits mitochondrial DNA (mtDNA) damage and depletion, but also causes mtDNA release, which has been also found in liver injury.

mtDNA is a double-stranded circular loop DNA of 16,569 bp by encoding for 37 genes in humans as well as 13 respiratory chain subunits and participating in synthesis of respiratory chain complexes I, III, IV, and V [4,5]. In general, quantitative polymerase chain reaction targeting some mitochondrial genes is used to test mtDNA copy number in plasma, serum or tissues, but there is no unified primer at present. Research has demonstrated that mtDNA acts as damage-associated molecular patterns (DAMPs) when released into the extracellular and cytosol environment [6]. Emerging evidence suggests that mtDNA can regulate inflammation through triggering Toll-like receptor 9 (TLR9) and cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING), playing a crucial role in sepsis, ischemia-reperfusion (I/R) injury, heart failure, and renal injury, etc. [7–10]. It also can activate inflammasomes, including the NOD-like receptor family pyrin domain containing-3 (NLRP3) inflammasomes, the NOD-like receptor family CARD domain-containing protein 4 (NLRC4) inflammasomes, and absent in melanoma 2 (AIM2) inflammasomes [11].

Investigators have demonstrated that during liver injury, necrotic hepatocytes release mtDNA, which acts as an agonist for TLR9 and cGAS-STING to induce neutrophil infiltration and liver inflammation that may further exacerbate hepatocellular damage [12,13]. mtDNA can induce type I interferon (IFN I) production through activating the cGAS-STING pathway [12,13]. Moreover, IFN I has been shown to be a crucial role in triggering oxidative stress and amplifying liver injury [14,15]. Aside from mtDNA release, mtDNA lesions are another feature of liver injury [16]. mtDNA participates in maintenance of mitochondria function and production of ATP. The damage and depletion of mtDNA induce oxidative stress and some mitochondrial diseases [2]. In

* Correspondence to: X. Wu, Research Institute of General Surgery, Jinling Hospital, Medical School of Nanjing University, Nanjing 210002, PR China.
** Correspondence to: J. Ren, Research Institute of General Surgery, Jinling Hospital, Nanjing Medical University, Nanjing 210002, PR China.
E-mail addresses: lygwxw@163.com (X. Wu), jiananr@gmail.com (J. Ren).

https://doi.org/10.1016/j.lfs.2019.05.020
Received 1 April 2019; Received in revised form 7 May 2019; Accepted 8 May 2019
Available online 09 May 2019
0024-3205/

addition, mtDNA also plays a vital role in hepatitis B virus (HBV)-related liver injury and hepatocellular carcinoma (HCC). The Review by Rawla et al. [17] emphasizes that there is a strong association between HBV and HCC with a 30 fold increased risk of HCC in chronic HBC carriers. Herein, we discuss the involvement of mtDNA in liver injury, its effects on liver inflammation and oxidative stress, and the association between mtDNA and HBV-related liver injury.

2. Mechanisms for hepatocellular mtDNA release

mtDNA contributes to disease pathogenesis through interaction with multiple signal pathways, which requires mtDNA to leave the confines of mitochondria to the extracellular or cytosolic environment. In general, mtDNA release involves two aspects, including cytosolic and extracellular. Up to now, mechanisms for mtDNA release from mitochondria mainly depend on mitochondrial permeability transition (MPT) pores, B-cell lymphoma 2 homologous antagonist killer (BAK) and Bcl-2-associated X protein (BAX). MPT pores are located in the inner mitochondrial membrane [18]. Inhibition of pore opening with cyclosporine A decreases leakage of mtDNA into cytosol [19]. A study has found that hepatocytes following severe shock show mitochondrial dysfunction characterized with opening of MPT pores, mitochondrial swelling, decreased mitochondrial membrane potential and reduced ATP levels [20]. BAK and BAX, two pro-apoptotic Bcl-2 family proteins, are required for rapid execution of Fas-mediated massive apoptosis in the liver [21]. When BAK and BAX are activated, they oligomerize in the mitochondrial outer membrane, causing its permeabilization and the release of mtDNA and cytochrome *c* [22]. Mechanically, these BAK/BAX macropores allow the inner mitochondrial membrane to herniate into the cytosol [22]. Once in the cytoplasm, the inner membrane increases permeability allowing mtDNA release, which is independent of MPT pores [23].

With respect to extracellular release, cellular stress and necrosis are primary factors in the non-discriminant release of mtDNA [24]. On the contrary to uncontrolled cell death, necroptosis, a newly discovered form of programmed necrosis, has been found to increase extracellular mtDNA [25]. There is accumulating evidence that necroptosis is a pathogenically relevant driver in nonalcoholic steatohepatitis (NASH) and liver cancer [26]. Moreover, red blood cell (RBC) transfusion and RBC-induced endothelial necroptosis were shown to increase the extracellular mtDNA, which points out a potential mechanism for transfusion-related lung injury [27]. Intriguingly, necroptosis was shown to be associated with MPT pores opening [28]. Another proposed mechanism by which mtDNA is translocated to the extracellular space is via extracellular vesicles. Extracellular vesicles have three forms, including exosomes, microparticles (MPs) and apoptotic bodies [29]. mtDNA has been observed in MPs [30] and exosomes [31]. Nevertheless, it is unclear whether free mtDNA and mtDNA in MP have different concentration, structure and immunostimulatory effects. Extracellular vesicles and necroptosis are not opposed to each other. A recent study has shown RAW 264.7 murine macrophage cells undergoing necroptosis release MPs [31]. The particles contain both chromosomal and mitochondrial DNA [32].

3. Liver inflammation induced by mtDNA release

As previously mentioned, mtDNA acts as DAMPs, which requires mtDNA to leave the confines of mitochondria to the extracellular and cytosolic environment. Circulating mtDNA levels are elevated in mice and humans during liver injury [33]. Actually, it is damaged hepatocytes that release mtDNA into extracellular environment and circulation. Emerging evidence suggest that the elevated mtDNA derived from liver may induce or aggravate liver inflammation through triggering TLR9, inflammasomes and cGAS-STING.

3.1. Acetaminophen-induced liver injury

Acetaminophen (APAP) is one of the most commonly used antipyretic and analgesic drug used to relieve symptoms of mild inflammatory conditions, but its overdose is also a primary cause of acute liver failure [34]. The overdose of APAP can deplete hepatic GSH and subsequently lead to intracellular oxidative stress, mitochondria dysfunction, DNA or mtDNA damage, finally contributing to hepatocyte injury or death [35]. Initially, it was thought that cell injury, apoptosis or necrosis resulted in the release of mtDNA out of the cell, leading to increased levels of extracellular mtDNA. However it is generally accepted that the mode of APAP-induced hepatocyte death in patients or mice is oncotic necrosis without caspase activation [36,37].

Recent studies have shown occurrence of MPT pore opening and mitochondrial membrane lysis after APAP treatment [38], which accounts for mtDNA release. McGill et al. [39] conducted clinical trials to investigate the association between serum mitochondrial biomarkers and outcome in APAP overdose patients. They showed that mtDNA is higher in serum from non-survivors of APAP-induced acute liver failure compared with survivors. Moreover, receiver operating characteristic curve analyses revealed mtDNA was predictive of outcome. Therefore, mtDNA may act as a biomarker for indicating APAP-induced liver injury.

It remains unclear whether other ways can also promote mtDNA release in APAP-induced liver injury. At present, free mtDNA, mtDNA in exosomes and mtDNA in MPs have been found in the circulation of APAP-induced liver injury. It is also unknown whether there are other existence forms of circulating mtDNA in APAP-induced liver injury. He et al. [12] found that injection of APAP remarkably elevated serum levels of mtDNA, and mtDNA were found in MPs and exosomes, with much higher levels in MPs. Furthermore, mtDNA from necrotic hepatocytes could trigger TLR9 in neutrophils, inducing expression of pro-inflammatory mediators and infiltration of hepatic neutrophil, and subsequently aggravating liver injury [12]. In addition, elevated mtDNA in circulation may trigger a systemic inflammatory response and remote lung injury [40].

Apart from TLR9, a recent study has shown that mice deficient in STING or cGAS were completely resistant to APAP-induced liver injury [15]. Furthermore, ablation of IFN I recognition in interferon α/β receptor ($IFNAR^{-/-}$) protected mice from APAP-induced liver injury [15], pointing out a potential mechanism for APAP-induce liver injury mediated by mtDNA via GAS-STING (Fig. 1). mtDNA can also activate the NLRP3 inflammasomes capable of recruiting and activating caspase-1, which cleaves pro-IL-1β and pro-IL-18 into IL-1β and IL-18 [8]. Imaeda et al. [8] showed reduced mortality and APAP-induced liver injury in mice lacking components of the NLRP3 inflammasomes. IL-1β and IL-18 may induce activation of liver non-parenchymal cells (NPCs) [41]. This demonstrates a critical role for the NLRP3 inflammasomes pathway in APAP-induced liver injury.

3.2. NASH

NASH is a multiple liver disorder characterized by steatosis, inflammation, ballooning injury and varying degrees of fibrosis [42,43]. Obesity, hyperglycemia, type 2 diabetes and hypertriglyceridemia are the most important risk factors [17,44]. Accumulating evidence indicates that mitochondria dysfunction exerts a pivotal role in the physiopathology of NASH, but the specific mechanisms underlying this dysfunction are still unclear. Circulating mtDNA levels were shown to be markedly enhanced in patients with NASH [30,45]. Gautheron et al. [46] demonstrated RIP3-dependent necroptosis controls NASH-induced liver fibrosis, which partly accounts for mtDNA release.

