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Pyroptosis, a Novel Player for Alcoholic Hepatitis?

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Alcoholic hepatitis (AH) is a clinical syndrome of jaundice and liver failure that develops over decades of excessive alcohol consumption, which represents 0.2% of all hospital admissions in US. (1) Patients with a severe episode of AH have a potential for 30–40% one month mortality. Recommended treatments for AH include corticosteroids, nutritional supplements and pentoxifylline. However, these treatments only provide limited survival benefit. (2) One of the main obstacles to develop effective therapeutic drugs to treat AH is due to the lack of understanding of the molecular mechanisms involved in the pathogenesis of AH. Difficulties to reproduce the histology and clinical features of AH in animal models play a major limiting step in advancing our knowledge on the pathogenesis of AH. Recently, Dr. Hidekazu Tsukamoto's group developed a hybrid feeding mouse model, which combined *ad libitum* Western diet feeding with intragastric ethanol diet, two risk factors of AH. This hybrid feeding mouse model can lead to severe chronic alcoholic steatohepatitis (ASH) in mice. When weekly binge was added to this model without changing the total alcohol intake, key histological and clinical changes of AH were reproduced. (3)

Hepatocyte death after alcohol consumption is known to contribute to the pathogenesis of alcoholic liver disease (ALD). Several types of cell death including apoptosis, necrosis, or necroptosis have been observed in cultured hepatocytes and livers of rodents exposed to alcohol as well as in livers of ALD patients. (4, 5) In this issue of *Hepatology*, Khanova et al. provided perspectives of a novel cell death named pyroptosis in the pathogenesis of AH. (6) Pyroptosis is a type of programmed necrosis that was long thought to depend on the activation of caspase-1, a pro-inflammatory caspase that is activated by inflammasome complexes. (7) Pyroptosis has been considered as an important innate immunity response to certain bacterial insults. Pyroptosis is associated with cell swelling and disruption of plasma membrane due to the formation of pores on the plasma membrane. In addition to caspase-1, caspase-11 (mouse) or caspase-4/5 (human) can sense intracellular lipopolysaccharide (LPS)

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to trigger pyroptosis. (7) Mechanistically, activated caspase-11 or caspase-4/5 cleaves gasdermin D (GSDMD) within its linking loop to release its autoinhibition on its GSDMD-N domain. The cleaved GSDMD-N domain binds to phosphoinositides on the plasma membrane and lyses it to cause cell death. (7) GSDMD is expressed in many other tissues and cell types, but it is predominantly expressed in the gastrointestinal tract and immune cells. (8) GSDMD belongs to the gasdermin family proteins that have more than 5 different members in human. All of them share similar structures with the most conserved gasdermin-N domain and the gasdermin-C domain. All the gasdermin proteins have similar pore-forming activity and thus pyroptosis is now considered as gasdermin-mediated program necrosis.(7)

In searching of the molecular mediators for the pathogenesis AH, Khanova et al. first performed a global unbiased RNA-seq and proteomic analyses in their newly established hybrid feeding mouse AH model and AH patients. (6) Through ontology and pathway analysis, a group of genes involved in the pathway activated by bacterial infection were consistently identified from the mouse AH model and human AH liver samples. Immunoblot and qPCR analyses further confirmed the upregulation of caspase-11 and GSDMD in the mouse AH model. Moreover, both the cleaved form and activities of caspase-11 significantly elevated in AH mouse livers but not in chronic alcoholic steatohepatitis (ASH) mouse livers. Surprisingly, Khanova et al found that caspase-1, the key player in the canonical inflammasome pathway, did not change in this AH mouse model. Consistent with the lack of caspase-1 activation, serum IL-1 β levels also tended to be lower in AH mice. Khanova et al further detected the mature GSDMD forms from the isolated hepatocytes and hepatic macrophages of the AH mouse model, suggesting the activation of GSDMD and a possible involvement of pyroptosis in both macrophages and hepatocytes in the pathogenesis of AH in mice. More importantly, Khanova et al found that levels of cleaved caspase-4 and membrane translocation of mature GSDMD were also increased in human AH liver samples but not in healthy human livers.(6) These data strongly support an association of caspase-11/4-GSDMD pathway and pyroptosis in mouse and human AH.

