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Mechanism of action of the nucleotide-binding oligomerization domain-like receptor protein 3 inflammasome and its regulation in liver injury

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Abstract

Nucleotide-binding oligomerization domain (NOD)-like receptor protein 3 (NLRP3) is a cytosolic pattern recognition receptor that recognizes multiple pathogen-associated molecular patterns and damage-associated molecular patterns. It is a cytoplasmic immune factor that responds to cellular stress signals, and it is usually activated after infection or inflammation, forming an NLRP3 inflammasome to protect the body. Aberrant NLRP3 inflammasome activation is reportedly associated with some inflammatory diseases and metabolic diseases. Recently, there have been mounting indications that NLRP3 inflammasomes play an important role in liver injuries caused by a variety of diseases, specifically hepatic ischemia/reperfusion injury, hepatitis, and liver failure. Herein, we summarize new research pertaining to NLRP3 inflammasomes in hepatic injury, hepatitis, and liver failure. The review addresses the potential mechanisms of action of the NLRP3 inflammasome, and its regulation in these liver diseases.

Keywords: Nucleotide-binding oligomerization domain-like receptor protein 3; Liver injury; Hepatic I/R injury; Non-alcoholic steatohepatitis; Acute liver failure

Introduction

Inflammation is one of the body's self-defense mechanisms, and it depends on the innate immune system. When stimulated by potentially harmful substances such as pathogens and dead cells, pattern recognition receptors (PRRs) participate in the development of inflammation, and are involved in the recognition of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs).[1] Inflammasomes were first proposed by Martinon *et al*[2] in 2002. They reported that nucleotide-binding oligomerization domain (NOD)-like receptor protein 1 (NLRP1) could combine with caspase-recruitment domain (CARD) and pro-caspase-1, forming a complex that played a key role in the activation of proinflammatory caspases. They named this protein complex the inflammasome, and suggested that it links pro-interleukin-1β (pro-IL-1β) to its upstream activator. Diverse families of PRRs have now been identified, including toll-like receptors (TLRs), C-type lectin receptors (CLRs), NOD-like receptors (NLRs), absent in melanoma 2 (AIM2)-like receptors (ALRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), and pyrin.[3,4]

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NLRP3 is the NLR that has been studied the most. Agostini *et al*[5] demonstrated that NLRP3 activates caspase-1 by forming an inflammasome with the CARD-containing protein Cardinal and apoptosis-associated speck-like protein containing a CARD (ASC). NLRP3 inflammasomes are composed of NLRP3, ASC, and pro-caspase-1. NLRP3 is a PRR, and it contains an amino-terminal pyrin domain (PYD), a central nucleotide-binding and oligomerization (NACHT) domain, and a C-terminal leucine-rich repeat (LRR) domain. The LRR domain can recognize DAMPs and PAMPs. The NACHT domain acts as a deoxyribonucleoside triphosphate (DNTP) enzyme and has ATPase activity. In the absence of immune activators such as PAMPs and DAMPs, the NACHT domain binds to the LRR domain, causing NLRP3 to enter a state of self-inhibition. When stimulated by activators, NLRP3 exposes its NACHT domain, and the PYD of NLRP3 can interact with the PYD of ASC.[6] ASC also recruits pro-caspase-1

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through CARD–CARD interactions, to complete assembly of the NLRP3 inflammasome (NLRP3-ASC-caspase-1 protein complex). After activation of the NLRP3 inflammasome, caspase-1 converts downstream substrates such as pro-IL-1β and pro-interleukin-18 (pro-IL-18) to their mature forms. Caspase-1 also cleaves gasdermin D (GSDMD) and releases its N-terminal domain, which is transferred to the cell membrane and forms pores, triggering a lytic and proinflammatory form of cell death called pyroptosis.[7,8]

NLRP3 inflammasome activation can produce an inflammatory response to protect the body, but excessive NLRP3 inflammasome activation can lead to a variety of diseases. NLRP3 inflammasomes are evidently involved in liver injuries caused by many diseases. Non-alcoholic fatty liver disease (NAFLD) affects a quarter of the global adult population and is the second-leading cause of end-stage liver disease and liver transplantation in Europe and America, after alcohol-associated liver disease. Non-alcoholic steatohepatitis (NASH) plays a significant role in the progress of NAFLD.[9] In developing countries, hepatitis viruses are the leading cause of liver disease, as well as acute liver failure (ALF).[10] At the end stage of liver disease, liver transplantation is currently the most effective treatment, but hepatic ischemia/reperfusion (I/R) injury is an inevitable problem. This review summarizes the activation mechanism of NLRP3 inflammasomes, and the role and regulation of NLRP3 inflammasomes in hepatic I/R injury, different kinds of hepatitis, and liver failure, in order to provide new insights into the mechanism and treatment of liver injury.

Mechanism of NLRP3 Inflammasome Activation

NLRP3 inflammasomes can be activated via three distinct pathways: the canonical or classical pathway, the non-canonical pathway, and the alternative pathway.[11]

Canonical or classical pathway

Classical NLRP3 inflammasome activation requires two steps, priming and activation [Figure 1]. The first priming signal provided by microbial components or endogenous cytokines activates nuclear factor kappa B (NF-κB) and induces the expression of NLRP3, pro-IL-1β, and pro-IL-18. The second activation signal comes from extracellular adenosine triphosphate (ATP), pore-forming toxins, or particulate matter. NLRP3 has not been observed to interact directly with any of these agonists, and due to their biochemical differences, it is suspected that they induce a common form of cell signaling. Multiple molecular and cellular signaling events induced by NLRP3 stimuli, including ionic flux (K^+ efflux, Cl^- efflux, Na^+ influx, and Ca^{2+} mobilization), mitochondrial dysfunction, endoplasmic reticulum (ER) stress, production of reactive oxygen species (ROS) and mitochondrial DNA (mtDNA), lysosomal damage, and trans-Golgi decomposition, have been shown to activate NLRP3 inflammasomes. K^+ efflux is reportedly a common upstream signal required for NLRP3 inflammasome activation.[12] However, NLRP3 inflammasome activation induced by *Staphylococcus aureus* cell wall peptidoglycan, imiquimod, and CL097 (a TLR7/8 agonist) is K^+ efflux-independent.[13,14] The alternative NLRP3 inflammasome activation