Recently, Luo et al. [47] demonstrated that NPCs of liver tissues from patients with non-alcoholic fatty liver disease (NAFLD) had increased levels of STING relative to liver tissues from patients without NAFLD. Yu et al. [13] found that mtDNA from hepatocytes of high fat

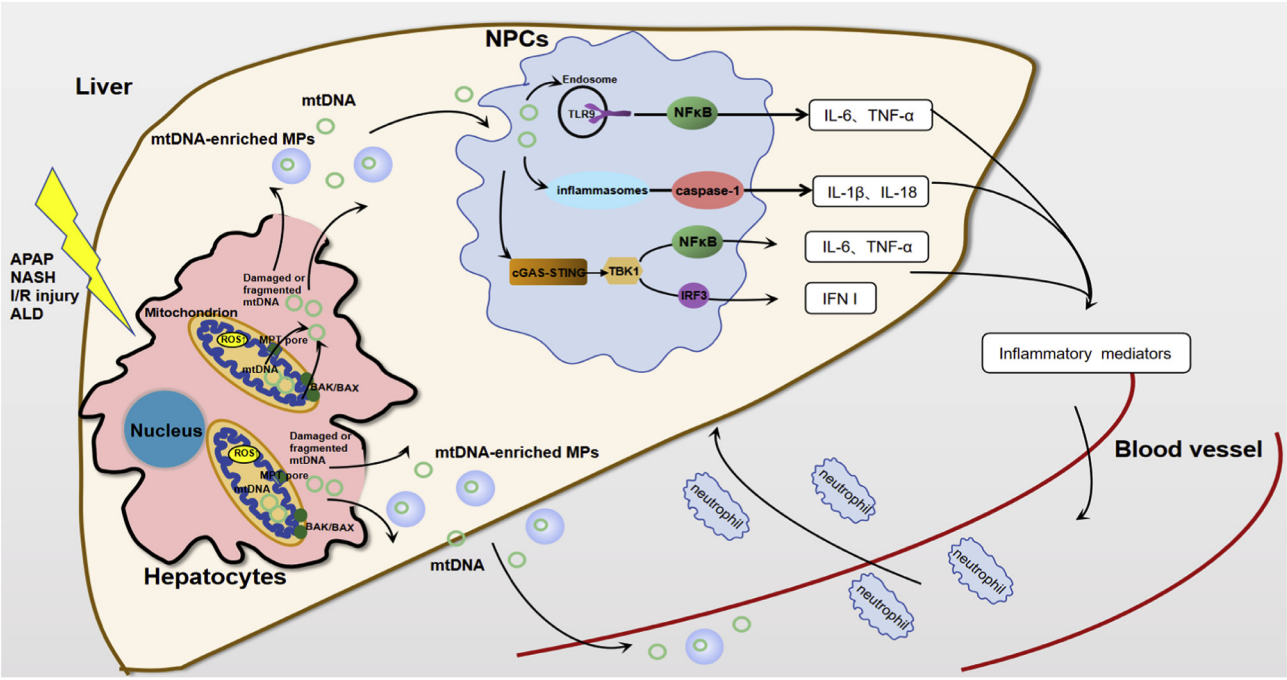

Fig. 1. Overview of liver inflammation mediated by mtDNA. Upon liver struck by APAP, I/R, ALD and NASH, increasing mitochondrial stress induces release of damaged or fragmented mtDNA into cytosol via MPT pore and Bak/Bax. Meanwhile, cellular stress, necrotic cells or cells undergoing necroptosis will expose free mtDNA or mtDNA-enriched MPs to extracellular space, subsequently diffusing into blood vessels. Free mtDNA and mtDNA-enriched MPs could be taken in by NPCs, including neutrophils, KCs and DCs, triggering the inflammation response. mtDNA can activate intracellular TLR9, inflammasomes and cGAS-STING, resulting in production of inflammatory mediators and infiltration of neutrophil, which, in turn, induces or exacerbates liver inflammation.

diet (HFD)-fed mice increased TNF-α and IL-6 expression in co-cultured Kupffer cells (KCs), which was associated with nuclear factor-κB (NF-κB) signal pathway and was attenuated by STING deficiency. Moreover, mice with disruption of STING and mice with STING disruption only in myeloid cells developed less methionine- and choline-deficient diet- or HFD-induced hepatic steatosis, fibrosis, and inflammation in mice [13,47]. The severity of steatosis and inflammation following a HFD could be restored by transplanting bone marrow cells from control mice to mice with disruption of STING [47]. Noteworthily, STING activation is associated with hepatocyte fat deposition and liver fibrosis [47]. Besides, the NLRP3 inflammasomes exert proinflammatory effects on the development and progression of NASH. It was reported that the NLRP3 inflammasomes triggered liver fibrosis by activating hepatic stellate cells [48]. Recently, Pan et al. [49] showed that using palmitic acid to stimulate KCs decreased the mitochondrial membrane potential and subsequently induced mtDNA release. Upon palmitic acid stimulation, KCs increased expression of NLRP3 inflammasomes and formation of mtDNA-NLRP3 inflammasome complexes accounting for activation of NLRP3 inflammasomes [49]. Similarly, KCs derived from the mouse NASH model had elevated expression of NLRP3 inflammasomes [49].

Garcia-Martinez et al. [30] demonstrated that plasma from mice and patients with NASH contained high mtDNA levels, and most of the plasma mtDNA was found in MPs of hepatocyte origin. Furthermore, the plasma from HFD-fed mice induced a significant increase in TLR9 activation using the TLR9 reporter cell line, compared with the plasma from the chow-fed control mice [30]. TLR9 is involved in hepatic steatosis and inflammation in the model of NASH [41,50,51]. Moreover, administration of the TLR9 antagonist IRS954 blocks the development of NASH showing a significant reduction in steatosis, ballooning and inflammation, serum transaminases, and inflammatory cytokine transcript levels [30], which supplies a new therapeutic for NASH. Similarly, there are remarkably increased serum levels of mtDNA-enriched MPs derived from hepatocytes stress in acute-on-chronic alcoholic use patients [52]. Moreover, release of mtDNA-enriched MPs depends on ER stress and leads to neutrophilia and liver injury via TLR9 pathway [52]. Thus, mtDNA is a potential biomarker predictive of outcome in NASH and alcoholic liver disease (ALD).

3.3. Hepatic I/R injury

Clinically, hepatic I/R injury remains a major complication of transplantation, liver resection, and hemorrhagic shock [53]. When hepatocytes are exposed to I/R, MPT pores will open [54], which allows mtDNA release. Emerging evidence indicates that mitochondrial DAMPs (MTDs), particularly mtDNA, play an important role in immune response during I/R injury [54]. Hu et al. [55] found that MTDs including mtDNA were released during hepatic I/R injury and were associated with the increased expression of pro-inflammatory cytokines (IL-6 and TNF-α). MTDs decrease hepatocytes viability in a concentration dependent manner, which indicates that MTDs are toxic mediators for amplifying liver damage. In addition, knockout of type I, but not type II, IFN receptor decreased intrahepatic pro-inflammatory response and protected mice against liver I/R injury [14]. Furthermore, IFN-α derived from plasmacytoid dendritic cells promoted liver I/R injury by increasing hepatocyte apoptosis as a consequence of induction of hepatocyte IFN regulatory factor 1 expression [56]. Supposedly, IFN I induced by mtDNA via cGAS-STING may contribute to pathogenesis of liver I/R injury. Mounting evidence indicates that NLRP3 signal is also involved in hepatic I/R injury. Zhu et al. [57] demonstrated that silencing of NLRP3 can protect the liver from I/R injury by reducing IL-1β and IL-18. Both NLRP3 and caspase-1 knockout mice are protected from liver I/R injury [58]. Moreover, inflammasome-mediated injury is dependent on caspase-1 expression in liver NPCs, including resident KCs, infiltrating neutrophils, and dendritic cells (DCs) [58]. So far, however, there is no research reporting mtDNA-mediated liver I/R injury via inflammasomes. Recently, a study found that reducing the mtDNA release and reactive oxygen species (ROS) overproduction by accelerating autophagy mediated by Nrf2/HO-1 pathway minimized hepatic I/R injury [59], as a potential therapeutic target for hepatic I/R

injury. Nevertheless, little is known about the association between hepatic I/R injury and free mtDNA.

While elevated circulating mtDNA derived from liver can induce or aggravate liver injury, it is unclear whether it can cause damage to extrahepatic tissue and organs. Some studies have demonstrated that the release of necrotic products from liver injury, including mtDNA, into the circulation may trigger a systemic inflammatory response like a sepsis-like state through identical innate immune pathways [33,60], and even induce remote lung injury [60]. This effect is well understood while mtDNA mainly activates three signaling pathways (TLR9, inflammasomes and cGAS-STING). However, occurrence of this effect may depend on the speed of mtDNA release, the concentration of circulating mtDNA and immunity condition of organism. Except that liver injury drives mtDNA releasing to circulation, some diseases, including trauma, sepsis, I/R injury, etc., also can remarkably elevate circulating mtDNA [33,61]. The model of hemorrhage-I/R-animals has displayed obvious oxidative mtDNA damage in all organs with the most prominent damage in the liver and accumulation of mtDNA in plasma [61]. Even so, there is little research to explore the association between elevated circulating mtDNA and liver injury in trauma, sepsis, I/R injury, etc.

