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Pyroptosis in liver disease

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ABSTRACT
Pyroptosis is an inflammatory cell death that is dependent on caspase. Pyroptosis is a specific form of programmed cell death with the morphological characteristics of formation of pores on the cell membrane, cell swelling and rupture of the plasma membrane. Recent studies have demonstrated that pyroptosis plays an important role in the occurrence and development of liver diseases. Here, we focus on the mechanisms of pyroptosis, as well as the relationship between pyroptosis and liver diseases.

Keywords
Pyroptosis; Liver disease; Mechanism

INTRODUCTION
Pyroptosis is a type of programmed cell death, which is characterized by pore formation on the plasma membrane, cell swelling and plasma membrane disruption [1,2]. It is mediated by caspase and is accompanied by the release of a large number of pro-inflammatory factors. In recent years, a trend of increasing incidence and mortality was observed in liver disease. Recent studies indicate that the typical feature of hepatocyte death is not only necrosis and apoptosis, but also pyroptosis. Pyroptosis plays an important role in chronic inflammation[3,4] and is closely related to the development of various liver diseases such as liver damage[5], fatty lesions[6], inflammation and fibrosis. This article reviews the research progress of the role of pyroptosis in liver diseases and its mechanism.

PYROPTOSIS AND ITS MECHANISM

Pyroptosis, a novel caspase-1 dependent form of programmed cell death, was characterized by Cookson and Brannan. Inflammasome-dependent caspase-1 activation initiates an inflammatory response, as pro-inflammatory cytokines pro-interleukin-1β (pro-IL-1β) and pro-interleukin-18 (pro-IL-18) are made active via cleavage. Additionally, caspase-1 introduces the formation of discretely sized ion-permeable pores in the plasma membrane, which leads to water influx, cell swelling and finally cell lysis due to increased osmotic pressure. It is closely related to the inflammatory process and is the key response of the innate immune system to pathogens[7]. Pyroptosis is a programmed cell death that is strictly regulated by inflammasomes and can be divided into canonical pathway and non-canonical pathway. In canonical pathway, pattern recognition receptors (PRR) such as Nucleotide-binding oligomerization domain-like receptor protein 1 (NLRP1) and nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) can bind to pro-caspase-1 to form inflammasomes. Under the stimulation of inflammatory factors, inflammasomes can process pro-caspase-1 into mature caspase-1 to promote the maturation and release of interleukin-1β (IL-1β) and interleukin-18 (IL-18), triggering an inflammatory response[8] . In non-canonical pathway, the lipopolysaccharide in Gram-negative bacteria can first bind to caspase-4, caspase-5 (human) or caspase-11 (mouse) and then initiate the process of pyroptosis[9] (Figure
Inflammasomes are polyprotein complex assembled by PRR to recognize pathogen-associated molecular pattern or damage-associated molecular pattern, capable of activating caspase (mainly caspase-1) to induce pyroptosis. Among these known inflammatory mediators, both NLRP4 and NLRP1 contain a caspase recruitment (CARD) domain that interacts with caspase-1 CARD. NLRP3 contains a pyrin signaling domain that binds the Pyrin domain of the adaptor protein ASC, which recruits caspase-1 through CARD–CARD interactions. Absent in melanoma 2 (AIM2) contains a HIN200 domain and a Pyrin domain that recruits apoptosis-associated speck-like protein containing a CARD (ASC) and activates caspase-1. Each of these inflammasomes can activate caspase-1 to trigger pyroptosis.

The major executor during pyroptosis is gasdermin D (GSDMD), which is a generic substrate of inflammatory caspases. GSDMD acts as a pyroptosis executor via its caspase-cleaved gasdermin-N domain (GSDMD-N). It triggers pyroptosis and causes IL-1β release. Gasdermin-C domain (GSDMD-C) is hydrophilic and exerts a self-inhibiting effect by binding to the N-terminus. Meanwhile, GSDMD can also affect the release of the inflammatory cytokine IL-1β / IL-18, which plays an important role in pyroptosis.