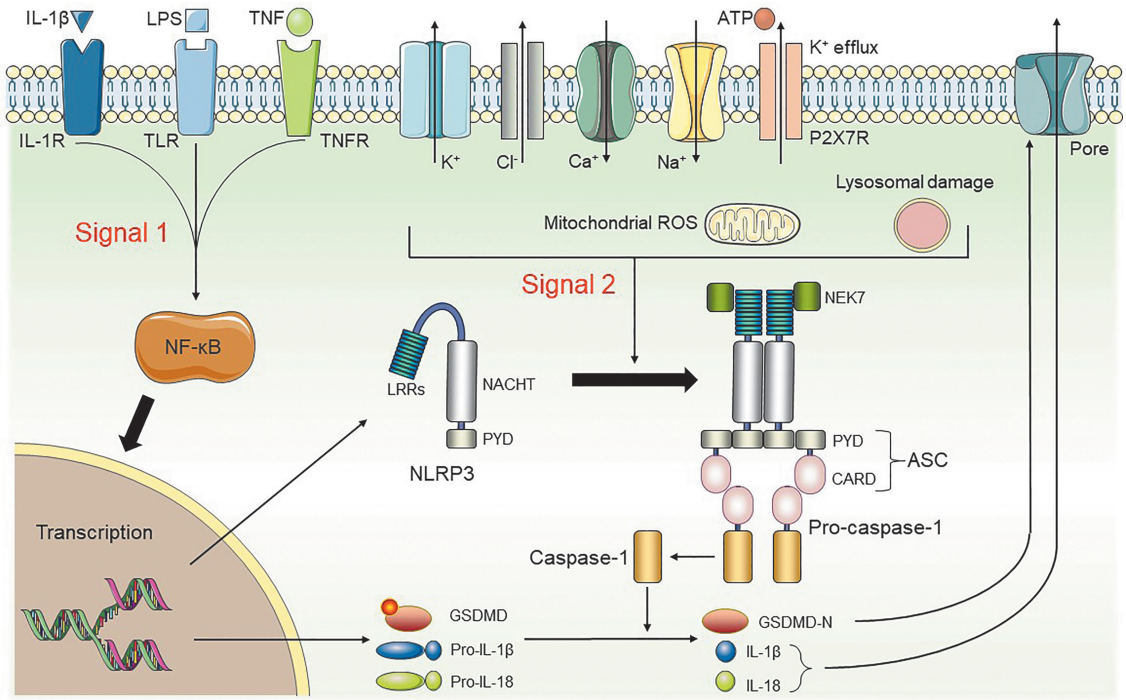

Figure 1: Classical NLRP3 inflammasome activation requires two steps, priming and activation. The first priming signal provided by microbial components or endogenous cytokines activates NF-κB and induces the expression of NLRP3, pro-IL-1β, and pro-IL-18. The second activation signal comes from ionic flux (K^+ efflux, Cl^- efflux, Na^+ influx, and Ca^{2+} mobilization), mitochondrial dysfunction, production of ROS and mtDNA, lysosomal damage, and trans-Golgi decomposition. ASC: Apoptosis-associated speck-like protein containing a caspase-recruitment domain; GSDMD: Gasdermin D; IL: Interleukin; LPS: Lipopolysaccharide; LRRs: Leucine-rich repeat; mtDNA: mitochondrial DNA; NACHT: Nucleotide-binding and oligomerization; NEK7: NIMA-associated kinase 7; NLRP3: NOD-like receptor protein 3; Pro-IL-18: IL-18 is produced as an inactive precursor; PYD: Pyrin domain; ROS: Reactive oxygen species; TLR: Toll-like receptor.

pathway is also K^+ efflux-independent.[15] After a priming step, NLRP3 is activated by a second signal, interacts with NIMA-associated kinase 7 (NEK7), oligomerizes to form complexes, forms ASC speck, and converts ASC proteins into filaments. Subsequently, the ASC protein recruits pro-caspase-1 through CARD–CARD interactions, and then pro-caspase-1 self-hydrolyzes into mature caspase-1, promoting the conversion of pro-IL-1β and pro-IL-18 to interleukin-1β (IL-1β) and interleukin-18 (IL-18), respectively. Caspase-1 also cleaves GSDMD and releases its N-terminal domain, which is transferred to the cell membrane and forms pores, inducing pyroptosis.[8,16]

Non-canonical pathway

Intracellular lipopolysaccharide (LPS) is sufficient to induce endotoxic shock independently of toll-like receptor 4 (TLR4) signaling. This is known as the non-canonical inflammasome activation pathway, which responds to Gram-negative bacteria but not to Gram-positive bacteria. The non-canonical pathway involves human caspase-4/5 and mouse caspase-11, rather than caspase-1. Similar to the canonical pathway, the priming process can enhance the inflammatory response in mice due to low baseline expression of caspase-11. In contrast, the human non-canonical pathway does not require the priming process for high levels of caspase-4 in cells.

During various bacterial infections, caspase-4/5/11 recognizes and binds to intracellular LPS, then oligomerizes and becomes activated. Mature caspase-4/5/11 cannot directly cleave IL-1β and IL-18, but causes cell membrane perforation and potassium efflux by cleaving GSDMD and pannexin-1 (Panx1), leading to pyroptosis [Figure 2]. Oxidized phospholipids (oxPAPC), a class of DAMPs, can bind to caspase-11 and induce inflammasome activation. However, oxPAPC induces caspase-11-dependent IL-1 release without inducing pyroptosis.[7,17]

Alternative pathway

Alternative NLRP3 inflammasome activation requires assembly of the NLRP3-ASC-caspase-1 complex, but there is no ASC speck formation, pyroptosis, or K^+ efflux. In murine dendritic cells, without other activating signals, prolonged exposure to LPS leads to NLRP3-mediated IL-1β processing and secretion in a P2X7-independent manner. TLR4-TRIF (toll-interleukin-1 receptor-domain-containing adaptor-inducing IFN-beta)-RIPK1 (receptor-interacting serine/threonine-protein kinase 1)-FADD (FAS-associated protein with death domain)-caspase-8 signaling plays a pivotal role in this alternative pathway [Figure 2].[7,15]

Post-translational modifications of NLRP3

Multiple post-translational modifications (PTMs) of NLRP3 can regulate NLRP3 inflammasome activation. Among various NLRP3 PTMs, phosphorylation, ubiquitination, and small ubiquitin-like modifier mediated modification (SUMOylation) have been extensively studied. These PTMs reportedly regulate NLRP3 inflammasome activation by

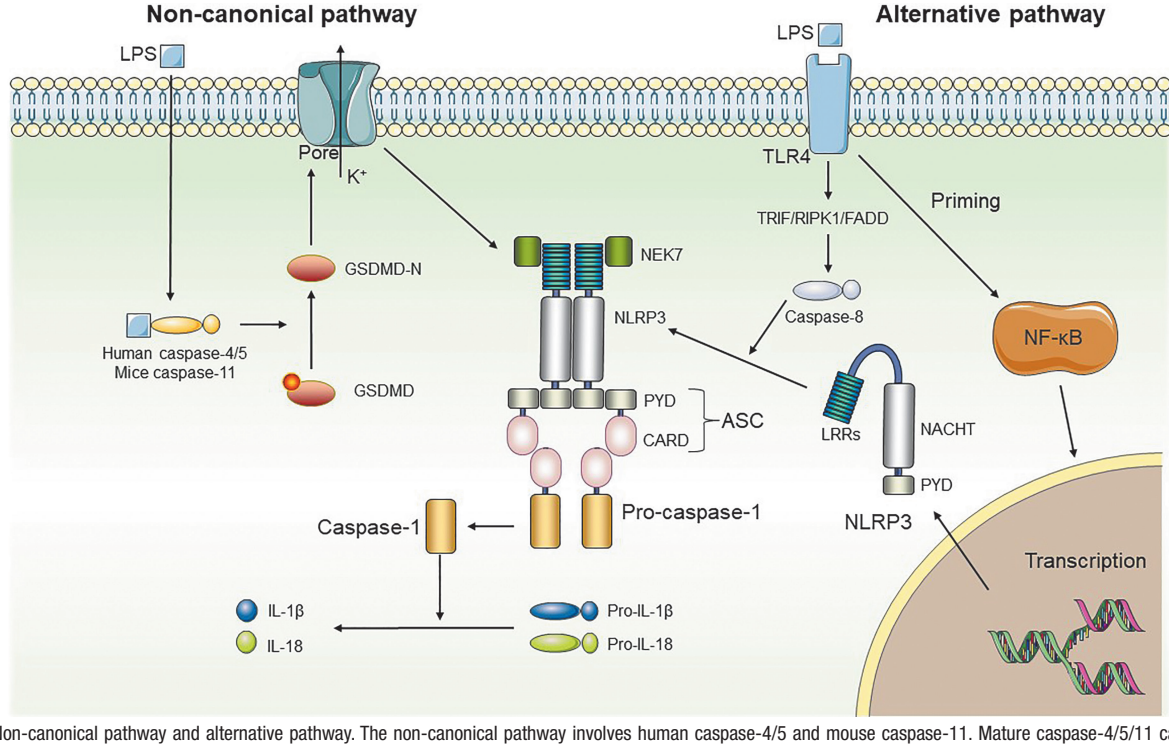

Figure 2: Non-canonical pathway and alternative pathway. The non-canonical pathway involves human caspase-4/5 and mouse caspase-11. Mature caspase-4/5/11 cannot directly cleave IL-1β and IL-18, but cause cell membrane perforation and potassium efflux by cleaving GSDMD. Alternative NLRP3 inflammasome activation requires assembly of the NLRP3-ASC-caspase-1 complex, but there is no ASC speck formation, pyroptosis, or K^+ efflux. LPS-induced TLR4-TRIF-RIPK1-FADD-caspase-8 signaling is important in this pathway. CARD: Caspase-recruitment domain; GSDMD: Gasdermin D; IL: Interleukin; LPS: Lipopolysaccharide; LRRs: Leucine-rich repeat; NACHT: Nucleotide-binding and oligomerization; NEK7: NIMA-associated kinase 7; NLRP3: NOD-like receptor protein 3; PYD: Pyrin domain; TLR: Toll-like receptor.