4. mtDNA-mediated liver oxidative stress injury

Mitochondria are the vital organelles that sustain intracellular biological processes by providing the high-energy phosphate molecule adenosine triphosphate [62]. Defective mitochondria contribute to shortage in ATP, increase and leakage in ROS, and activation of intrinsic apoptotic cell death pathway [63]. Mitochondria dysfunction induces mtDNA lesions and mtDNA depletion [64]. Moreover, mtDNA damage is not only the manifestation of mitochondrial dysfunction, but also aggravates mitochondrial dysfunction and cell lesions. The liver is an organ with high energy requirements. Most hepatic disorders are characterized by profound mitochondrial dysfunction and mtDNA damage [62,65]. As previously mentioned, mtDNA can encode 13 respiratory chain subunits, involving in synthesis of respiratory chain complexes I, III, IV, and V. Moreover, mitochondrial oxidative respiratory chain is associated with the production of ATP and ROS [66]. Once ROS give damage to mtDNA beyond a certain threshold, damaged mtDNA can increase ROS and amplify oxidative stress by encoding deficient subunits for the respiratory chain, which aggravates the oxidative damage to mitochondrial function until cell death occur [67]. Therefore, mtDNA may make important contributions to the pathogenesis of liver oxidative stress injury.

4.1. ALD

Excessive alcohol consumption contributes to ALD [68,69]. ALD represents a broad spectrum of hepatic pathology including alcoholic steatosis, alcoholic hepatitis, and alcoholic cirrhosis, which may ultimately progress to hepatocellular carcinoma [68]. Alcohol consumption induces oxidative stress with increasing ROS formation in hepatocyte mitochondria [70]. It has been known that ROS participate in the transition of simple steatosis to advanced stage of ALD [71,72]. A consequence of increased ROS production is oxidation of mtDNA manifested by increased amounts of 8-hydroxy-2′-deoxyguanosine, mutations of mtDNA, strand breaks of mtDNA, and mitochondrial dysfunction [73–76], which in turn worsens ROS production and decreases hepatic ATP. Replication of damaged mtDNA molecules, together with mtDNA strand breaks, causes mtDNA deletion [77].

Multiple mtDNA deletions have been detected in liver tissues from alcoholic patients. Mansouri et al. [78] found that 24% of all alcoholic patients and 85% of the 13 alcoholic patients with microvesicular steatosis exhibited either single or multiple 4977, 5385, 5039 and 5556-base pair mtDNA deletions, while only 3% of the non-alcoholic controls carried a mtDNA deletion. Although hepatocytes contain hundreds of copies of mtDNA, it is possible that the accumulation of diverse mtDNA damage and deletions, together with mtDNA strand breaks, could reach a threshold sufficient to reduce mitochondrial respiration and ATP synthesis, resulting in mitochondrial dysfunction and even hepatocyte injury in patients with ALD [77]. mtDNA mutations and damage located in the D-loop region interfere with replication and maintenance of mtDNA, and dysfunctional mitochondria also reduces expression of mtDNA replication-related proteins, such as mitochondrial single-stranded DNA-binding protein and mitochondrial transcription factor A (TFAM) [79–81]. In addition, extensive mtDNA lesions may enhance the susceptibility to nucleolytic attacks from mitochondrial endonuclease G [70,82,83], thereby decreasing mtDNA levels, which may impair the synthesis of the mtDNA-encoded proteins that are key proteins of the oxidative phosphorylation system [2]. mtDNA depletion in hepatocytes impairs mitochondrial function and causes hepatic steatosis [84]. During oxidative stress induced by alcohol consumption, peroxynitrite, the reactive species derived from the spontaneous reaction of nitric oxide with superoxide, is involved in the mtDNA depletion effects [70]. As previously mentioned, increasing serum levels of mtDNA-enriched MPs happen in acute-on-chronic alcoholic use patients [52]. It is highly possible that mtDNA in MPs or free mtDNA is partly damaged. Interestingly, it is unclear what effects damaged mtDNA has on innate immunity.

There are some repair mechanisms on mtDNA damage and mtDNA depletion in alcohol-induced liver injury. Manganese superoxide dismutase (MnSOD) can prevent mtDNA depletion and mitochondrial dysfunction after acute alcohol binge in mice [70]. Sun et al. [71] demonstrated chronic alcohol feeding decreased hepatic mitochondrial respiratory complex, ATP, TFAM and mtDNA contents, companied with reducing zinc level. They also found that alcohol exposure-induced hepatic zinc deficiency could inactivate mitochondrial biogenesis pathway and decrease mitochondrial DNA replication, which, in turn, lowers mitochondrial complex proteins expression and sequentially worsens alcohol-induced ROS production, finally aggravating liver injury. Zhang et al. [73] showed that IL-6 activates mtDNA repair enzymes and induces cell cycle arrest allowing time for mtDNA repair after alcoholic liver injury in mice. Meanwhile, IL-6 treatment remarkably improved survival associated with fatty liver transplants from alcohol-fed rats [85]. In addition, mitophagy can also be induced by mtDNA damage, while activation of mitophagy alleviates alcohol-induced liver injury in ethanol-fed mice [86]. Oxidative stress plays a crucial role in ALD. Sources of ROS generation in liver include hepatic cytochrome P450 2E1, mitochondrial oxidative respiratory chain, and NADPH oxidase (NOX) [63,69,87]. Sun et al. [63] found that pharmacological inhibition of NOX4 activity protects against alcohol-induced liver injury in mice through improving oxidative stress and mitochondrial function with hepatic increasing mtDNA and ATP. Therefore, removal of hepatic ROS could be an effective strategy in reducing mtDNA lesions and preventing the development of ALD.

4.2. Hepatic I/R injury

Hepatocellular damage induced by I/R also involves the generation of ROS and the activation of inflammatory pathways [88]. The mitochondrion is a major source of ROS generation. Mitochondrial dysfunction can induce oxidative stress, inflammation and cell death, all of which could exacerbate I/R injury. mtDNA, as important DAMPs from mitochondria, plays a key role in I/R injury through triggering TLR9, inflammasomes and cGAS-STING [8]. However, there is little recognition of mtDNA function in hepatic I/R injury. Apart from elevated circulating mitochondrial DAMPs [58,59], hepatic I/R injury also induces hepatocellular mtDNA damage [88]. Emerging evidence indicates that mtDNA lesions and mtDNA depletion may contribute to the process of I/R injury. Improving hepatic mitochondrial function and increasing mtDNA replication are the therapeutic strategies to minimize hepatic I/R injury. A study found that the phosphodiesterase inhibitor

cilostazol increases mtDNA and mitochondria content, reduces mtDNA damage and protects against hepatic I/R injury [88]. In addition, mitochondrial transplantation may be a valued treatment of varied ischemic disorders, mitochondrial diseases and related disorders [89]. Lin et al. [90] showed that an intrasplenic infusion of viable mitochondria isolated from the donor before reperfusion significantly reduced hepatic I/R injury.

4.3. Lipopolysaccharide-induced liver injury

Lipopolysaccharide (LPS) treatment can induce a "sepsis-like" inflammation state [82]. As we know, LPS has been identified as one of the main factors causing acute hepatocyte lesions through macrophage activation [91]. LPS increases mitochondrial ROS formation, causes mtDNA lesions, and decreases complex I activity in mice [92]. Mitochondrial dysfunction and mtDNA damage play a key role in LPS-induced liver injury. As early as 2003, Hagir et al. [93] found that LPS led to decreasing liver mtDNA copy number and oxidant-dependent 3.8-kb liver mtDNA deletion in the region encoding NADH dehydrogenase subunits 1 and 2 and cytochrome *c* oxidase subunit I, which was associated with mitochondrial glutathione depletion. Furthermore, Choumar et al. [93] demonstrated that MnSOD, NOS inhibitors, and superoxide or peroxynitrite scavengers protected against LPS-induced mtDNA depletion, pointing out a role of the superoxide anion reacting with NO to form mtDNA- and protein-damaging peroxynitrite. In addition, glucocorticoid receptor was found to be involved in protecting against LPS-induced liver injury via an increased binding to the mtDNA D-loop region, thereby regulating the expression of mtDNA-encoded genes [94]. Taken together, these studies indicate that LPS administration impairs mtDNA integrity and transcriptional capacity, which shows the link between LPS-mediated liver injury and oxidative mtDNA deletion.

4.4. Cholestatic liver injury

Cholestasis is a condition that results in bile stasis and the accumulation of toxic bile acids in the liver and the systemic circulation, leading to hepatocyte death and eventually to liver fibrosis and cirrhosis [95,96]. It is described as a prominent feature of primary biliary cirrhosis, primary sclerosing cholangitis, biliary atresia, and iatrogenic obstruction of bile ducts [95,97]. Evidence from in vivo and in vitro experiments demonstrates that oxidative stress, mitochondrial dysfunction and mtDNA damage are the major pathophysiologic mechanism in cholestatic liver diseases. It was shown that selective defects of complexes I, III and IV, coded by mtDNA, were detected in liver tissue of two siblings with neonatal cholestasis and early liver insufficiency [98]. In a study of rats, mtDNA copy number significantly decreases 72 h after bile duct ligation [99]. Long-term cholestasis leads to mtDNA severe depletion and deletions, which is likely to be consequence of loss TFAM [95]. Furthermore, there are a novel 11, 194-bp mtDNA deletion and fewer mtDNA copies detected in hepatocytes of rats with obstructive jaundice [100]. These changes are consistent with damage to hepatic and mitochondrial function. Therefore, mtDNA damage is tightly associated with cholestatic liver injury. In addition, Xu et al. [67] demonstrated that mtDNA damage is involved in liver damage in extrahepatic cholestatic patients and TFAM has mtDNA-protective effects. Recently, Koh et al. [96] performed a mitochondrial genome-wide analysis to investigate the extent of mtDNA mutations in the liver of 14 biliary atresia patients and five choledochal cyst, suggesting that the mutations in liver mtDNA protein-coding genes are highly associated with biliary atresia. Nevertheless, it is still unclear whether mtDNA alterations are the cause of cholestatic liver injury or just its manifestation.