PYROPTOSIS AND LIVER DISEASE

Pyroptosis and Liver inflammation

Pyroptosis and non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) has become the most common form of chronic liver disease, which is widely-distributed in countries like America, Europe and the Asian-Pacific region. It currently affecting 20–30 % of the general population and 75–100 % of obese individuals. Steatosis is an extremely common disorder affecting nearly 30 % of the US population, among which 25 % develop non-alcoholic steatohepatitis (NASH) with an inherent risk for progression to cirrhosis and hepatocarcinoma. Due to the lack of effective treatments, NAFLD has become a serious public health problem. Previously, the role of cell necrosis and apoptosis in NAFLD has been emphasized, but it has recently been recognized that pyroptosis
may also play an important role.

Endoplasmic reticulum stress causes activation of NLRP3 inflammasome, causing inflammation of the liver and pyroptosis. Gonzalez-Rodriguez et al. observed that NASH patients displayed more elevated endoplasmic reticulum stress markers, namely C/EBP homologous protein (CHOP) and glucose-regulated protein78 (GRP78), reinforcing the notion that enhanced endoplasmic reticulum (ER) stress within liver cells may be relevant in the progression from steatosis to NASH[15].

The NLRP3 inflammasome senses obesity-associated danger signals, including endotoxin[16], hyperglycemia and free fatty acids (FFAs), and mediates caspase-1-dependent maturation of the pro-inflammatory cytokines IL-1β and IL-18. NLRP3 inflammasome may have a deleterious role in steatosis and NASH pathogenesis. Indeed, a deficiency in caspase-1, NLRP3 or ASC in mice results in protection from high-fat diet (HFD)-induced steatosis[17]. Similarly, a deficiency in IL-1β, IL-1 receptor protects mice from methionine and choline-deficient (MCD) diet-induced steatohepatitis. NLRP3 inhibitor MCC950 was used in atherogenic diet-fed mice with NASH. We showed that NLRP3 blockade abolishes liver inflammation during the development of NASH, with beneficial effects on liver fibrogenesis[18]. Therefore, pyroptosis induced by NLRP3 inflammasome may be the main cause of cell death and inflammation in the progression of NASH.

In patients with NAFLD, GSDMD can induce the expression of pro-inflammatory cytokines (IL-1β, tumor necrosis factor-alpha (TNF-α) and monocyte chemotactic protein 1 (MCP-1)), causing activation of the nuclear factor-kappa B (NF-κB) signaling pathway and subsequent macrophage recruitment to trigger pyroptosis. This indicates an important role in the pathogenic effects of GSDMD in steatohepatitis. Xu et al. reported that protein levels of GSDMD and its pyroptosis inducing fragment GSDMD-N were increased in liver tissues of human NAFLD/NASH when compared with control samples from healthy donors. In addition, the authors demonstrated that GSDMD-N levels were correlated with the NAFLD activity score (NAS) and fibrosis[19]. In line with these results, in an experimental model of NAFLD, GSDMD -/- mice fed a MCD were protected from steatohepatitis and fibrosis, suggesting a causal role for GSDMD in NAFLD.
**Pyroptosis and Alcoholic liver disease**

Excessive alcohol drinking can result in alcoholic liver disease (ALD), including steatosis, steatohepatitis, fibrosis, cirrhosis as well as hepatocellular carcinoma, which is the leading cause of death among all chronic liver diseases. Alcoholic hepatitis (AH) with liver cirrhosis accounts for approximately 50% of the mortality in Western countries[20]. It is an acute-on-chronic liver disease characterized by hepatic steatosis, ballooned hepatocytes with Mallory-Denk bodies, neutrophilic infiltration, and liver fibrosis or cirrhosis and clinically presented with decompensated liver functions with hypoalbuminemia, cholestasis, coagulopathy, and portal hypertension. Patients with severe AH develop sepsis, liver failure, and multiorgan dysfunction, leading to rapid progression to death. In the past few years, research has disclosed several key mechanisms of alcohol-mediated hepatocellular damage, including cytochrome P450 2E1-mediated oxidant stress, mitochondrial glutathione stress, endoplasmic reticulum stress, malondialdehyde acetaldehyde protein adducts, centrilobular hypoxia, suppressed autophagy, and lysosomal dysfunction[20-22]. Recent studies have shown that pyroptosis is also involved.