affecting the activity and stability of NLRP3.[18–20] Qin *et al*[20] reported that tripartite motif-containing protein 28 (TRIM28) could inhibit NLRP3 ubiquitination and proteasomal degradation by promoting small ubiquitin-like modifier (SUMO) 1, SUMO2, and SUMO3 modification of NLRP3. Other less known PTMs such as ADP (adenosine diphosphate)-ribosylation,[21] S-nitrosylation,[22] S-glutathionylation,[23] alkylation,[24] acetylation,[25] prenylation,[26] neddylation,[27] and citrullination[28] also play roles in inflammasome activation, but the exact mechanisms involved are unclear. Recently, Qin *et al*[29] described ISGylation (interferon-stimulated gene 15 covalently binds to the target protein) as a completely new PTM that can facilitate NLRP3 inflammasome activation by stabilizing the NLRP3 protein. Interestingly, besides NLRP3, PTMs of other inflammasome components such as ASC and caspase-1 also regulate NLRP3 inflammasome activation.[30,31]

Role of NLRP3 in Liver Diseases

Hepatic I/R injury

Kupffer cells (KCs) evidently play a key role in aseptic inflammation induced by I/R. M1 macrophages activated by immune interferon-γ (IFN-γ) and LPS are the dominant players in I/R injury as they secrete a variety of inflammatory cytokines that exacerbate the injury and recruit other types of immune cells via the circulation. In contrast, M2 macrophages activated by IL-4 can ameliorate I/R injury via unregulated anti-inflammatory factors.[32] Asiatic acid can reportedly effectively mitigate hepatic I/R injury through attenuation of KCs activation via the peroxisome proliferator-activated receptor gamma (PPARγ)/NLRP3 inflammasome signaling pathway. Mechanistically, asiatic acid-mediated PPARγ upregulation suppresses the ROS/ mitogen-activated protein kinases (MAPK) and ROS/NF-κB signaling pathways.[33]

In one study, expression of vacuolar-type proton ATPase (V-ATPase) D2 subunit (ATP6V0D2) in liver macrophages was upregulated after hepatic I/R. Knockdown of *ATP6V0D2* results in increased secretion of proinflammatory factors and chemokines, which enhances activation of NLRP3 and aggravation of liver injury. Exacerbated activation of NLRP3 is related to the autophagic flux regulated by ATP6V0D2. Knocking down *ATP6V0D2* impairs the formation of autophagolysosomes and aggravates hepatic I/R injury via non-specific V-ATPase activation.[34]

SET domain-containing protein 8 (SET8) can mitigate hepatic I/R injury by suppressing the microtubule-affinity regulating kinase 4 (MARK4)/NLRP3 inflammasome pathway.[35] Cytidine monophosphate kinase 2 (CMPK2) reportedly accelerates hepatic I/R injury by activating the NLRP3 inflammasome.[36] The TLR4/NF-κB/NLRP3 pathway can evidently induce inflammation in hepatic I/R injury.[37,38] In another study, silencing long noncoding RNA (lncRNA) KCNQ1 overlapping transcript 1 (*KCNQ1OT1*) reduced hepatic I/R injury-induced pyroptosis by regulating the miR-142a-3p/high mobility group box 1 (HMGB1) axis. Mechanistically, *KCNQ1OT1* functions as a competing endogenous RNA, which binds to miR-142a-3p, thus promoting HMGB1 expression which activates the TLR4/NF-κB signaling pathway in hepatic I/R injury.[39]

Increased *Panx1* expression has been observed after reperfusion. Interaction between Panx1 and caspase-1 or ASC is increased in an ischemic liver. Pretreatment with the Panx1-specific inhibitor PBN (probenecid) and *Panx1* gene silencing attenuates liver injury and IL-1β production. This study also reported increased levels of cytosolic cathepsin B (Cat B) after reperfusion, indicating release of Cat B from lysosomes. Furthermore, intravenous administration of Cat B monoclonal antibody and *Cat B* gene silencing dramatically reduce I/R injury and serum IL-1β.[40] In another study, hydroxychloroquine attenuated renal I/R injury by inhibiting cathepsin-mediated NLRP3 inflammasome activation.[41] We speculate that the above-described silencing of *Panx1* and *Cat B* may also reduce hepatic I/R injury by inhibiting NLRP3 inflammasomes, but this speculation requires verification.

In one study, PTEN-induced putative kinase 1 (PINK1)-mediated mitophagy protected against hepatic I/R injury by restraining KC-mediated NLRP3 inflammasome activation. Kinase-dead mutation and *PINK1* silencing completely abolished these protective effects. Treatment with different autophagic inhibitors also consistently reverses these PINK1-mediated effects.[42] These results indicate that PINK1-mediated mitophagy is critical in hepatic I/R injury. Interestingly, 25-hydroxycholesterol reportedly inhibits NLRP3 inflammasomes and mitigates hepatic I/R injury via the same pathway.[43]

Knocking out *NLRP3* and its downstream target caspase-1 can evidently reduce hepatic I/R injury.[44] However, in another study, NLRP3 regulated neutrophil functions and contributed to hepatic I/R injury independently of inflammasomes. *NLRP3*$^{-/-}$ mice, but not *ASC*$^{-/-}$ and *caspase-1*$^{-/-}$ mice, exhibit significantly less liver injury after hepatic I/R. It has also been reported that NLRP3 regulates chemokine-mediated function and neutrophil recruitment in an inflammasome-independent manner, thereby participating in hepatic I/R injury.[45] These two completely opposite conclusions require more experimental data for validation.

Downregulation of transient receptor potential melastatin 2 (*TRPM2*) attenuates hepatic I/R injury via activation of autophagy and inhibition of NLRP3. The activation of autophagy also negatively regulates the NLRP3 inflammasome pathway in this process.[46] Dexmedetomidine (Dex) increases miR-494 expression and miR-494-targeted JunD (a versatile transcription factor of the activating protein-1). Upregulation of *JunD* activates the phosphatidylinositol-3 kinase/protein kinase B/nuclear factor E2-related factor 2 (PI3K/AKT/Nrf2) pathway, and therefore inhibits NLRP3 inflammasomes and reduces hepatic I/R injury. Regrettably, researchers have not reported further studies on the mechanisms of Nrf2 regulation of NLRP3.[47]

Fisetis reportedly inhibits the inhibitory effect of glycogen synthase kinase 3β (GSK3β) on AMP-activated protein kinase (AMPK), and AMPK can inhibit NLRP3 inflammasomes and protect the liver by boosting M2 macrophage polarization via mTOR.[48] In contrast, in another study, AMPK could regulate GSK3β activity in macrophages.[49] Pharmacological inhibition of AMPK did not influence the inhibitory effects of fisetin on GSK3β, nor did it entirely reverse the effects of fisetin on NLRP3 inflammasomes. This further suggests that other pathways must be involved.[48,50] A recent study indicates that NLRP3 stimuli initiate GSK3β activation with subsequent binding to NLRP3, facilitating NLRP3 recruitment to mitochondria and transition to the Golgi network (TGN). GSK3β activation also phosphorylates phosphatidylinositol 4-kinase 2A (PI4k2A) in TGN, promoting sustained NLRP3 oligomerization.[51]

Enhanced activation of NLRP3 inflammasomes in aged macrophages stimulated by I/R and mtDNA has been reported. Furthermore, the stimulator of interferon genes (STING)/TANK-binding kinase 1 (TBK1) signaling pathway is over-activated in aged macrophages. Over-activation of NLRP3 signaling and excessive secretion of proinflammatory cytokines vanished after blocking STING. Zhong *et al*[52] reveal that, elderly recipients had much higher levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-1β, and IL-18 post-transplantation, indicating increased NLRP3 activation in I/R-stressed livers in elderly recipients. That study demonstrated that the STING-NLRP3 axis plays a key role in the proinflammatory response of aged macrophages [Table 1].

In addition to classical NLRP3 inflammasome activation, the caspase-11-mediated non-canonical pathway and pyroptosis have been reported in hepatic I/R injury.[53] In that study, hepatic I/R injury was successfully alleviated by inhibiting caspase-11-mediated non-canonical pyroptosis via ghrelin.