5. mtDNA in hepatitis B virus infection and hepatocellular carcinoma

Hepatitis B virus (HBV) is a primary cause of chronic viral hepatitis, and chronic hepatitis B infection (CHB) can progress to liver cirrhosis and hepatocellular carcinoma (HCC) [101]. Accumulating evidence indicates that alterations of mitochondrial metabolism and oxidative/nitrosative stress contribute to initiation and progression of chronic HBV infection [1]. mtDNA alterations have been identified in human cancers, including HCC. mtDNA change is also closely related to different stages of HBV-related liver injury. In 2011, Zhao et al. [102] conducted a case-control study by collecting peripheral blood leukocyte samples from 274 HBV-related HCC cases, 126 non-cancer patient controls with HBV-related chronic liver diseases (CLD), and 258 healthy controls. They showed that HCC cases have a significant lower mtDNA content in peripheral blood leukocytes samples than CLD controls and mtDNA content from HBV-related HCC is significantly associated with HCC. Recently, Chen et al. [103] published a case-control study to test mtDNA content in peripheral blood leukocyte samples from 76 CHB cases naive to antivirus therapy and 96 healthy controls. They found that mtDNA content of CHB cases without antivirus therapy is significantly higher than healthy controls and mtDNA content is negatively associated with hepatitis B surface antigen. They also showed that a decreasing trend of levels of HBV viral load and alanine aminotransferase is associated with the increase of mtDNA content.

In 2015, Wang et al. [79] published a case-control study including 136 cirrhotic HBV cases and 136 frequency-matched non-cirrhotic HBV controls, and found that cirrhotic HBV patients had significantly lower mtDNA content in serum than non-cirrhotic HBV controls. To explore the relationship between circulating mtDNA content and HCC, Li et al. [104] collected blood from 116 HBV-related HCC cases and 232 frequency-matched cancer-free HBV controls. HCC patients had significantly lower mtDNA content in serum than HBV patients. Moreover, compared to HBV patients with higher mtDNA content in serum, those with lower mtDNA content had a remarkably increased risk of HCC with an odds ratio of 2.19 (95% confidence interval: 1.28–3.72, $P = 0.004$). Therefore, mtDNA content in peripheral blood leukocyte or in serum may serve as a potential noninvasive biomarker of HBV-related liver diseases (Fig. 2), which may need more studies to confirm. Nevertheless, it remains unclear why mtDNA content alters during different stages of HBV infection. But the association between mtDNA and HBV infection is evident only in never-drinkers [79,102]. Actually, increased production of mitochondrial ROS and decreased glutathione content were found in chronically ethanol-fed animals, which might induce cellular death, therefore preventing malignant transformation of hepatocytes in heavy drinkers [102,105].

Increasing evidence has indicated that mtDNA alterations play a crucial role in HCC development. mtDNA content is required to maintain normal mitochondrial respiratory function. The alteration of mtDNA copy number is correlated with tumor progression and patient prognosis [106]. Previous studies have reported that mtDNA content is significantly lower in tumor tissue than that of non-tumorous tissue in HCC [80,107,108]. Moreover, it was shown that mtDNA content was not only remarkably reduced in HCC tissues, but also in cirrhotic tissues [109]. Actually, both mtDNA mutations and mtDNA copies are associated with cancer development and progression [107,110,111]. mtDNA mutations located in the D-loop region not only induce mtDNA depletion [80], but also are positively related to poor HCC differentiation grade [112]. Mechanically, increased production of ROS induced by mtDNA depletion impairs oxidative phosphorylation capacity and leads to DNA damage and subsequent malignant cellular transformation [113,114]. Although mtDNA alterations have been described in HCC, whether mtDNA alterations are correlated with the initiation and progression of HCC remains controversial.

To some extent, cells with low mtDNA content could be resistant to apoptosis and thus more vulnerable to malignant transformation [115].

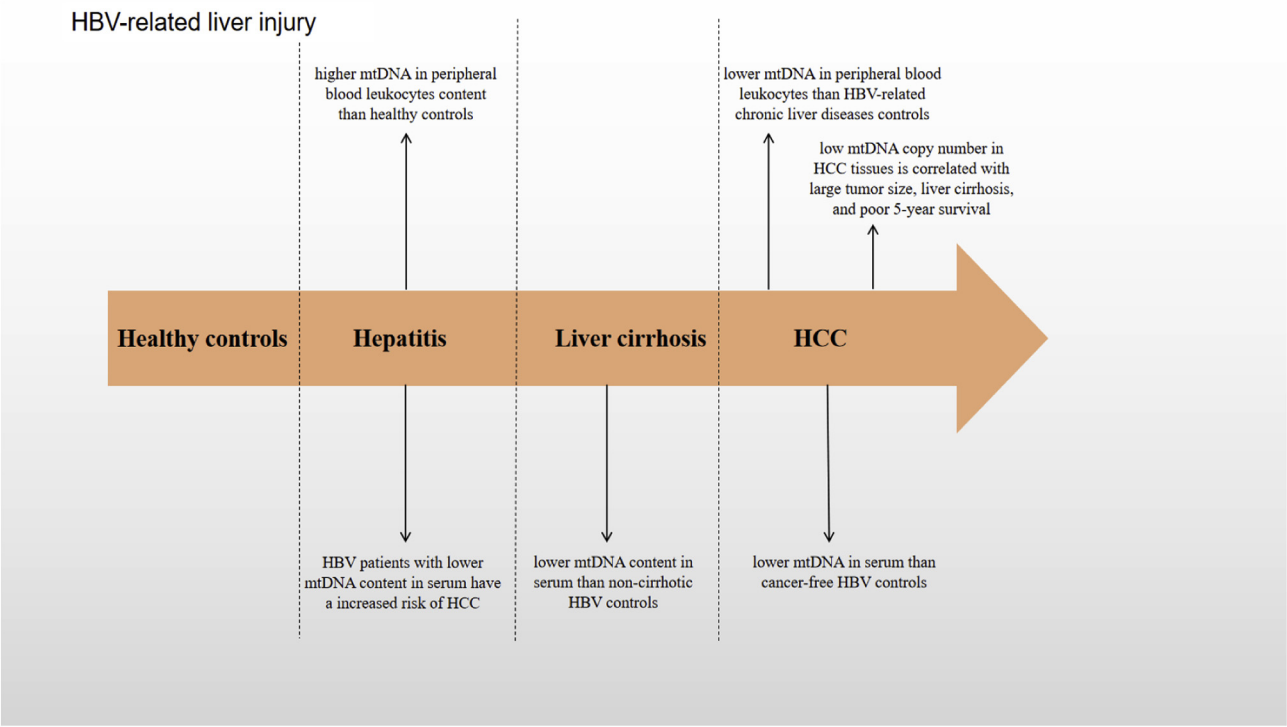

Fig. 2. Alteration of mtDNA content in different stages of HBV-related liver injury.

Yamada et al. [116] demonstrated that low mtDNA copy number in HCC was correlated with large tumor size, liver cirrhosis, and poor 5-year survival. Furthermore, low mtDNA content was found to stimulate NF-κB or Rel factors by suppressing NF-κB inhibitor, while dysregulation of NF-κB has been associated with various cancers [117]. However, once the mtDNA content decreases to a certain threshold, at which production of energy is insufficient to keep the normal cellular metabolism, the cell will turn to cell death but not tumorigenesis [102]. It was also shown that mtDNA binding to High Mobility Group Box-1 (HMGB1) activates TLR9 signaling to induce tumor growth during hypoxia [118]. HBV X protein (HBx) encoded by the HBV genome is a 154 amino acids, non-structural protein that is known to regulate calcium signaling and ROS and participate in activation of potential transcription factors such as STAT-3 and NF-κB [119,120]. A study has found that ROS production mediated by the C-terminal region of HBx results in mtDNA damage, which may play a role in HBV-related HCC development [121].

6. Conclusions

In this review, we demonstrate that liver injury not only induces hepatocyte mtDNA damage, but also releases mtDNA. mtDNA participates in maintenance of mitochondria function and production of ATP. Cells with extensive mtDNA lesions may selectively undergo apoptosis [122]. Mechanically, damage and depletion of mtDNA increase ROS in hepatocytes and subsequently impair liver normal function. Reduction of mtDNA lesions and increase in mtDNA content could be effective strategies in alleviating liver injury. No matter how mtDNA mutations induce liver oxidative stress injury or even cancer, it remains unclear what degree or type of mutation is involved in these processes. Furthermore, emerging evidence has indicated circulating mtDNA is elevated in NASH, ALD, hepatic I/R injury, etc. circulating mtDNA acts as DAMPs inducing neutrophil infiltration and liver inflammation via TLR9 and STING, which may exacerbate hepatocellular damage. Notably, liver NPCs, namely neutrophils, KCs, DCs and lymphocyte, express remarkably higher levels of DNA sensors, including cGAS, STING, TLR9 and AIM2, than hepatocytes during homeostasis [15], suggesting that liver NPCs are the main DNA sensors within the liver microenvironment. Moreover, NPCs have been shown to be crucial to production of IFN I and amplification of liver injury [15]. Therefore, mtDNA amplifies inflammation by irritating liver NPCs (Fig. 1), when damaged hepatocytes release mtDNA. Taken together, mounting studies indicate that mtDNA contributes to pathological and physiological changes and may be a biomarker in liver injury. However, it remains unclear whether mtDNA actively contributes to pathogenesis of liver injury through interaction with inflammasomes or other potential signaling pathways. Additional studies are needed to further explore the role of mtDNA in liver inflammation, liver oxidative stress injury and HCC.