The microRNAs (miRNAs) regulate diverse biological functions in the liver. Alteration in the levels of specific miRNAs promotes the incidence and progression of liver diseases. In ALD patients, alcohol decreases miR-148a expression in hepatocytes through forkhead box 01 (FoxO1), facilitating thioredoxin-interacting Protein (TXNIP) overexpression and NLRP3 inflammasome activation, which induces hepatocyte pyroptosis. In a four-week ethanol feeding, mice deficient in NLRP3 were protected from ethanol-induced liver injury, inflammation and steatosis[23]. Particularly important was the absence of inflammasome activation after ethanol administration in these NLRP3-KO mice. It suggests that NLRP3 plays a critical role in inflammasome activation in alcoholic liver disease. In addition, certain metabolites such as reactive oxygen species after alcohol intake, can activate the NLRP3 inflammasome pathway[24].

GSDMD-induced pyroptosis plays an important role in the pathogenesis of AH. In ALD, especially AH patients, pyroptosis is a form of hepatocyte death and its
mechanism is associated with polymorphonuclear cell inflammation and bacteremia. Increased levels of pro-GSDMD in hepatic macrophages and hepatocytes isolated from the AH model suggest a programmed death pathway for pyroptosis. Khanova et al. identified the noncanonical inflammasome caspase, caspase -11/4, which is upregulated in both mouse and patient AH livers. Caspase -11/4 cleaves and activates GSDMD to induce programmed lytic cell death, called pyroptosis[23]. This indicates that pyroptosis has a certain role in AH mice and patients.

**Pyroptosis and Autoimmune hepatitis**

Autoimmune hepatitis (AIH) is a progressive immune mediated liver disease of unknown etiology, characterized by immune-mediated destruction of hepatocytes and massive production of cytokines. IL-1β is a pro-inflammatory cytokine involved in pyroptosis and is closely related to a variety of autoimmune diseases. In ConA-treated mice, pathogenic elevated NLRP3, caspase-1 and IL-1β levels, as well as an inflammatory cell death indicate pyroptosis predominantly occurred in the livers. NLRP3−/− and caspase-1−/− mice were broadly protected from hepatitis as determined by decreased histological liver injury. In vivo intervention with recombinant human interleukin-1 receptor antagonist (rhIL-1Ra) strongly suppressed ConA-induced hepatitis by decreasing TNF-α and IL-17 secretion, and inflammatory cell infiltration into livers, which shed light on the development of promising therapeutic strategies for AIH by blocking NLRP3 inflammasome and IL-1β[26]. Chen et al. reported that long-term high-fat diet feeding AIH mice can make excessive lipid deposition in the body and then activate inflammasome. The excessive activation of inflammasome makes the inflammatory-immune system activation increased, resulting in increased pyroptosis, leading to liver injury aggravated[27].

**Pyroptosis and HCV Infection**

Hepatitis C virus (HCV) infection continues to be one of the major health challenges in the modern world. An estimated 185 million people are infected globally, which constitutes approximately 3% of the world’s population[28]. In untreated individuals, HCV infection progresses to chronicity in 70-85% of new cases, putting those
chronically infected patients at risk of developing severe liver disease. The mechanisms by which these HCV-associated liver diseases develop are poorly understood, but evidence suggests that induction of programmed cell death in the HCV-infected liver plays a critical role in this pathogenic process. The pro-inflammatory nature of pyroptosis suggests that this form of cell death may be related to the pathogenesis of HCV infection.

HCV can activate inflammasome complex in monocytes and macrophages and induce IL-1β production. NLRP3, ASC and caspase -1 are involved in HCV-mediated IL-1β activation in human THP-1 bone marrow cells[^29]. These data indicate that inflammasome activation and pyroptosis exist in the liver of HCV infected persons. To further verify this point of view, Kofahi et al. Explained the view from the following aspects: 1) Lactate dehydrogenase (LDH) is released extracellularly only in the event of cell lysis. LDH release is one of the predominant characteristics of cells undergoing pyroptosis. 2) We can observe the cells cracked due to pyroptosis after examining the images of cells representing HCV infection. 3) We stained virus-infected cells with FAM-YVAD-FMK FLICA reagent, which specifically recognizes the active form of caspase-1. The result showed that HCV infection caused a significant increase in the proportion of active caspase-1-positive cells. 4) Treating the HCV-infected or control cells with the caspase-1-specific inhibitor Z-WEHD-FMK, we found inhibiting caspase-1 rescued more than half of the cells undergoing HCV-induced programmed cell death, confirming that pyroptosis is induced in the HCV-infected cell population[^30].