Hepatitis

Hepatitis is an inflammation of the liver tissue. It is commonly caused by hepatoviruses, heavy alcohol use, NASH, and autoimmune diseases. Herein, we focus on NASH and viral hepatitis.

NASH

NAFLD is defined by hepatic steatosis without significant alcohol consumption. NASH, which refers to steatosis combined with inflammasomes and fibrosis, is the progressive form of NAFLD and the key cause of end-stage liver diseases.

In one study, rhubarb-free anthraquinones could treat NAFLD by inhibiting NLRP3 inflammasomes.[54] The specific mechanism involved blocking ASC speck formation and the combining of NLRP3 and ASC. Polysaccharides derived from *Gynostemma pentaphyllum* (GPPs) reportedly ameliorate NASH, possibly via the TLR2 (Toll-like receptor-2)/NLRP3 signaling pathway. High doses of GPPs could inhibit the expression of *TLR2* and downregulate NLRP3 inflammasome activation.[55]

Lymphocyte antigen 6 family member D (*LY6D*) is upregulated in NASH livers, and blocking it can reduce NASH-associated hepatocyte pyroptosis. Fos-like 2 (FOSL2), the upstream transcription factor of *LY6D*, can promote *LY6D* transcription by binding to the *LY6D* promoter. The effect of FOSL2/LY6D is significantly attenuated by inhibiting NLRP3. Therefore, the FOSL2/LY6D axis may delay NASH progression by regulating NLRP3 inflammasomes.[56]

Type 4 sphingosine-1-phosphate receptor (*S1PR4*) was found to be overexpressed in a group of NASH patients. Depletion of S1PR4 in hepatic macrophages inhibits LPS-mediated Ca^{2+} release and downregulates NLRP3 inflammasomes. Sphingosine 1-phosphate (S1P) increases *S1PR4* expression via the inositol 1,4,5-trisphosphate/inositol 1,4,5-trisphosphate receptor (IP3/IP3R) signaling pathway, thereby activating NLRP3 inflammasomes.[57] S1PR4 may be a promising target for NASH.

High tripartite motif-containing protein 31 (*TRIM31*) expression reportedly suppresses NLRP3 inflammasomes and the NF-κB signaling pathway, thus restraining hepatic inflammation. Mulberrin, an important component of the traditional Chinese medicine Romulus Mori, was found to improve *TRIM31* expression, further inhibiting the activation of NLRP3 inflammasomes and NF-κB signaling.[58] Notably, however, it has also been reported that TRIM31 plays the opposite role. In that study, *TRIM31* upregulation activated the NF-κB signaling pathway, and TRIM31 could sustain NF-κB signaling pathway activation.[59] Because the two studies were performed in completely different models, this discrepancy may be related to different cells and different physiological environments.

Blocking Ca^{2+} signaling and the induction of mitochondrial reactive oxygen species (mtROS), which are important upstream activating signals of NLRP3 inflammasomes, attenuates caspase-1 activation.[60] Those researchers considered it a promising target for NASH. NADPH oxidase 4 (NOX4), a source of cellular superoxide anions, was found to be the upstream signal of NLRP3, and knocking out *NOX4* could significantly inhibit *NLRP3* expression.[61] Ursolic acid (UA) could reverse liver fibrosis by inhibiting the NOX4/NLRP3 inflammasome pathway.[62] We speculate that NOX4 may affect NLRP3 inflammasomes by regulating its upstream signal ROS, as NOXs are enzymes that produce ROS.[63]

G-protein-coupled bile acid receptor 1 (TGR5), a type of cell surface receptor involved in many metabolic reactions, has been shown to relieve NASH by inhibiting NLRP3 inflammasome activation and caspase-1 cleavage.[64] In another study, bile acids inhibited NLRP3 inflammasome activation via the Takeda G protein-coupled receptor 5/cyclic adenosine monophosphate/protein kinase A (TGR5/cAMP/PKA) signaling pathway axis in several inflammation models.[65] Therefore, we have reason to speculate that the TGR5/cAMP/PKA signaling pathway may be the underlying mechanism by which TGR5 treated NASH, but further studies are needed to verify that.

Table 1: NLRP3 regulation in hepatic I/R injury.

Change in upstream target activity	Pathway	Effect on NLRP3	Effect on hepatic I/R injury	Conclusion	References
PPARγ↑ (treat with asiatic acid)	PPARγ → ROS → MAPK or NF-κB → NLRP3	NLRP3 protein↓	↓	Asiatic acid is effective in mitigating hepatic I/R injury through attenuation of KCs activation via PPARγ/NLRP3 inflammasome signaling pathway	[33]
ATP6V0D2↑	ATP6V0D2 → autophagolysosomes → NLRP3	NLRP3 protein↓	↓	Knocking down ATP6V0D2 impairs autophagolysosome formation and aggravates hepatic I/R injury via non-specific VATPase activation	[34]
SET8↓	SET8 → MARK4 → NLRP3	NLRP3 protein↑	↑	SET8 could mitigate hepatic I/R injury by suppressing the MARK4/NLRP3 inflammasome pathway	[35]
CMPK2↑	CMPK2 → NLRP3	NLRP3 activation↑	↑	CMPK2 could accelerate hepatic I/R injury by activating NLRP3 inflammasomes	[36]
HMGB1↑	HMGB1 → TLR4 → NF-κB → NLRP3	NLRP3 protein↑	↑	TLR4/NF-κB/NLRP3 pathway induces inflammation in hepatic I/R injury	[38]
lncRNA KCNQ1OT1↑	lncRNA KCNQ1OT1 → miR-142a-3p → HMGB1 → TLR4 → NF-κB → NLRP3	NLRP3 protein↑	↑	KCNQ1OT1 functions as a competing endogenous RNA which binds to miR-142a-3p, and therefore promotes HMGB1 expression, activating the TLR4/NFκB signaling pathway in hepatic I/R injury	[39]
Panx1↑	Panx1 → ASC, caspase-1	NLRP3 activation↑	↑	Interaction between Panx1 and caspase-1 or ASC increases in ischemic liver	[40]
PINK1↑	PINK1 → Parkin → mitophagy → NLRP3	NLRP3 mRNA↓ NLRP3 activation↓	↓	PINK1-mediated mitophagy protects against hepatic I/R injury	[42]
TRPM2↓ (knockout *TRPM2* gene)	TRPM2 → autophagy → NLRP3	NLRP3 protein↓	↓	TRPM2 downregulation attenuates hepatic I/R injury via activation of autophagy and inhibition of NLRP3	[46]
miR-494↑ (treat with Dex)	miR-494 → JunD → PI3K → AKT → Nrf2 → NLRP3	NLRP3 protein↓	↓	Dex increases miR-494 expression, and miR-494 targets JunD. JunD upregulation activates the PI3K/AKT/Nrf2 pathway, thereby inhibiting NLRP3 inflammasomes, reducing hepatic I/R injury	[47]
GSK3β↓ (treat with fisetin)	GSK3β → AMPK → NLRP3	NLRP3 protein↓	↓	Fisetin inhibits the inhibitory effect of GSK3β on AMPK, and AMPK can inhibit NLRP3 inflammasomes and protect the liver by boosting M2 macrophage polarization via mTOR	[48]
STING↓ (treat with STING siRNA)	STING, TBK-1 → NLRP3	NLRP3 protein↓	↓	The STING-NLRP3 axis plays a key role in the proinflammatory response of aged macrophages	[52]

AKT: Protein kinase B; AMPK: AMP-activated protein kinase; ASC: Apoptosis-associated speck-like protein containing a CARD; ATP6V0D2: Vacuolar-type proton ATPase D2 subunit; CMPK2: Cytidine monophosphate kinase 2; Dex: Dexmedetomidine; GSK3β: Glycogen synthase kinase 3β; HMGB1: High mobility group box 1; I/R: Ischemia/reperfusion; JunD: A versatile transcription factor of the activating protein-1; KCNQ1OT1: KCNQ1 overlapping transcript 1; KCs: Kupffer cells; lncRNA: Long noncoding RNA; MAPK: Mitogen-activated protein kinases; MARK4: Microtubule-affinity regulating kinase 4; mRNA: Messenger RNA; NF-κB: Nuclear factor kappa B; NLRP3: NOD-like receptor protein 3; Nrf2: Nuclear factor E2-related factor 2; Panx1: Pannexin 1; PINK1: PTEN-induced putative kinase 1; PI3K: Phosphatidylinositol-3 kinase; PPARγ: Peroxisome proliferator-activated receptor gamma; ROS: Reactive oxygen species; SET8: SET domain-containing protein 8; siRNA: Small interfering RNA; STING: Stimulator of interferon genes; TBK-1: TANK-binding kinase; TLR: Toll-like receptor; TRPM2: Transient receptor potential melastatin 2; V-ATPase: Vacuolar-type proton ATPase.