Author contributions

Conceptualization, Xufei Zhang and Xiuwen Wu; bibliographic investigation, Xufei Zhang, Qiongyuan Hu and Zhiwu Hong; writing-original draft preparation, Xufei Zhang and Jie Wu; writing-review and editing, Jianan Ren and Xiuwen Wu; visualization, Xufei Zhang and Gefei Wang.

Funding

This study was supported by grants from National Natural Science Foundation of China (81801971, 81571881 and 81772052).and Key Project of Science Foundation of the 12th Five-Year Plan (BNJ13J002).

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

The authors are grateful to Haohao Xie and Canwen Chen for critical reading and valuable suggestions.

References

[1] K. Begriche, A. Igoudjil, D. Pessayre, B. Fromenty, Mitochondrial dysfunction in NASH: Causes, consequences and possible means to prevent it, Mitochondrion 6 (2006) 1–28.
[2] J. Nunnari, A. Suomalainen, Mitochondria: in sickness and in health, Cell 148 (2012) 1145–1159.
[3] L. Galluzzi, O. Kepp, C. Trojel-Hansen, G. Kroemer, Mitochondrial control of cellular life, stress, and death, Circ. Res. 111 (2012) 1198–1207.
[4] E.M.V. de Cavanagh, I. Flores, M. Ferder, F. Inserra, L. Ferder, Renin–angiotensin system inhibitors protect against age-related changes in rat liver mitochondrial DNA content and gene expression, Exp. Gerontol. 43 (2008) 919–928.
[5] G.S. Shadel, D.A. Clayton, Mitochondrial DNA maintenance in vertebrates, Annu. Rev. Biochem. 66 (1997) 409–435.
[6] C. Fang, X. Wei, Y. Wei, Mitochondrial DNA in the regulation of innate immune responses, Protein Cell 7 (2016) 11–16.
[7] J.S. Harrington, A.M.K. Choi, K. Nakahira, Mitochondrial DNA in sepsis, Curr. Opin. Crit. Care 23 (2017) 284–290.
[8] Q. Hu, Q. Zhou, J. Wu, X. Wu, J. Ren, The role of mitochondrial DNA in the development of ischemia reperfusion injury, Shock 51 (2019) 52–59.
[9] T. Oka, S. Hikoso, O. Yamaguchi, M. Taneike, T. Takeda, T. Tamai, J. Oyabu, T. Murakawa, H. Nakayama, K. Nishida, S. Akira, A. Yamamoto, I. Komuro, K. Otsu, Mitochondrial DNA that escapes from autophagy causes inflammation and heart failure, Nature 485 (2012) 251–255.
[10] W. Bao, H. Xia, Y. Liang, Y. Ye, Y. Lu, X. Xu, A. Duan, J. He, Z. Chen, Y. Wu, X. Wang, C. Zheng, Z. Liu, S. Shi, Toll-like receptor 9 can be activated by endogenous mitochondrial DNA to induce podocyte apoptosis, Sci. Rep.-UK 6 (2016) 22579.
[11] A.P. West, G.S. Shadel, Mitochondrial DNA in innate immune responses and inflammatory pathology, Nat. Rev. Immunol. 17 (2017) 363–375.
[12] Y. He, D. Feng, M. Li, Y. Gao, T. Ramirez, H. Cao, S. Kim, Y. Yang, Y. Cai, C. Ju, H. Wang, J. Li, B. Gao, Hepatic mitochondrial DNA/Toll-like receptor 9/MicroRNA-223 forms a negative feedback loop to limit neutrophil overactivation and acetaminophen hepatotoxicity in mice, Hepatology 66 (2017) 220–234.
[13] Y. Yu, Y. Liu, W. An, J. Song, Y. Zhang, X. Zhao, STING-mediated inflammation in Kupffer cells contributes to progression of nonalcoholic steatohepatitis, J. Clin. Invest. 129 (2019) 546–555.
[14] Y. Zhai, B. Qiao, F. Gao, X. Shen, A. Vardanian, R.W. Busuttil, J.W. Kupiec-Weglinski, Type I, but not type II, interferon is critical in liver injury induced after ischemia and reperfusion, Hepatology 47 (2008) 199–206.
[15] A. Araujo, M. Antunes, M. Mattos, A. Diniz, D. Alvarenga, B. Nakagaki, É. Carvalho, V. Lacerda, R. Carvalho-Gontijo, J. Goulart, K. Mafra, M. Freitas-Lopes, H. Oliveira, C. Dutra, B. David, A. Mendes Silva, V. Quesniaux, B. Ryffel, S. Oliveira, G. Barber, D. Mansur, T. Cunha, R. Rezende, A. Oliveira, G. Menezes, Liver immune cells release type 1 interferon due to DNA sensing and amplify liver injury from acetaminophen overdose, Cells-Basel 7 (2018) 88.
[16] A. Mansouri, I. Gaou, C. De Kerguenec, S. Amsellem, D. Haouzi, A. Berson, A. Moreau, G. Feldmann, P. Letteron, D. Pessayre, B. Fromenty, An alcoholic binge causes massive degradation of hepatic mitochondrial DNA in mice, Gastroenterology 117 (1999) 181–190.
[17] P. Rawla, T. Sunkara, P. Muralidharan, J.P. Raj, Update in global trends and aetiology of hepatocellular carcinoma, Contemp. Oncol. (Pozn) 22 (2018) 141–150.
[18] A.P. Halestrap, What is the mitochondrial permeability transition pore? J. Mol. Cell. Cardiol. 46 (2009) 821–831.
[19] M. Patrushev, V. Kasymov, V. Patrusheva, T. Ushakova, V. Gogvadze, A. Gaziev, Mitochondrial permeability transition triggers the release of mtDNA fragments, Cell. Mol. Life Sci. 61 (2004) 3100–3103.
[20] P. Li, X. Wang, M. Zhao, R. Song, K. Zhao, Polydatin protects hepatocytes against mitochondrial injury in acute severe hemorrhagic shock via SIRT1-SOD2 pathway, Expert Opin. Ther. Tar. 19 (2015) 997–1010.
[21] H. Hikita, T. Takehara, T. Kodama, S. Shimizu, M. Shigekawa, A. Hosui, T. Miyagi, T. Tatsumi, H. Ishida, W. Li, T. Kanto, N. Hiramatsu, S. Shimizu, Y. Tsujimoto, N. Hayashi, Delayed-onset caspase-dependent massive hepatocyte apoptosis upon Fas activation in Bak/Bax-deficient mice, HEPATOLOGY 54 (2011) 240–251.
[22] K. McArthur, L.W. Whitehead, J.M. Heddleston, L. Li, B.S. Padman, V. Oorschot, N.D. Geoghegan, S. Chappaz, S. Davidson, C.H. San, R.M. Lane, M. Dramicanin, T.L. Saunders, C. Sugiana, R. Lessene, L.D. Osellame, T.L. Chew, G. Dewson, M. Lazarou, G. Ramm, G. Lessene, M.T. Ryan, K.L. Rogers, M.F. van Delft, B.T. Kile, BAK/BAX macropores facilitate mitochondrial herniation and mtDNA efflux during apoptosis, Science 359 (2018).
[23] J.S. Riley, G. Quarato, C. Cloix, J. Lopez, J. O'Prey, M. Pearson, J. Chapman, H. Sesaki, L.M. Carlin, J.F. Passos, A.P. Wheeler, A. Oberst, K.M. Ryan, S.W. Tait, Mitochondrial inner membrane permeabilisation enables mtDNA release during apoptosis, EMBO J. 37 (2018) e99238-n/a.
[24] R.K. Boyapati, A. Tamborska, D.A. Dorward, G.T. Ho, Advances in the understanding of mitochondrial DNA as a pathogenic factor in inflammatory diseases, F1000Res 6 (2017) 169.
[25] A. Kaczmarek, P. Vandenabeele, D.V. Krysko, Necroptosis: the release of damage-associated molecular patterns and its physiological relevance, Immunity 38 (2013) 209–223.
[26] R.F. Schwabe, T. Luedde, Apoptosis and necroptosis in the liver: a matter of life and death, Nat. Rev. Gastroenterol. Hepatol. 15 (2018) 738–752.
[27] M. N, Q. D, H. M, Mitochondrial DNA released following necroptosis accumulates on RBCs, Am. J. Respir. Crit. Care Med. 193 (2016) A4309.
[28] I. Gan, J. Jiang, D. Lian, X. Huang, B. Fuhrmann, W. Liu, A. Haig, A.M. Jevnikar, Z.X. Zhang, Mitochondrial permeability regulates cardiac endothelial cell necroptosis and cardiac allograft rejection, Am. J. Transplant. 19 (2018) 686–698.
[29] P. Raeven, J. Zipperle, S. Drechsler, Extracellular vesicles as markers and mediators in sepsis, Theranostics 8 (2018) 3348–3365.
[30] I. Garcia-Martinez, N. Santoro, Y. Chen, R. Hoque, X. Ouyang, S. Caprio, M.J. Shlomchik, R.L. Coffman, A. Candia, W.Z. Mehal, Hepatocyte mitochondrial DNA drives nonalcoholic steatohepatitis by activation of TLR9, J. Clin. Invest. 126 (2016) 859–864.
[31] M. Guescini, S. Genedani, V. Stocchi, L.F. Agnati, Astrocytes and glioblastoma cells release exosomes carrying mtDNA, J. Neural Transm. 117 (2010) 1–4.
[32] D.M. Spencer, J.R. Dye, C.A. Piantadosi, D.S. Pisetsky, The release of microparticles and mitochondria from RAW 264.7 murine macrophage cells undergoing necroptotic cell death in vitro, Exp. Cell Res. 363 (2018) 151–159.
[33] Q. Zhang, M. Raoof, Y. Chen, Y. Sumi, T. Sursal, W. Junger, K. Brohi, K. Itagaki, C.J. Hauser, Circulating mitochondrial DAMPs cause inflammatory responses to injury, Nature 464 (2010) 104–107.
[34] H. Jaeschke, Acetaminophen: dose-dependent drug hepatotoxicity and acute liver failure in patients, Dig. Dis. 33 (2015) 464–471.
[35] C. Bunchorntavakul, K.R. Reddy, Acetaminophen-related hepatotoxicity, Clin. Liver Dis. 17 (2013) 587–607.
[36] J.S. Gujral, T.R. Knight, A. Farhood, M.L. Bajt, H. Jaeschke, Mode of cell death after acetaminophen overdose in mice: apoptosis or oncotic necrosis? Toxicol. Sci. 67 (2002) 322–328.
[37] M.R. McGill, M.R. Sharpe, C.D. Williams, M. Taha, S.C. Curry, H. Jaeschke, The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation, J. Clin. Invest. 122 (2012) 1574–1583.
[38] H. Jaeschke, A. Ramachandran, Oxidant stress and lipid peroxidation in acetaminophen hepatotoxicity, React. Oxyg. Species (Apex) 5 (2018) 145–158.
[39] M.R. McGill, V.S. Staggs, M.R. Sharpe, W.M. Lee, H. Jaeschke, Serum mitochondrial biomarkers and damage-associated molecular patterns are higher in acetaminophen overdose patients with poor outcome, Hepatology 60 (2014) 1336–1345.
[40] M.J. Hotz, D. Qing, M.G.S. Shashaty, P. Zhang, H. Faust, N. Sondheimer, S. Rivella, G.S. Worthen, N.S. Mangalmurti, Red blood cells homeostatically bind mitochondrial DNA through TLR9 to maintain quiescence and to prevent lung injury, Am. J. Resp. Crit. Care 197 (2018) 470–480.
[41] A.B. Imaeda, A. Watanabe, M.A. Sohail, S. Mahmood, M. Mohamadnejad, F.S. Sutterwala, R.A. Flavell, W.Z. Mehal, Acetaminophen-induced hepatotoxicity in mice is dependent on Tlr9 and the Nalp3 inflammasome, J. Clin. Invest. 119 (2009) 305–314.
[42] T. Hardy, F. Oakley, Q.M. Anstee, C.P. Day, Nonalcoholic fatty liver disease: pathogenesis and disease spectrum, Annu. Rev. Pathol. 11 (2016) 451–496.
[43] C.A. Matteoni, Z.M. Younossi, T. Gramlich, N. Boparai, Y.C. Liu, A.J. McCullough, Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity, Gastroenterology 116 (1999) 1413–1419.
[44] M.E. Rinella, A.J. Sanyal, Management of NAFLD: a stage-based approach, Nat. Rev. Gastroenterol. Hepatol. 13 (2016) 196–205.
[45] F. Chiappini, A. Barrier, R. Saffroy, M.C. Domart, N. Dagues, D. Azoulay, M. Sebagh, B. Franc, S. Chevalier, B. Debuire, S. Dudoit, A. Lemoine, Exploration of global gene expression in human liver steatosis by high-density oligonucleotide microarray, Lab. Investig. 86 (2006) 154–165.
[46] J. Gautheron, M. Vucur, F. Reisinger, D.V. Cardenas, C. Roderburg, C. Koppe, K. Kreggenwinkel, A.T. Schneider, M. Bartneck, U.P. Neumann, A. Canbay, H.L. Reeves, M. Luedde, F. Tacke, C. Trautwein, M. Heikenwalder, T. Luedde, A positive feedback loop between RIP3 and JNK controls non-alcoholic steatohepatitis, Embo. Mol. Med. 6 (2014) 1062–1074.
[47] X. Luo, H. Li, L. Ma, J. Zhou, X. Guo, S.L. Woo, Y. Pei, L.R. Knight, M. Deveau, Y. Chen, X. Qian, X. Xiao, Q. Li, X. Chen, Y. Huo, K. McDaniel, H. Francis, S. Glaser, F. Meng, G. Alpini, C. Wu, Expression of STING is increased in liver tissues from patients with NAFLD and promotes macrophage-mediated hepatic inflammation and fibrosis in mice, Gastroenterology 155 (2018) 1971–1984.e4.
[48] M.E. Inzaugarat, C.D. Johnson, T.M. Holtmann, M.D. McGeough, C. Trautwein, B.G. Papouchado, R. Schwabe, H.M. Hoffman, A. Wree, A.E. Feldstein, NLR family pyrin domain-containing 3 inflammasome activation in hepatic stellate cells induces liver fibrosis in mice, Hepatology 69 (2019) 845–859.
[49] J. Pan, Z. Ou, C. Cai, P. Li, J. Gong, X.Z. Ruan, K. He, Fatty acid activates NLRP3 inflammasomes in mouse Kupffer cells through mitochondrial DNA release, Cell. Immunol. 332 (2018) 111–120.
[50] A. Watanabe, A. Hashmi, D.A. Gomes, T. Town, A. Badou, R.A. Flavell, W.Z. Mehal, Apoptotic hepatocyte DNA inhibits hepatic stellate cell chemotaxis via toll-like receptor 9, Hepatology 46 (2007) 1509–1518.
[51] K. Miura, Y. Kodama, S. Inokuchi, B. Schnabl, T. Aoyama, H. Ohnishi, J.M. Olefsky, D.A. Brenner, E. Seki, Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1beta in mice, Gastroenterology 139 (2010) 323–334 (e7).
[52] Y. Cai, M.J. Xu, E.H. Koritzinsky, Z. Zhou, W. Wang, H. Cao, P.S. Yuen, R.A. Ross, R.A. Star, S. Liangpunsakul, B. Gao, Mitochondrial DNA-enriched microparticles promote acute-on-chronic alcoholic neutrophilia and hepatotoxicity, JCI Insight 2 (2017).
[53] K.L. Go, S. Lee, I. Zendejas, K.E. Behrns, J. Kim, Mitochondrial dysfunction and autophagy in hepatic ischemia/reperfusion injury, Biomed. Res. Int. 2015 (2015) 1–14.
[54] T. Qian, A.L. Nieminen, B. Herman, J.J. Lemasters, Mitochondrial permeability transition in pH-dependent reperfusion injury to rat hepatocytes, Am. J. Phys. 273 (1997) C1783–C1792.