**Pyroptosis and Acute liver failure (ALF)**

ALF is often accompanied by severe hepatocyte dysfunction, with an overall mortality rate of 90%. Hepatocyte death can be divided into necrosis, programmed death (apoptosis), and pyroptosis, which is discovered as a new pattern of death. Acute liver failure results in high stress levels of a large number of antigens, immune cells, inflammatory factors and chemokines.

Liver failure is known to be closely related to pyroptosis. Patients with ALF are often
accompanied by severe intestinal microecological imbalances and impaired intestinal mucosal barrier function, leading to intestinal bacterial translocation, which induces hepatocytes pyroptosis. Excessive hepatocytes pyroptosis produces more inflammatory mediators, which forms a positive feedback loop. The inflammatory mediators cause aggravate hepatocytes necrosis. Hepatic endoplasmic reticulum stress could activate NLRP3 inflammasome, leading to inflammation-mediated liver damage and pyroptosis. Early studies demonstrated that mesenchymal stem cells can release anti-inflammatory factor IL-10, reducing the level of NLRP3-caspase-1 inflammasome, thereby playing an important role in the anti-pyroptosis treatment of ALF. Recent studies have also shown that both the caspase family inhibitor and the caspase-1 inhibitor can increase protection against liver damage in the ALT model induced by ConA.

**Pyroptosis and Liver fibrosis**

Liver fibrosis is an intrinsic response to chronic persistent liver injury. For the reason of no clinical symptoms is found in early liver fibrosis, liver biopsy is still the mainly useful tool for the clinical diagnosis of early liver fibrosis. In most cases, liver fibrosis can progress to cirrhosis, and normal liver parenchyma is replaced by scar tissue, leading to complications such as portal hypertension, liver failure, and liver cancer. Of note, pyroptosis is involved in this process. Continuous activation of NLRP3 inflammasome in mice clearly results in severe liver inflammation, fibrosis, and hepatocyte pyroptosis. P2X7 activation is a trigger for NLRP3 inflammasome. Treatment of CCl4-induced liver fibrosis in mice with P2X7-specific inhibitor (A438079) could significantly reduce collagen formation in the liver and down-regulate the expression of α-smooth muscle actin and transforming growth factor (TGF)-β1. In this study, it has also been confirmed that inhibition of NLRP3 inflammasomes could reduce liver fibrosis in mouse models. Therefore, pyroptosis caused by NLRP3 inflammasome activation may have a direct effect on liver fibrosis. Lacking any of the three components of NLRP3 inflammasome (caspase-1, NLRP3, ASC) in mice has also shown protection against liver fibrosis. In the Lieber-DeCarli ALD model, knockout of mouse caspase-1 significantly reduces liver fibrosis and liver
histological fibrosis area, indicating that caspase-1 plays a role in liver fibrosis mice. Of the various cytokines involved in liver fibrosis, IL-1β is the most widely explored. IL-1β promotes proliferation and differentiation of HSC (hepatic stellate cells) with a significant increase in the level of fibrin. Knockout of mouse IL-1β or IL-1 receptor type I (IL-1RI) gene can prevent liver fibrosis progression, as well as in NASH and AL models. These findings clearly suggest that IL-1 mediated pyroptosis can promote transformation of liver injury to liver fibrosis.

**Pyroptosis and Liver cancer**

Liver cancer is the leading cause of death in patients with liver disease, and liver cancer-related mortality is on the rise. The occurrence of liver cancer is a complex process involving multiple genes, multiple pathways and multiple steps. The top priority is to clarify the molecular mechanisms of liver cancer and to develop effective treatment program. Pyroptosis can induce cancer cell death. Therefore, activation of pyroptosis may become a potential direction for the treatment of liver cancer.