Extracellular ATP is one of the DAMPs that can activate NLRP3 inflammasomes, and ATP exerts its effects through purinergic ligand-gated ion channel 7 receptor (P2X7R).[66] Damaged cells release ATP, then activate P2X7R. P2X7R activation induces K^+ efflux, thereby promoting NLRP3 inflammasome activation.[17] Substantial evidence indicates that the ATP/P2X7R/NLRP3 signaling pathway is involved in the induction of NASH.[67,68]

P2X4R was found to perform the same role as P2X7R in liver inflammasomes.[67]

The ROS/thioredoxin-interacting protein (TXNIP) pathway participates in the pathogenesis of fructose-induced NAFLD.[69] Under excessive ROS generation, TXNIP separates from thioredoxin 1 (TRX-1), links to NLRP3, and further activates NLRP3 inflammasomes.[70] In another study, ER stress activated NLRP3 inflammasomes in a similar way. Farnesoid X receptor (FXR) inhibits NLRP3 and *TXNIP* expression via the p-PERK (phosphor-protein kinase R-like ER kinase)/CHOP (C/EBP homologous protein) pathway. miR-186 and its potential target non-catalytic region of tyrosine kinase adaptor protein 1 (NCK1) are also involved in the pathway (FXR/miR-186/NCK1/p-PERK/CHOP/TXNIP/NLRP3) [Table 2].[71]

Viral hepatitis

NLRP3 inflammasomes are involved in hepatitis virus-induced liver injury. Of the numerous different hepatitis viruses, hepatitis B virus (HBV) and hepatitis C virus (HCV) are the most studied. Relevant research pertaining to NLRP3 in this context is scarce; however, so herein we only briefly describe the latest research progress.

The hepatitis B e antigen significantly inhibits NLRP3 inflammasome activation and IL-1β production by inhibiting the NF-κB signaling pathway and ROS generation.[72] Conversely, the hepatitis B core antigen and HBV x protein (HBx) can facilitate liver inflammation and hepatocellular pyroptosis by promoting NLRP3 inflammasome activation.[73,74] Mechanistically, HBx localizes to mitochondria and induces the production of mtROS, thereby promoting NLRP3 inflammasomes.[74] Green tea polyphenol epigallocatechin-3-gallate (EGCG) can reportedly inhibit HBV and the HMGB1/NLRP3 signaling pathway induced by autophagic flux. Therefore, the HMGB1/NLRP3 pathway is involved in HBV-induced liver injury.[75] Hepatic knock-down of *NLRP3* significantly inhibits HBV replication and hepatitis B surface antigen expression,[76] indicating that NLRP3 plays a key

Table 2: Regulation of NLRP3 in NASH.

Change in upstream target activity	Pathway	Effect on NLRP3	Effect on NASH	Conclusion	References
TLR2↓ (treat with high dose of GPP)	TLR2 → NLRP3	NLRP3 mRNA↓	↓	High dose of GPP could inhibit TLR2 expression and down-regulate NLRP3 inflammasome activation	[55]
FOSL2↑	FOSL2 → LY6D → NLRP3	NLRP3 protein↑	↑	FOSL2/LY6D promoted NASH-associated hepatocyte pyroptosis by regulating NLRP3 inflammasome activation	[56]
S1PR4↑	SK1 → S1P → S1PR4 → NLRP3	NLRP3 activation↑	↑	S1P increased S1PR4 expression via the IP3/IP3R signaling pathway, thereby activating NLRP3 inflammasomes	[57]
TRIM31↑ (treat with mulberrin)	TRIM31 → NLRP3	NLRP3 mRNA↓ NLRP3 protein↓	↓	Mulberrin could restrain hepatic inflammation by suppressing NLRP3 and NF-κB signaling pathways via improvement of TRIM31 signaling	[58]
NOX4↑	NOX4 → CPT1A → fatty acid oxidation → NLRP3	NLRP3 activation↑	↑	NOX4 is the upstream signal of NLRP3, and knocking out NOX4 significantly inhibited NLRP3 expression	[61]
TGR5↓	TGR5 → NLRP3	NLRP3 mRNA↑ NLRP3 protein↑ NLRP3 activation↑	↑	TGR5 relieved NASH by inhibiting NLRP3 inflammasome activation and caspase1 cleavage	[64,65]
P2X7R↑	ATP → P2X7R → NLRP3	NLRP3 mRNA↑	↑	The ATP/P2X7R/NLRP3 signaling pathway is involved in NASH induction	[67,68]
TXNIP↑	ROS → Nrf2 → TXNIP → NLRP3	NLRP3 protein↑	↑	Under excessive ROS generation, TXNIP separates from TRX-1, links to NLRP3, and further activates NLRP3 inflammasomes	[69]

ATP: Adenosine triphosphate; CPT1A: Carnitine palmitoyl transferase 1A; FOSL2: Fos-like 2; GPP: *Gynostemma pentaphyllum*; IP3: Inositol 1,4,5-trisphosphate; IP3R: Inositol 1,4,5-trisphosphate receptor; LY6D: Lymphocyte antigen 6 family member D; mRNA: Messenger RNA; NASH: Non-alcoholic steatohepatitis; NF-κB: Nuclear factor kappa B; NLRP3: NOD-like receptor protein 3; NOX4: NADPH oxidase 4; Nrf2: Nuclear factor E2-related factor 2; P2X7R: Purinergic ligand-gated ion channel 7 receptor; ROS: Reactive oxygen species; SK1: Sphingosine kinase 1; S1P: Sphingosine 1-phosphate; S1PR4: Type 4 sphingosine-1-phosphate receptor; TGR5: G-protein-coupled bile acid receptor 1; TLR: Toll-like receptors; TRIM31: Tripartite motif-containing protein 31; TRX-1: Thioredoxin 1; TXNIP: Thioredoxin-interacting protein.

role in HBV-induced hepatic injury. NLRP3 also plays an important role in liver disease progression during HCV infection via caspase-1 activation.[77] Mechanistically, HCV core protein promotes NLRP3 inflammasome activation by inducing phospholipase C-mediated calcium flux.[78]

Liver failure

ALF is severe liver damage caused by a variety of factors, and there is currently no effective treatment other than artificial liver support systems and/or a liver transplant. NLRP3 inflammasomes play an important role in the pathogenesis of LPS/D-galactosamine (D-GalN) (L/D)-induced ALF.[79–83] Mechanistically, NLRP3 inflammasomes mediate liver failure by activating pro-caspase-1 and pro-IL-1β, and regulating downstream CD40-CD40L signaling.[84] However, in one study, NLRP3 inflammasomes had limited effects on L/D-induced ALF. After L/D treatment *NLRP3*$^{-/-}$ mice and wild-type mice had similar survival rates. The study also suggested it was TNF-α that mediated L/D-induced ALF, rather than caspase-1 and IL-1β, the downstream factors of NLRP3 inflammasomes.[85] In another study, TNF-α-pretreated exosomes derived from human umbilical cord mesenchymal stem cells could alleviate ALF by inhibiting NLRP3 inflammasome activation in macrophages.[83] The reason for these discrepancies is unknown. We suspect that they may be due to different physiological environments, mouse species, and reagent (LPS and D-GalN) doses, but the exact cause requires further research.

In one study, daphnetin inhibited the TXNIP/NLRP3 axis by enhancing the Keap1 (Kelch-like ECH-associated protein 1)-Nrf2/TRX-1 axis, thereby effectively relieving acetaminophen-induced ALF.[70] In L/D-induced ALF models, formation of the TXNIP/NLRP3 complex was increased, whereas the TXNIP/thioredoxin (TRX) complex was decreased. Verapamil treatment could significantly inhibit formation of the TXNIP/NLRP3 complex, but not formation of the TXNIP/TRX complex.[81] In other researches, luteolin mitigated LPS-induced ALF by inhibiting TXNIP production.[86] This suggests that the TXNIP/NLRP3 axis is a reliable target in the treatment of ALF.