To determine the causal role of caspase-11/4-GSDMD-mediated pyroptosis in the pathogenesis of AH, Khanova et al elegantly utilized two genetic approaches to modulate this pathway. In the first approach, Khanova et al compared the liver pathogenesis of the caspase-11 knockout (KO) mice with their matched wild type mice in the AH model. They found that the activation of GSDMD, rate of hepatocellular cell death and liver bacterial load in experimental AH was blunted in capase-11 KO mice compared with the wild type mice. However, no significant changes were found in the histology grading for steatosis and inflammation as well as the serum ALT and AST levels between capase-11 KO mice and their matched wild type mice. It should be noted that caspase-11 KO mice are also defective for caspase-1. Because Khanova et al found that caspase-1 was not active in AH mice, thus the phenotypes observed in the caspase-11 KO mice might be due primarily to the lack of caspase-11. In a second approach, Khanova et al overexpressed the N-terminal active form of GSDMD into the mouse hepatocytes *in vivo* using an adeno-associated virus (AAV) system followed by AH model. GSDMD was driven by a Ttr (transthyretin) promoter and thus GSDMD was presumably only expressed in hepatocytes but not in other hepatic non-parenchymal cells. Compared to the control mice that received the AAV-Ttr-eGFP, mice with

overexpression of GSDMD in hepatocytes had significantly increased hepatocyte death and hepatic PMN infiltration. Similar to the caspase-11 KO mice, liver histology such as parameters of macro- or micro-vesicular steatosis were not different between the mice received AAV-Ttr-eGFP and AAV-Ttr-GSDMD after AH model. Moreover, the serum ALT and AST was only slightly elevated in the mice received AAV-Ttr-GSDMD compared to the mice received AAV-Ttr-eGFP. (6) Together, these data from the genetic modulation of caspase-11 and GSDMD support a role of caspase-11-GSDMD in contributing to the death of hepatocytes and infiltration of PMN but is dispensable for hepatic steatosis during the pathogenesis of AH.

In addition to the key finding of activation of Caspase-11/4-GSDMD pathway in AH, Khanova et al. also found that the elevated levels of serum interleukin-18 (IL-18) were associated with increased liver bacterial load in mouse AH model. (6) IL-18 is a proinflammatory cytokine that belongs to the IL-1 cytokine family. Although IL-18 is initially identified as interferon- γ -inducing factor, IL-18 also acts as an anti-bacterial cytokine and plays a pivotal role in defense against bacterial pathogens. (9) Indeed, Khanova et al found that IL-18 KO mice displayed increased liver bacterial load and GSDMD-mediated pyroptosis of hepatocytes in AH mouse model. (6) These results are consisted with the hypothesis that endotoxin induces non-canonical inflammasome caspase11/4-GSDMD-pyroptosis pathway in AH.

In conclusion, this current study highlights a new role of pyroptosis in AH. While this study has largely expanded our knowledge to further understand the pathogenesis of ALD, many questions remain unanswered. As discussed above, GSDMD mainly expresses in immune cells and gastrointestinal tract, it is likely that its expression levels in hepatocytes are lower. Therefore, it is possible that the pyroptotic death of macrophages may contribute more to the pathogenesis of AH than the pyroptotic death of hepatocytes. Future work to use tissue-specific GSDMD KO mice in immune cells vs hepatocytes will be helpful to define the role of pyroptosis in immune cells vs hepatocytes in the pathogenesis of AH. Finally, small molecule GSDMD inhibitors are urgently needed to be developed, perhaps in combination with inhibitors for other modes of cell death, to further explore the translational potential of targeting cell death for treating AH.

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Abbreviations

ALD	alcoholic liver disease
AH	alcoholic hepatitis
ALT	alanine aminotransferase
ASH	alcoholic steatohepatitis
AST	aspartate transaminase

GSDMD	gasdermin D
IL-1	interleukin-1
IL-18	interleukin-18
LPS	lipopolysaccharide

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