

Role of ER Stress in Inflammasome Activation and Non-Alcoholic Fatty Liver Disease Progression

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Abstract

With the prevalence of obesity increasing worldwide, nonalcoholic fatty liver disease (NAFLD) has become the most common form of chronic liver disease. Despite this, knowledge about the molecular mechanisms involved in NAFLD progression is still limited. Recent findings have shown that endoplasmic reticulum (ER) stress links inflammation and hepatocyte death, inherent to the transition from simple steatosis to nonalcoholic steatohepatitis (NASH). Here, we emphasize the central role of the ER stress response and its crosstalk with the inflammasome. We hope to provide new insight on the identification of ER stress-dependent pathways that contribute substantially to chronic liver disease progression as important triggers of cell death and inflammation, and therefore may represent potential therapeutic strategies.

Keywords: Liver; Inflammation; Inflammasome; Unfolded protein response; Apoptosis

Abbreviations

NAFLD: Nonalcoholic Fatty Liver Disease; NASH: Nonalcoholic Steatohepatitis; ER: Endoplasmic Reticulum; NLRP: NOD-like Receptor family, Pyrin domain containing; IL: Interleukin; IRE1α: Inositol-Requiring Enzyme 1; PERK: PKR-like ER Kinase; UPR: Unfolded Protein Response; CHOP: C/EBP Homologous Protein; LPS: Lipopolysaccharide (Endotoxin); TXNIP: Thioredoxin-Interacting Protein; ROS: Reactive Oxygen Species; TUDCA: Taurooursodeoxycholic Acid

Commentary

Nonalcoholic fatty liver disease (NAFLD) encompasses a broad spectrum of liver conditions ranging from simple steatosis to steatohepatitis, cirrhosis and finally even hepatocellular carcinoma. Little is known about the natural history or prognostic significance of NAFLD. While hepatic steatosis is characterized by benign triglyceride accumulation in hepatocytes, nonalcoholic steatohepatitis (NASH) is defined as a combination of triglyceride accumulation, hepatocyte death, inflammation and fibrosis. Therefore, it is important to elucidate the molecular etiology of NASH, a multifactorial and progressive form of NAFLD, to propose new therapeutic avenues [1-3].

Endoplasmic reticulum (ER) stress has been linked to obesity, type 2 diabetes and the pathogenesis of NAFLD. Obesity results in liver ER stress, insulin resistance and hepatic steatosis in obese mice and humans [4-7]. Although it is increasingly evident that the ER stress pathway is also an important trigger of hepatocyte death (apoptotic and potentially necrotic death) [8,9], and hence a potential accelerator of inflammation leading to steatohepatitis, its link to inflammasomes in hepatic disorders has just begun to emerge.

In particular, the NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome is a multi-protein complex that ignites inflammation [10] and insulin resistance [11] in response to metabolic danger signals. NLRP3 activation induces the recruitment and assembly with its adaptor protein ASC and the procaspases-1 and -11. Formation of this inflammasome complex leads to the autocatalytic activation of the cysteine proteases caspase-1 and caspase-11 and maturation of the proinflammatory cytokines interleukin (IL)-1β and IL-18. Sustained NLRP3 inflammasome activation has also been shown to trigger pyroptosis, a form of programmed cell death, in hepatocytes [12].

Previous studies have shown that human liver biopsies obtained from NASH patients present significantly increased inflammasome (NLRP3, ASC, Caspase-1 [13] or NLRP3, IL-1β, IL-18 [14]) or ER stress [15] gene expression. We, Lebeaupin et al., recently provided further evidence that NLRP3 inflammasome and ER stress component transcripts correlate with liver injury severity (NAFLD activity score and alanine and aspartate transaminase levels) in NASH patients [16]. With our study, we were the first to show that ER stress and inflammasome markers were also positively correlated, suggesting that ER stress and the inflammasome signalling pathways cooperate and exacerbate steatohepatitis progression. We demonstrated that ER stress leads to proinflammatory, pyroptotic death through NLRP3 inflammasome activation specifically in hepatocytes. Using an experimental mouse model of liver disease and primary hepatocytes, we demonstrated that the overwhelmed inositol-requiring enzyme 1 (IRE1α) and PKR-like ER kinase (PERK) branches of the unfolded protein response (UPR) converge on C/EBP homologous protein (CHOP) activation, leading to NLRP3 inflammasome activation through the increased activations of caspase-1 and caspase-11, triggering hepatic pyroptosis and apoptosis [16].