F-actin can evidently downregulate NLRP3 inflammasomes via the Flightless-1-LRRFIP2 (leucine-rich repeat Fli-I-interacting protein 2)-NLRP3 inflammasome complex. Transient receptor potential (TRP) channels mediated increased intracellular concentrations of Ca^{2+} and abrogated the negative regulation of F-actin with respect to NLRP3 inflammasomes by promoting the severing of Flightless-1 to F-actin. In this process, Ca^{2+} is not the second signal of NLRP3 inflammasome activation, but the regulator of its activity.[87] The ataxia telangiectasia-mutated (ATM) signaling pathway may be the upstream signaling pathway that regulates F-actin in ALF.[88]

During NLRP3 inflammasome assembly, Bruton tyrosine kinase (Btk) promotes the linking of NLRP3 and ASC, which facilitates the activation of NLRP3 inflammasomes. Btk inhibitor can reportedly alleviate ALF by downregulating NLRP3 inflammasomes.[89] Therefore, the Btk/NLRP3 pathway is a promising target for the treatment of ALF.

One study investigated the treatment of ALF by targeting transcription of NLRP3 messenger RNA (mRNA).[80] Phosphorylated Y-box-binding protein 1 (YB-1) could activate NLRP3 mRNA transcription by binding to its promoter region. Soyasaponin II significantly reduced YB-1 phosphorylation, thus downregulating NLRP3 inflammasomes in ALF [Table 3].

Conclusions

Recently, a growing number of studies have begun to investigate NLRP3 inflammasomes. As mediators of inflammatory responses, NLRP3 inflammasomes play an important role in liver injury. Inhibitors targeting

Table 3: Regulation of NLRP3 in ALF.

Change in upstream target activity	Pathway	Effect on NLRP3	Effect on ALF	Conclusion	References
TXNIP↓ (treat with daphnetin)	Keap1-Nrf2 → TRX-1 → ROS → TXNIP → NLRP3	NLRP3 protein↓	↓	The TXNIP/NLRP3 axis is a reliable target in the treatment of ALF	[70,81,86]
F-actin↑ (treat with HDAC6 inhibitor ACY1215)	ATM → Factin → NLRP3	NLRP3 protein↓ NLRP3 activation↓	↓	The HDAC6 inhibitor ACY1215 inhibits NLRP3 activation in ALF by regulating the ATM/F-actin signaling pathway	[87,88]
Btk↓ (treat with acalabrutinib)	Btk → NLRP3, ASC	NLRP3 mRNA↓ NLRP3 protein↓ NLRP3 activation↓	↓	Btk could promote the linking of NLRP3 and ASC, which facilitates NLRP3 inflammasome activation	[89]
p-YB-1↓ (phosphorylated YB-1) (treat with soyasaponin II)	AKT → pYB1 → NLRP3	NLRP3 mRNA↓ NLRP3 protein↓	↓	Phosphorylated YB-1 could activate NLRP3 mRNA transcription by binding to its promoter region	[80]

ACY1215: Ricolinostat; AKT: Protein kinase B; ALF: Acute liver failure; ASC: Apoptosis-associated speck-like protein containing a CARD; ATM: Ataxia telangiectasia-mutated; Btk: Bruton tyrosine kinase; HDAC6: Histone deacetylase 6; Keap1: Kelch-like ECH-associated protein 1; mRNA: Messenger RNA; NLRP3: NOD-like receptor protein 3; Nrf2: Nuclear factor E2-related factor 2; p-YB-1: Phosphorylated YB-1; ROS: Reactive oxygen species; TRX-1: Thioredoxin 1; TXNIP: Thioredoxin-interacting protein; YB-1: Y-box-binding protein 1.

NLRP3 inflammasomes are a promising research subject for the treatment of liver injury. Substances such as asiatic acid, fisetin, ghrelin, dex, octreotide, and melatonin can reportedly reduce liver injury by inhibiting NLRP3 inflammasomes. Notably, however, the regulatory mechanisms of action of NLRP3 inflammasomes are quite complex. At present, studies on the role and regulation of NLRP3 inflammasomes in liver injuries caused by different diseases are scant, and there has been no in-depth investigation of the underlying mechanisms involved. This has led to poor therapeutic effects of singular strategies targeting NLRP3 inflammasomes or associated upstream and downstream proteins. The drugs now entering clinical trials include RRx-001 (Nibrozetone, developed by EpicentRx), OLT-1177 (Dapansutrile, developed by Olatec), IFM-2427 (DFV890, developed by IFMTRE), IZD-174 (MCC7840, Inzomelid, developed by Inflazome), IZD-334 (Developed by Inflazome), VTX-2735 (Developed by Ventyx Biosciences), ZYIL-1 (Developed by Zydus Pharmaceuticals), Somalix (Developed by Inflazome), VTX-3232 (Developed by Ventyx Biosciences), NT-0167 (Developed by Nodthera), NT-0249 (Developed by Nodthera), NT-0796 (Developed by Nodthera), IPS-07004 (Developed by InnoPharmaScreen), and HT-6184 (Developed by Halia Therapeutics). OLT-1177 is currently in a phase IIa clinical trial for treating gout flares,[90] and RRx-001 has prioritized entry into a phase III clinical trial called REPLATINUM (NCT03699956). Although many drugs have entered clinical trials, few have been specifically targeted at liver diseases. Only DFV890 and NT-0167 have been investigated for the treatment of NAFLD and liver fibrosis.[91] Furthermore, some drugs are reportedly hepatotoxic. A phase II clinical trial investigating the use of MCC-950 (a kind of diarylsulfonylurea-containing compound developed by Pfizer) to treat rheumatoid arthritis ultimately ended in failure due to clinically increased serum liver enzyme levels. The reason for its hepatotoxicity remains elusive.[92] Similarly, administration of GDC-2394 ((S)-N-((1,2,3,5,6,7-Hexahydro-s-indacen-4-yl)carbamoyl)-6-(methylamino)-6,7-dihydro-5H-pyrazolo[5,1-b][1,3]oxazine-3-sulfonamide), another NLRP3 inhibitor, was also halted in a phase I clinical trial due to hepatotoxicity.[93] Hence, the current outlook with respect to the use of NLRP3 inhibitors for treating liver diseases is not optimistic. Further research is required to clarify the roles of NLRP3 inflammasomes in liver injury, and the regulatory mechanisms involved. The quest for more effective targeted inhibitors is ongoing.

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Conflicts of interest

None.