AIM2 plays a critical role in the pyroptosis of hepatocellular carcinoma (HCC) by regulating the mammalian target of rapamycin (mTOR) signaling pathway. An excessive activation of mTOR signaling pathway results in excessive proliferation of cells and cell cycle disorder, and involvement in HCC. Several studies have shown that mTOR molecules are overexpressed in 67% of HCC tumor tissues, and mTOR signaling pathways are overactivated in 40% to 45% of liver cancer patients. Blocking the conduction of mTOR signaling pathway can effectively inhibit the synthesis of hepatocarcinoma cell proteins and proliferation of cancer cells, induce pyroptosis of liver cancer cells and inhibit angiogenesis. Ma et al. reported that AIM2 exerts an antitumor effect in liver cancer by activating AIM2-ASC-caspase-1 inflammasome and inhibiting mTOR signaling pathway.

Recent studies have found that caspase-1 immunostaining is significantly reduced in liver cancer tissues compared to adjacent normal tissues. The mRNA and protein levels of caspase-1 are down-regulated in liver cancer tissues, indicating that the loss
of caspase-1 expression is involved in the pathogenesis of HCC, namely, pyroptosis may be inhibited in patients with HCC. In response to this mechanism, some scholars believe that berberine has an inhibitory effect on liver cancer. Chu et al. showed that berberine can upregulate the mRNA and protein expression levels of caspase-1 in HepG2 cells, inhibit the viability, migration and invasion of HepG2 cells, and these effects can be weakened by caspase-1 inhibitors. That is, the process of pyroptosis is inhibited in HCC, and berberine can reactivate the program by increasing caspase-1 in cancer cells.

It is worth noting that the inflammasome in pyroptosis pathway may be a "double-edged sword" for the growth of liver cancer cells. On the one hand, inflammasome can suppress the proliferation of tumor cells by inducing pyroptosis. On the other hand, the cumulative effect of inflammasome can also form a microenvironment which is suitable for tumor cells and promote tumor growth. Studies have found that in hepatoma cells, hypoxia-induced caspase-1 activity and subsequent production of a variety of inflammatory factors can promote cancer cell invasion and metastasis.

Pyroptosis was discovered as a programmed cell death that relies on caspase-1 in recent years. Under the action of stimulating factors, NLRP3, caspase-1, GSDMD, AIM2 and so on participate in the occurrence and progress of liver diseases. However, whether inflammasome, caspase-1 and inflammatory factors can be used as predictors of liver disease, and whether new drugs can be developed through pyroptosis research are issues that should be paid attention to in the future. With the deepening of research, pyroptosis as an emerging way of cell death, will become a new target for the diagnosis and treatment of liver diseases.

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**Abbreviations**

AIM2, absent in melanoma 2
ASC, apoptosis-associated speck-like protein containing a CARD
ALD, alcoholic liver disease
AH, alcoholic hepatitis
AIH, autoimmune hepatitis
CHOP, C/EBP homologous protein
ER, endoplasmic reticulum
FFAs, free fatty acids
FoxO1, forkhead box 01
GSDMD, gasdermin D
GSDMD-N, gasdermin-N domain
GSDMD-C, gasdermin-C domain
GRP78, glucose-regulated protein78
HCV, hepatitis C virus
HCC, hepatocellular carcinoma
HFD, high-fat diet
LDH, lactate dehydrogenase
mTOR, mammalian target of rapamycin
MCD, methionine and choline deficient
MCP-1, monocyte chemotactic protein 1
NAFLD, non-alcoholic fatty liver disease
NASH, non-alcoholic steatohepatitis
NAS, nactivity score
NF-kB, nuclear factor-kappa B
TXNIP, thioredoxin-interacting Protein
TNF-α, tumor necrosis factor-alpha
TGF, transforming growth factor
rhIL-1Ra, recombinant human interleukin-1 receptor antagonist
Figure 1. In canonical pathway, inflammasomes can process pro-caspase-1 into mature caspase-1 to promote the maturation and release of interleukin-1β (IL-1β) and interleukin-18 (IL-18), triggering an inflammatory response. In non-canonical pathway, the lipopolysaccharide in Gram-negative bacteria can first bind to caspase-4, caspase-5 (human) or caspase-11 (mouse) and then initiate the process of pyroptosis, which has a higher similarity to the canonical pathway.