Our results support a deleterious role of CHOP, and suggest that CHOP may be the critical link between inflammasome activation and hepatocyte death in the progression from steatosis to NASH. In line

with this hypothesis, a previous study showed that lipopolysaccharide (LPS)-induced inflammation activated the ER stress-CHOP pathway that was crucial to caspase-11 activation. This then induced caspase-1 activation and led to the maturation and activation of IL-1 β [17]. Consistent with a connection through CHOP, a study using an ER stress-driven steatohepatitis mouse model has shown that a deficiency in IL-1 α leads to attenuated ER stress-induced hepatocyte apoptosis and inflammation through decreased CHOP expression, thus alleviating NASH. This study showed that ER stress induced the expression of IL-1 α , and further confirmed IL-1 β secretion in both macrophages and hepatocytes. The secretion of the lesser studied IL-1 α proinflammatory cytokine has also been suggested to be caspase-1 and inflammasome-dependent [18]. However, the molecular mechanisms involved in the direct activation of the NLRP3 inflammasome through CHOP remain to be demonstrated.

Other links between ER stress and NLRP3 inflammasome activation have been made through the thioredoxin-interacting protein (TXNIP), known to increase concentrations of mitochondrial reactive oxygen species (ROS) and recruit NLRP3, subsequently leading to procaspase-1 cleavage and IL-1 β secretion [19,20]. More specifically, these studies respectively showed that the IRE1 α -dependent decay of the microRNA mir17 [19] and the PERK mediator ATF5 [20] upregulated the levels of TXNIP under irremediable ER stress conditions promoting programmed cell death of pancreatic β cells. In line with this data, a recent study also emphasized that ER stress modulates inflammatory responses by showing that during a bacterial infection, IRE1 α acts through TXNIP to induce ROS-dependent NLRP3 activation, promoting mitochondrial damage via caspase-2 and BH3-only protein Bid [21]. The function of TXNIP in inflammasome activation in chronic liver diseases is quite limited. One study reported a significant increase in TXNIP expression in NAFLD patients. They also went on to show that TXNIP-deficient mice fed a high fat diet were protected from hepatic steatosis development [22]. Nevertheless, the function of TXNIP in inflammasome activation in chronic liver diseases needs to be more thoroughly explored.

Another NLR protein, NLRP1 has also been shown to participate in pyroptosis [23] and IL-1 β secretion [24], but its involvement in UPR signalling was not reported. Recently, both the IRE1 α and PERK branches were found to stimulate NLRP1 gene transcription through ATF4, providing further evidence that links ER stress with inflammasome activation [25].

Our recent results are opening up translational implications for the biological knowledge, and eventually clinical treatment, of chronic liver diseases. We indeed showed that a treatment with tauroursodeoxycholic acid (TUDCA), a hydrophilic bile acid, in obese mice challenged with LPS dramatically reduced NLRP3 inflammasome activation and protected against liver injury and hepatocyte death, improving the NASH-pathological features [16]. In the past decade, many reports have shown that ER stress can be alleviated by chemical compounds, such as with the chemical chaperones 4-phenyl butyric acid and TUDCA. Indeed, Hotamisligil et al. showed that the treatment of obese and diabetic mice with these compounds resulted in the resolution of hepatic steatosis and enhancement of insulin action in liver, muscle, and adipose tissues, suggesting its potential application in the case of the metabolic syndrome [26].

More specifically, Lerner et al. showed that a small-molecule called STF-083010 that covalently inhibits IRE1 α endoribonuclease activity, effectively abrogated secretion of IL-1 β [19]. Also targeting an IRE1 α -mediated pathway, a recent study using a cell-permeable-specific

inhibitor of GSK-3 β , called SB216763, resulted in decreased transcription, and consequently the secretion, of IL-1 β [27]. It would therefore be interesting to further explore the mechanisms of these novel small-molecule inhibitors in the liver to test their potential therapeutic effects on NASH progression.

It was reported that the inflammasome and IL-1 signalling were required for the development of alcohol-induced inflammation, steatosis, liver damage and fibrosis, and that the human IL-1 receptor antagonist anakinra ameliorated inflammasome-dependent alcoholic steatohepatitis in mice [28]. A recent study also supported a key role for IL-1 signalling in the pathogenesis of excessive inflammation by showing that anakinra prevented septic shock and improved survival in LPS-challenged mice [29].

In light of these results, combining therapeutic strategies through ER stress inhibition and inflammasome-response suppression could be an attractive strategy in restraining NASH development.

Concluding Remarks

Because the ER stress response is a critical mediator of inflammation, apoptosis and insulin resistance, it could play a central role in the progression from steatosis to NASH, and more advanced stages of the disease. With our results, we also suggest that targeting ER-dependent inflammation and cell death pathways may represent a novel approach to the treatment of chronic liver diseases.

There are of course major challenges associated with the translation of findings from animal models of obesity and liver disease to human NASH. Therefore, the potential clinical relevance of this ER stress-inflammasome pathway must be further demonstrated. Consequently, studies that aim to understand and ultimately prevent the progression from steatosis to NASH through this signalling mechanism may have real therapeutic promise for the treatment of chronic liver diseases, but also for the treatment of diseases in which extensive cell death may cause organ failure and expose organisms to further dangers.

Conflict of Interest

The authors declared no conflict of interest.

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