[55] Q. Hu, C.R. Wood, S. Cimen, A.B. Venkatachalam, I.P.J. Alwayn, Mitochondrial damage-associated molecular patterns (MTDs) are released during hepatic ischemia reperfusion and induce inflammatory responses, PLoS One 10 (2015) e0140105.

[56] A. Castellaneta, O. Yoshida, S. Kimura, S. Yokota, D.A. Geller, N. Murase, A.W. Thomson, Plasmacytoid dendritic cell-derived IFN-α promotes murine liver ischemia/reperfusion injury by induction of hepatocyte IRF-1, Hepatology 60 (2014) 267–277.

[57] P. Zhu, L. Duan, J. Chen, A. Xiong, Q. Xu, H. Zhang, F. Zheng, Z. Tan, F. Gong, M. Fang, Gene silencing of NALP3 protects against liver ischemia–reperfusion injury in mice, Hum. Gene Ther. 22 (2011) 853–864.

[58] H. Huang, H.W. Chen, J. Evankovich, W. Yan, B.R. Rosborough, G.W. Nace, Q. Ding, P. Loughran, D. Beer-Stolz, T.R. Billiar, C.T. Esmon, A. Tsung, Histones activate the NLRP3 inflammasome in Kupffer cells during sterile inflammatory liver injury, J. Immunol. 191 (2013) 2665–2679.

[59] D. Xu, L. Chen, X. Chen, Y. Wen, C. Yu, J. Yao, H. Wu, X. Wang, Q. Xia, X. Kong, The triterpenoid CDDO-imidazolide ameliorates mouse liver ischemia-reperfusion injury through activating the Nrf2/HO-1 pathway enhanced autophagy, Cell Death Dis. 8 (2017) e2983.

[60] P.E. Marques, S.S. Amaral, D.A. Pires, L.L. Nogueira, F.M. Soriani, B.H. Lima, G.A. Lopes, R.C. Russo, T.V. Avila, J.G. Melgaco, A.G. Oliveira, M.A. Pinto, C.X. Lima, A.M. De Paula, D.C. Cara, M.F. Leite, M.M. Teixeira, G.B. Menezes, Chemokines and mitochondrial products activate neutrophils to amplify organ injury during mouse acute liver failure, Hepatology 56 (2012) 1971–1982.