References

1. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell 2010;140:805–820. doi: 10.1016/j.cell.2010.01.022.
2. Martinon F, Burns K, Tschopp J. The inflammasome: A molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell 2002;10:417–426. doi: 10.1016/S1097-2765(02)00599-3.
3. Lamkanfi M, Dixit VM. Mechanisms and functions of inflammasomes. Cell 2014;157:1013–1022. doi: 10.1016/j.cell.2014.04.007.
4. Sharma D, Kanneganti TD. The cell biology of inflammasomes: Mechanisms of inflammasome activation and regulation. J Cell Biol 2016;213:617–629. doi: 10.1083/jcb.201602089.
5. Agostini L, Martinon F, Burns K, McDermott MF, Hawkins PN, Tschopp J. NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. Immunity 2004;20:319–325. doi: 10.1016/S1074-7613(04)00046-9.
6. Shao BZ, Xu ZQ, Han BZ, Su DF, Liu C. NLRP3 inflammasome and its inhibitors: A review. Front Pharmacol 2015;6:262. doi: 10.3389/fphar.2015.00262.
7. Kelley N, Jeltema D, Duan Y, He Y. The NLRP3 Inflammasome: An overview of mechanisms of activation and regulation. Int J Mol Sci 2019;20:3328. doi: 10.3390/ijms20133328.
8. Zhang WJ, Li KY, Lan Y, Zeng HY, Chen SQ, Wang H. NLRP3 Inflammasome: A key contributor to the inflammation formation. Food Chem Toxicol 2023;174:113683. doi: 10.1016/j.fct.2023.113683.
9. Tilg H, Byrne CD, Targher G. NASH drug treatment development: Challenges and lessons. Lancet Gastroenterol Hepatol 2023;8:943–954. doi: 10.1016/S2468-1253(23)00159-0.
10. Devarbhavi H, Asrani SK, Arab JP, Nartey YA, Pose E, Kamath PS. Global burden of liver disease: 2023 update. J Hepatol 2023;79:516–537. doi: 10.1016/j.jhep.2023.03.017.
11. Chen MY, Ye XJ, He XH, Ouyang DY. The signaling pathways regulating NLRP3 inflammasome activation. Inflammation 2021;44:1229–1245. doi: 10.1007/s10753-021-01439-6.
12. Muñoz-Planillo R, Kuffa P, Martínez-Colón G, Smith Brenna L, Rajendiran Thekkelnaycke M, Núñez G. K^+ efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. Immunity 2013;38:1142–1153. doi: 10.1016/j.immuni.2013.05.016.
13. Groß CJ, Mishra R, Schneider KS, Médard G, Wettmarshausen J, Dittlein DC, *et al*. K^+ efflux-independent NLRP3 inflammasome activation by small molecules targeting mitochondria. Immunity 2016;45:761–773. doi: 10.1016/j.immuni.2016.08.010.
14. Wolf AJ, Reyes CN, Liang W, Becker C, Shimada K, Wheeler ML, *et al*. Hexokinase is an innate immune receptor for the detection of bacterial peptidoglycan. Cell 2016;166:624–636. doi: 10.1016/j.cell.2016.05.076.
15. Gaidt MM, Ebert TS, Chauhan D, Schmidt T, Schmid-Burgk JL, Rapino F, *et al*. Human monocytes engage an alternative inflammasome pathway. Immunity 2016;44:833–846. doi: 10.1016/j.immuni.2016.01.012.
16. Wang L, Hauenstein AV. The NLRP3 inflammasome: Mechanism of action, role in disease and therapies. Mol Aspects Med 2020;76:100889. doi: 10.1016/j.mam.2020.100889.
17. Zhan X, Li Q, Xu G, Xiao X, Bai Z. The mechanism of NLRP3 inflammasome activation and its pharmacological inhibitors. Front Immunol 2022;13:1109938. doi: 10.3389/fimmu.2022.1109938.
18. Song N, Li T. Regulation of NLRP3 inflammasome by phosphorylation. Front Immunol 2018;9:2305. doi: 10.3389/fimmu.2018.02305.
19. Lopez-Castejon G. Control of the inflammasome by the ubiquitin system. FEBS J 2019;287:11–26. doi: 10.1111/febs.15118.
20. Qin Y, Li Q, Liang W, Yan R, Tong L, Jia M, *et al*. TRIM28 SUMOylates and stabilizes NLRP3 to facilitate inflammasome activation. Nat Commun 2021;12:4794. doi: 10.1038/s41467-021-25033-4.
21. Bose S, Segovia JA, Somarajan SR, Chang TH, Kannan TR, Baseman JB. ADP-ribosylation of NLRP3 by Mycoplasma pneumoniae CARDS toxin regulates inflammasome activity. mBio 2014;5:e2186–14. doi: 10.1128/mBio.02186-14.
22. Tang X, Pan L, Zhao S, Dai F, Chao M, Jiang H, *et al*. SNO-MLP (S-nitrosylation of muscle LIM Protein) Facilitates myocardial hypertrophy through TLR3 (toll-like receptor 3)-mediated RIP3 (receptor-interacting protein kinase 3) and NLRP3 (NOD-like receptor pyrin domain containing 3) inflammasome activation. Circulation 2020;141:984–1000. doi: 10.1161/CIRCULATIONAHA.119.042336.
23. Guglielmo A, Sabra A, Elbery M, Cerveira MM, Ghenov F, Sunasee R, *et al*. A mechanistic insight into curcumin modulation of the