[61] G.E. Black, K.K. Sokol, D.M. Moe, J.D. Simmons, D. Muscat, V. Pastukh, G. Capley, O. Gorodnya, M. Ruchko, M.B. Roth, M. Gillespie, M.J. Martin, Impact of a novel phosphoinositol-3 kinase inhibitor in preventing mitochondrial DNA damage and damage-associated molecular pattern accumulation, J. Trauma Acute Care 83 (2017) 683–689.

[62] A. Al-Hussaini, E. Faqeih, A.W. El-Hattab, M. Alfadhel, A. Asery, B. Alsaleem, E. Bakhsh, A. Ali, A. Alasmari, K. Lone, A. Nahari, W. Eyaid, M. Al Balwi, K. Craig, A. Butterworth, L. He, R.W. Taylor, Clinical and molecular characteristics of mitochondrial DNA depletion syndrome associated with neonatal cholestasis and liver failure, J. Pediatr. 164 (2014) 553–559.e2.

[63] Q. Sun, W. Zhang, W. Zhong, X. Sun, Z. Zhou, Pharmacological inhibition of NOX4 ameliorates alcohol-induced liver injury in mice through improving oxidative stress and mitochondrial function, Biochim. Biophys. Acta Gen. Subj. 1861 (2017) 2912–2921.

[64] I. Shokolenko, N. Venediktova, A. Bochkareva, G.L. Wilson, M.F. Alexeyev, Oxidative stress induces degradation of mitochondrial DNA, Nucleic Acids Res. 37 (2009) 2539–2548.

[65] J. Müller-Höcker, R. Horvath, S. Schäfer, H. Hessel, W. Müller-Felber, J. Kühr, W.C. Copeland, P. Seibel, Mitochondrial DNA depletion and fatal infantile hepatic failure due to mutations in the mitochondrial polymerase γ (POLG) gene: a combined morphological/enzyme histochemical and immunocytochemical/biochemical and molecular genetic study, J. Cell. Mol. Med 15 (2011) 445–456.

[66] S.E. Siegmund, R. Grassucci, S.D. Carter, E. Barca, Z.J. Farino, M. Juanola-Falgarona, P. Zhang, K. Tanji, M. Hirano, E.A. Schon, J. Frank, Z. Freyberg, Three-dimensional analysis of mitochondrial crista ultrastructure in a patient with Leigh syndrome by in situ cryoelectron tomography, iScience 6 (2018) 83–91.

[67] S. Xu, Y. Chen, H. Lin, H. Pi, N. Zhang, C. Zhao, L. Shuai, M. Zhong, Z. Yu, Z. Zhou, P. Bie, Damage to mtDNA in liver injury of patients with extrahepatic cholestasis: the protective effects of mitochondrial transcription factor a, Free Radic. Biol. Med. 52 (2012) 1543–1551.

[68] B. Gao, R. Bataller, Alcoholic liver disease: pathogenesis and new therapeutic targets, Gastroenterology 141 (2011) 1572–1585.

[69] M.G. Neuman, S. Malnick, Y. Maor, R.M. Nanau, E. Melzer, P. Ferenci, H.K. Seitz, S. Mueller, H. Mell, D. Samuel, L.B. Cohen, K.K. Kharbanda, N.A. Osna, M. Ganesan, K.J. Thompson, I.H. McKillop, A. Bautista, R. Bataller, S.W. French, Alcoholic liver disease: clinical and translational research, Exp. Mol. Pathol. 99 (2015) 596–610.

[70] I. Larosche, P. Letteron, A. Berson, B. Fromenty, T.T. Huang, R. Moreau, D. Pessayre, A. Mansouri, Hepatic mitochondrial DNA depletion after an alcohol binge in mice: probable role of peroxynitrite and modulation by manganese superoxide dismutase, J. Pharmacol. Exp. Ther. 332 (2010) 886–897.

[71] Q. Sun, W. Zhong, W. Zhang, Z. Zhou, Defect of mitochondrial respiratory chain is a mechanism of ROS overproduction in a rat model of alcoholic liver disease: role of zinc deficiency, Am. J. Physiol. Gastrointest. Liver Physiol. 310 (2016) G205–G214.

[72] H. Zhu, Y.R. Li, Oxidative stress and redox signaling mechanisms of inflammatory bowel disease: updated experimental and clinical evidence, Exp. Biol. Med. (Maywood) 237 (2012) 474–480.

[73] X. Zhang, S. Tachibana, H. Wang, M. Hisada, G.M. Williams, B. Gao, Z. Sun, Interleukin-6 is an important mediator for mitochondrial DNA repair after alcoholic liver injury in mice, Hepatology 52 (2010) 2137–2147.

[74] J.B. Hoek, A. Cahill, J.G. Pastorino, Alcohol and mitochondria: a dysfunctional relationship, Gastroenterology 122 (2002) 2049–2063.

[75] P.H. Lin, S.H. Lee, C.P. Su, Y.H. Wei, Oxidative damage to mitochondrial DNA in atrial muscle of patients with atrial fibrillation, Free Radic. Biol. Med. 35 (2003) 1310–1318.

[76] M. Kakimoto, T. Inoguchi, T. Sonta, H.Y. Yu, M. Imamura, T. Etoh, T. Hashimoto, H. Nawata, Accumulation of 8-hydroxy-2′-deoxyguanosine and mitochondrial DNA deletion in kidney of diabetic rats, Diabetes 51 (2002) 1588–1595.

[77] A. Mansouri, C. Gattolliat, T. Asselah, Mitochondrial dysfunction and signaling in chronic liver diseases, Gastroenterology 155 (2018) 629–647.

[78] A. Mansouri, B. Fromenty, A. Berson, M. Robin, S. Grimbert, M. Beaugrand, S. Erlinger, D. Pessayre, Multiple hepatic mitochondrial DNA deletions suggest premature oxidative aging in alcoholic patients, J. Hepatol. 27 (1997) 96–102.

[79] C. Wang, H.W. Hann, R.S. Hann, S. Wan, R.E. Myers, Z. Ye, J. Xing, H. Yang, Circulating mitochondrial DNA content associated with the risk of liver cirrhosis: a nested case-control study, Dig. Dis. Sci. 60 (2015) 1707–1715.

[80] H.C. Lee, S.H. Li, J.C. Lin, C.C. Wu, D.C. Yeh, Y.H. Wei, Somatic mutations in the D-loop and decrease in the copy number of mitochondrial DNA in human hepatocellular carcinoma, Mutat. Res. 547 (2004) 71–78.

[81] C.C. Hsu, H.C. Lee, Y.H. Wei, Mitochondrial DNA alterations and mitochondrial dysfunction in the progression of hepatocellular carcinoma, World J. Gastroenterol. 19 (2013) 8880–8886.

[82] S. Deng, L. Zhang, Y. Ai, P. Pan, S. Zhao, X. Su, D. Wu, H. Tan, L. Zhang, A. Tsung, Role of interferon regulatory factor-1 in lipopolysaccharide-induced mitochondrial damage and oxidative stress responses in macrophages, Int. J. Mol. Med 40 (2017) 1261–1269.

[83] S. Ikeda, K. Ozaki, Action of mitochondrial endonuclease G on DNA damaged by L-ascorbic acid, peplomycin, and cis-diamminedichloroplatinum (II), Biochem. Biophys. Res. Commun. 235 (1997) 291–294.

[84] C. Demeilliers, C. Maisonneuve, A. Grodet, A. Mansouri, R. Nguyen, M. Tinel, P. Lettéron, C. Degott, G. Feldmann, D. Pessayre, B. Fromenty, Impaired adaptive resynthesis and prolonged depletion of hepatic mitochondrial DNA after repeated alcohol binges in mice, Gastroenterology 123 (2002) 1278–1290.

[85] Z. Sun, A.S. Klein, S. Radaeva, F. Hong, O. El-Assal, H. Pan, B. Jaruga, S. Batkai, S. Hoshino, Z. Tian, G. Kunos, A.M. Diehl, B. Gao, In vitro interleukin-6 treatment prevents mortality associated with fatty liver transplants in rats1 1The authors thank Tim Lee (Moor Instruments Ltd., Millwey, United Kingdom) for assistance with laser Doppler image analysis, Gastroenterology 125 (2003) 202–215.

[86] J. Williams, W. Ding, A mechanistic review of mitophagy and its role in protection against alcoholic liver disease, Biomolecules 5 (2015) 2619–2642.

[87] S. Altenhofer, P.W. Kleikers, K.A. Radermacher, P. Scheurer, H.J. Rob, P. Schiffers, H. Ho, K. Wingler, H.H. Schmidt, The NOX toolbox: validating the role of NADPH oxidases in physiology and disease, Cell. Mol. Life Sci. 69 (2012) 2327–2343.

[88] Y. Joe, M. Zheng, H.J. Kim, M.J. Uddin, S. Kim, Y. Chen, J. Park, G.J. Cho, S.W. Ryter, H.T. Chung, Cilostazol attenuates murine hepatic ischemia and reperfusion injury via heme oxygenase-dependent activation of mitochondrial biogenesis, Am J Physiol-Gastr L 309 (2015) G21–G29.

[89] J.D. McCully, S. Levitsky, N.P. Del, D.B. Cowan, Mitochondrial transplantation for therapeutic use, Clin. Transl. Med. 5 (2016) 16.

[90] H. Lin, S. Liu, H. Lai, I. Lai, Isolated mitochondria infusion mitigates ischemia-reperfusion injury of the liver in rats, Shock 39 (2013) 304–310.

[91] X. Zeng, G. Liu, W. Peng, J. He, C. Cai, W. Xiong, S. Chen, M. Yang, Z. Dong, Combined deficiency of SLAMF8 and SLAMF9 prevents endotoxin-induced liver inflammation by downregulating TLR4 expression on macrophages, Cell. Mol. Immunol. (2018), https://doi.org/10.1038/s41423-018-0191-z.

[92] A. Choumar, A. Tarhuni, P. Lettéron, F. Reyl-Desmars, N. Dauhoo, J. Damasse, N. Vadrot, P. Nahon, R. Moreau, D. Pessayre, A. Mansouri, Lipopolysaccharide-induced mitochondrial DNA depletion, Antioxid. Redox Signal. 15 (2011) 2837–2854.

[93] H.B. Suliman, M.S. Carraway, C.A. Piantadosi, Postlipopolysaccharide oxidative damage of mitochondrial DNA, Am. J. Respir. Crit. Care 167 (2003) 570–579.

[94] Z. Liu, J. Guo, H. Sun, Y. Huang, R. Zhao, X. Yang, α-Lipoic acid attenuates LPS-induced liver injury by improving mitochondrial function in association with GR mitochondrial DNA occupancy, Biochimie 116 (2015) 52–60.

[95] A. Arduini, G. Serviddio, J. Escobar, A.M. Tormos, F. Bellanti, J. Viña, M. Monsalve, J. Sastre, Mitochondrial biogenesis fails in secondary biliary cirrhosis in rats leading to mitochondrial DNA depletion and deletions, Am. J. Phys. Gastrointest. Liver 301 (2011) G119–G127.

[96] H. Koh, G.S. Park, S.M. Shin, C.E. Park, S. Kim, S.J. Han, H.Q. Pham, J.H. Shin, D.W. Lee, Mitochondrial mutations in cholestatic liver disease with biliary atresia, Sci. Rep. 8 (2018) 905.

[97] P. Rawla, H. Samant, Primary Sclerosing Cholangitis, StatPearls Publishing, Treasure Island (FL), 2019.

[98] I. Goncalves, D. Hermans, D. Chretien, P. Rustin, A. Munnich, J.M. Saudubray, F. Van Hoof, R. Reding, J. de Ville De Goyet, J.B. Otte, Mitochondrial respiratory chain defect: a new etiology for neonatal cholestasis and early liver insufficiency, J. Hepatol. 23 (1995) 290–294.

[99] M. Tiao, T. Lin, C. Liou, P. Wang, J. Chen, F. Kuo, C. Huang, Y. Chou, J. Chuang, Early transcriptional deregulation of hepatic mitochondrial biogenesis and its consequent effects on murine cholestatic liver injury, Apoptosis 14 (2009) 890–899.

[100] C. Tang, H. Lin, C.L. Feng, Q. Wu, Y.J. Zhang, P. Bie, Mitochondrial DNA damage and its effect on mitochondrial function in rats with obstructive jaundice, Mol. Med. Rep. 6 (2012) 1393–1398.

[101] C. Brechot, D. Kremsdorf, P. Soussan, P. Pineau, A. Dejean, P. Paterlini-Brechot, P. Tiollais, Hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC): molecular mechanisms and novel paradigms, Pathol. Biol. (Paris) 58 (2010) 278–287.

[102] S. Zhao, Y. Yang, J. Liu, H. Liu, N. Ge, H. Yang, H. Zhang, J. Xing, Association of mitochondrial DNA content in peripheral blood leukocyte with hepatitis B virus-related hepatocellular carcinoma in a Chinese Han population, Cancer Sci. 102 (2011) 1553–1558.

[103] T. Chen, Z. Xun, J. Lin, Y. Fu, W. Wu, X. Fu, Y. Hu, Y. Zeng, Q. Ou, Association between mitochondrial DNA content and baseline serum levels of HBsAg in chronic hepatitis B infection, J. Med. Virol. 89 (2017) 1958–1962.

[104] L. Li, H.W. Hann, S. Wan, R.S. Hann, C. Wang, Y. Lai, X. Ye, A. Evans, R.E. Myers, Z. Ye, B. Li, J. Xing, H. Yang, Cell-free circulating mitochondrial DNA content and risk of hepatocellular carcinoma in patients with chronic HBV infection, Sci. Rep.

6 (2016) 23992.
[105] T. Radosavljevic, D. Mladenovic, D. Vucevic, The role of oxidative stress in alcoholic liver injury, Med. Pregl. 62 (2009) 547–553.
[106] M. Yu, Y. Zhou, Y. Shi, L. Ning, Y. Yang, X. Wei, N. Zhang, X. Hao, R. Niu, Reduced mitochondrial DNA copy number is correlated with tumor progression and prognosis in Chinese breast cancer patients, IUBMB Life 59 (2007) 450–457.
[107] P.H. Yin, H.C. Lee, G.Y. Chau, Y.T. Wu, S.H. Li, W.Y. Lui, Y.H. Wei, T.Y. Liu, C.W. Chi, Alteration of the copy number and deletion of mitochondrial DNA in human hepatocellular carcinoma, Br. J. Cancer 90 (2004) 2390–2396.
[108] P. Vivekanandan, H. Daniel, M.M. Yeh, M. Torbenson, Mitochondrial mutations in hepatocellular carcinomas and fibrolamellar carcinomas, Mod. Pathol. 23 (2010) 790–798.
[109] C. Tang, Y. Hou, H. Wang, K. Wang, H. Xiang, X. Wan, Y. Xia, J. Li, W. Wei, S. Xu, Z. Lei, T.M. Pawlik, H. Wang, M. Wu, F. Shen, Aspartate β-hydroxylase disrupts mitochondrial DNA stability and function in hepatocellular carcinoma, Oncogenesis 6 (2017) e362-e362.
[110] M. Yu, Generation, function and diagnostic value of mitochondrial DNA copy number alterations in human cancers, Life Sci. 89 (2011) 65–71.
[111] J. Xing, M. Chen, C.G. Wood, J. Lin, M.R. Spitz, J. Ma, C.I. Amos, P.G. Shields, N.L. Benowitz, J. Gu, M. de Andrade, G.E. Swan, X. Wu, Mitochondrial DNA content: its genetic heritability and association with renal cell carcinoma, J. Natl. Cancer Inst. 100 (2008) 1104–1112.
[112] M. Nishikawa, S. Nishiguchi, S. Shiomi, A. Tamori, N. Koh, T. Takeda, S. Kubo, K. Hirohashi, H. Kinoshita, E. Sato, M. Inoue, Somatic mutation of mitochondrial DNA in cancerous and noncancerous liver tissue in individuals with hepatocellular carcinoma, Cancer Res. 61 (2001) 1843–1845.
[113] H.C. Lee, P.H. Yin, C.Y. Lu, C.W. Chi, Y.H. Wei, Increase of mitochondria and mitochondrial DNA in response to oxidative stress in human cells, Biochem. J. 348 (Pt 2) (2000) 425–432.
[114] H. Simonnet, N. Alazard, K. Pfeiffer, C. Gallou, C. Beroud, J. Demont, R. Bouvier, H. Schagger, C. Godinot, Low mitochondrial respiratory chain content correlates with tumor aggressiveness in renal cell carcinoma, Carcinogenesis 23 (2002) 759–768.
[115] N.S. Chandel, P.T. Schumacker, Cells depleted of mitochondrial DNA (rho0) yield insight into physiological mechanisms, FEBS Lett. 454 (1999) 173–176.
[116] S. Yamada, S. Nomoto, T. Fujii, T. Kaneko, S. Takeda, S. Inoue, N. Kanazumi, A. Nakao, Correlation between copy number of mitochondrial DNA and clinicopathologic parameters of hepatocellular carcinoma, Eur. J. Surg. Oncol. 32 (2006) 303–307.
[117] G. Biswas, H.K. Anandatheerthavarada, M. Zaidi, N.G. Avadhani, Mitochondria to nucleus stress signaling: a distinctive mechanism of NFkappaB/Rel activation through calcineurin-mediated inactivation of IkappaBbeta, J. Cell Biol. 161 (2003) 507–519.
[118] Y. Liu, W. Yan, S. Tohme, M. Chen, Y. Fu, D. Tian, M. Lotze, D. Tang, A. Tsung, Hypoxia induced HMGB1 and mitochondrial DNA interactions mediate tumor growth in hepatocellular carcinoma through Toll-like receptor 9, J. Hepatol. 63 (2015) 114–121.
[119] G. Waris, K.W. Huh, A. Siddiqui, Mitochondrially associated hepatitis B virus X protein constitutively activates transcription factors STAT-3 and NF-kappa B via oxidative stress, Mol. Cell. Biol. 21 (2001) 7721–7730.
[120] M. Khan, G.H. Syed, S. Kim, A. Siddiqui, Mitochondrial dynamics and viral infections: a close nexus, Biochim. Biophys. Acta (BBA) - Mol. Cell Res. 1853 (2015) 2822–2833.
[121] S. Jung, Y. Kim, C-terminal region of HBx is crucial for mitochondrial DNA damage, Cancer Lett. 331 (2013) 76–83.
[122] G. Cortopassi, E. Wang, Modelling the effects of age-related mtDNA mutation accumulation; complex I deficiency, superoxide and cell death, BBA-Mol. Basis Dis. 1271 (1995) 171–176.