IL-1β secretion and NLRP3 S-glutathionylation induced by needle-like cationic cellulose nanocrystals in myeloid cells. Chem Biol Interact 2017;274:1–12. doi: 10.1016/j.cbi.2017.06.028.
24. Juliana C, Fernandes-Alnemri T, Wu J, Datta P, Solorzano L, Yu JW, *et al*. Anti-inflammatory compounds parthenolide and bay 11-7082 are direct inhibitors of the inflammasome. J Biol Chem 2010;285:9792–9802. doi: 10.1074/jbc.M109.082305.
25. Zhao K, Zhang Y, Xu X, Liu L, Huang L, Luo R, *et al*. Acetylation is required for NLRP3 self-aggregation and full activation of the inflammasome. bioRxiv 2019:891556. doi: 10.1101/2019.12.31.891556.
26. Skinner OP, Jurczyluk J, Baker PJ, Masters SL, Rios Wilks AG, Clearwater MS, *et al*. Lack of protein prenylation promotes NLRP3 inflammasome assembly in human monocytes. J Allergy Clin Immunol 2019;143:2315–2317.e3. doi: 10.1016/j.jaci.2019.02.013.
27. Segovia JA, Tsai SY, Chang TH, Shil NK, Weintraub ST, Short JD, *et al*. Nedd8 regulates inflammasome-dependent caspase-1 activation. Mol Cell Biol 2015;35:582–597. doi: 10.1128/MCB.00775-14.
28. Mishra N, Schwerdtner L, Sams K, Mondal S, Ahmad F, Schmidt RE, *et al*. Cutting edge: Protein arginine deiminase 2 and 4 regulate NLRP3 inflammasome-dependent IL-1β maturation and ASC speck formation in macrophages. J Immunol 2019;203:795–800. doi: 10.4049/jimmunol.1800720.
29. Qin Y, Meng X, Wang M, Liang W, Xu R, Chen J, *et al*. Post-translational ISGylation of NLRP3 by HERC enzymes facilitates inflammasome activation in models of inflammation. J Clin Invest 2023;133:e161935. doi: 10.1172/JCI161935.
30. Liang Z, Damianou A, Di Daniel E, Kessler BM. Inflammasome activation controlled by the interplay between post-translational modifications: Emerging drug target opportunities. Cell Commun Signal 2021;19:23. doi: 10.1186/s12964-020-00688-6.
31. Xia J, Jiang S, Dong S, Liao Y, Zhou Y. The role of post-translational modifications in regulation of NLRP3 inflammasome activation. Int J Mol Sci 2023;24:6126. doi: 10.3390/ijms24076126.
32. Ye L, He S, Mao X, Zhang Y, Cai Y, Li S. Effect of hepatic macrophage polarization and apoptosis on liver ischemia and reperfusion injury during liver transplantation. Front Immunol 2020;11:1193. doi: 10.3389/fimmu.2020.01193.
33. Xu Y, Yao J, Zou C, Zhang H, Zhang S, Liu J, *et al*. Asiatic acid protects against hepatic ischemia/reperfusion injury by inactivation of Kupffer cells via PPARγ/NLRP3 inflammasome signaling pathway. Oncotarget 2017;8:86339–86355. doi: 10.18632/oncotarget.21151.
34. Wang Z, Wang H, Chen X, Han S, Zhu Y, Wang H, *et al*. Inhibiting ATP6V0D2 aggravates liver ischemia-reperfusion injury by promoting NLRP3 activation via impairing autophagic flux independent of Notch1/Hes1. J Immunol Res 2021;2021:6670495. doi: 10.1155/2021/6670495.
35. Luo Y, Huang Z, Mou T, Pu J, Li T, Li Z, *et al*. SET8 mitigates hepatic ischemia/reperfusion injury in mice by suppressing MARK4/NLRP3 inflammasome pathway. Life Sci 2021;273:119286. doi: 10.1016/j.lfs.2021.119286.
36. Luo Y, Zheng D, Mou T, Pu J, Huang Z, Chen W, *et al*. CMPK2 accelerates liver ischemia/reperfusion injury via the NLRP3 signaling pathway. Exp Ther Med 2021;22:1358. doi: 10.3892/etm.2021.10793.
37. El-Sisi AEE, Sokar SS, Shebl AM, Mohamed DZ, Abu-Risha SE. Octreotide and melatonin alleviate inflammasome-induced pyroptosis through inhibition of TLR4-NF-κB-NLRP3 pathway in hepatic ischemia/reperfusion injury. Toxicol Appl Pharmacol 2021;410:115340. doi: 10.1016/j.taap.2020.115340.
38. Mcdonald KA, Huang H, Tohme S, Loughran P, Ferrero K, Billiar T, *et al*. Toll-like receptor 4 (TLR4) antagonist eritoran tetrasodium attenuates liver ischemia and reperfusion injury through inhibition of high-mobility group box protein B1 (HMGB1) signaling. Mol Med 2015;20:639–648. doi: 10.2119/molmed.2014.00076.
39. Liang C, Peng Y, Sun H, Wang L, Jiang L, Zou S. Silencing lncRNA KCNQ1OT1 reduced hepatic ischemia reperfusion injury-induced pyroptosis by regulating miR-142a-3p/HMGB1 axis. Mol Cell Biochem 2023;478:1293–1305. doi: 10.1007/s11010-022-04586-y.
40. Kim HY, Kim SJ, Lee SM. Activation of NLRP3 and AIM2 inflammasomes in Kupffer cells in hepatic ischemia/reperfusion. FEBS J 2015;282:259–270. doi: 10.1111/febs.13123.
41. Tang TT, Lv LL, Pan MM, Wen Y, Wang B, Li ZL, *et al*. Hydroxychloroquine attenuates renal ischemia/reperfusion injury by inhibiting cathepsin mediated NLRP3 inflammasome activation. Cell Death Dis 2018;9:351. doi: 10.1038/s41419-018-0378-3.
42. Xu Y, Tang Y, Lu J, Zhang W, Zhu Y, Zhang S, *et al*. PINK1-mediated mitophagy protects against hepatic ischemia/reperfusion injury by restraining NLRP3 inflammasome activation. Free Radic Biol Med 2020;160:871–886. doi: 10.1016/j.freeradbiomed.2020.09.015.
43. Cao Q, Luo J, Xiong Y, Liu Z, Ye Q. 25-Hydroxycholesterol mitigates hepatic ischemia reperfusion injury via mediating mitophagy. Int Immunopharmacol 2021;96:107643. doi: 10.1016/j.intimp.2021.107643.
44. Huang H, Chen HW, Evankovich J, Yan W, Rosborough BR, Nace GW, *et al*. Histones activate the NLRP3 inflammasome in Kupffer cells during sterile inflammatory liver injury. J Immunol 2013;191:2665–2679. doi: 10.4049/jimmunol.1202733.
45. Inoue Y, Shirasuna K, Kimura H, Usui F, Kawashima A, Karasawa T, *et al*. NLRP3 regulates neutrophil functions and contributes to hepatic ischemia-reperfusion injury independently of inflammasomes. J Immunol 2014;192:4342–4351. doi: 10.4049/jimmunol.1302039.
46. Zhang T, Huang W, Ma Y. Down-regulation of TRPM2 attenuates hepatic ischemia/reperfusion injury through activation of autophagy and inhibition of NLRP3 inflammasome pathway. Int Immunopharmacol 2022;104:108443. doi: 10.1016/j.intimp.2021.108443.
47. Wu Y, Qiu G, Zhang H, Zhu L, Cheng G, Wang Y, *et al*. Dexmedetomidine alleviates hepatic ischaemia-reperfusion injury via the PI3K/AKT/Nrf2-NLRP3 pathway. J Cell Mol Med 2021;25:9983–9994. doi: 10.1111/jcmm.16871.
48. Pu JL, Huang ZT, Luo YH, Mou T, Li TT, Li ZT, *et al*. Fisetin mitigates hepatic ischemia-reperfusion injury by regulating GSK3beta/AMPK/NLRP3 inflammasome pathway. Hepatobiliary Pancreat Dis Int 2021;20:352–360. doi: 10.1016/j.hbpd.2021.04.013.
49. Ci X, Zhou J, Lv H, Yu Q, Peng L, Hua S. Betulin exhibits anti-inflammatory activity in LPS-stimulated macrophages and endotoxin-shocked mice through an AMPK/AKT/Nrf2-dependent mechanism. Cell Death Dis 2017;8:e2798. doi: 10.1038/cddis.2017.39.
50. Zhou H, Wang H, Ni M, Yue S, Xia Y, Busuttil RW, *et al*. Glycogen synthase kinase 3beta promotes liver innate immune activation by restraining AMP-activated protein kinase activation. J Hepatol 2018;69:99–109. doi: 10.1016/j.jhep.2018.01.036.
51. Arumugam S, Qin Y, Liang Z, Han SN, Boodapati SLT, Li J, *et al*. GSK3beta mediates the spatiotemporal dynamics of NLRP3 inflammasome activation. Cell Death Differ 2022;29:2060–2069. doi: 10.1038/s41418-022-00997-y.
52. Zhong W, Rao Z, Rao J, Han G, Wang P, Jiang T, *et al*. Aging aggravated liver ischemia and reperfusion injury by promoting STING-mediated NLRP3 activation in macrophages. Aging Cell 2020;19:e13186. doi: 10.1111/acel.13186.
53. Tong L, Liu R, Yang Y, Zhao J, Ye S, Wang X, *et al*. Ghrelin protects against ischemia/reperfusion-induced hepatic injury via inhibiting Caspase-11-mediated noncanonical pyroptosis. Transpl Immunol 2023;80:101888. doi: 10.1016/j.trim.2023.101888.
54. Wu C, Bian Y, Lu B, Wang D, Azami NLB, Wei G, *et al*. Rhubarb free anthraquinones improved mice nonalcoholic fatty liver disease by inhibiting NLRP3 inflammasome. J Transl Med 2022;20:294. doi: 10.1186/s12967-022-03495-4.
55. Yue SR, Tan YY, Zhang L, Zhang BJ, Jiang FY, Ji G, *et al*. *Gynostemma pentaphyllum* polysaccharides ameliorate non-alcoholic steatohepatitis in mice associated with gut microbiota and the TLR2/NLRP3 pathway. Front Endocrinol (Lausanne) 2022;13:885039. doi: 10.3389/fendo.2022.885039.
56. Hu PX, Sheng MY, Liu YP, Zhang CQ. FOSL2 deficiency delays nonalcoholic steatohepatitis progression by regulating LY6D-mediated NLRP3 activation. Hum Cell 2022;35:1752–1765. doi: 10.1007/s13577-022-00760-y.
57. Hong CH, Ko MS, Kim JH, Cho H, Lee CH, Yoon JE, *et al*. Sphingosine 1-phosphate receptor 4 promotes nonalcoholic steatohepatitis by activating NLRP3 inflammasome. Cell Mol Gastroenterol Hepatol 2022;13:925–947. doi: 10.1016/j.jcmgh.2021.12.002.
58. Ge C, Tan J, Lou D, Zhu L, Zhong Z, Dai X, *et al*. Mulberrin confers protection against hepatic fibrosis by Trim31/Nrf2 signaling. Redox Biol 2022;51:102274. doi: 10.1016/j.redox.2022.102274.
59. Yu C, Chen S, Guo Y, Sun C. Oncogenic TRIM31 confers gemcitabine resistance in pancreatic cancer via activating the NF-κB

signaling pathway. Theranostics 2018;8:3224–3236. doi: 10.7150/thno.23259.
60. Liu H, Zhan X, Xu G, Wang Z, Li R, Wang Y, *et al*. Cryptotanshinone specifically suppresses NLRP3 inflammasome activation and protects against inflammasome-mediated diseases. Pharmacol Res 2021;164:105384. doi: 10.1016/j.phrs.2020.105384.
61. Moon JS, Nakahira K, Chung KP, DeNicola GM, Koo MJ, Pabón MA, *et al*. NOX4-dependent fatty acid oxidation promotes NLRP3 inflammasome activation in macrophages. Nat Med 2016;22:1002–1012. doi: 10.1038/nm.4153.
62. Nie Y, Liu Q, Zhang W, Wan Y, Huang C, Zhu X. Ursolic acid reverses liver fibrosis by inhibiting NOX4/NLRP3 inflammasome pathways and bacterial dysbiosis. Gut Microbes 2021;13:1972746. doi: 10.1080/19490976.2021.1972746.
63. Gabbia D, Cannella L, De Martin S. The role of oxidative stress in NAFLD-NASH-HCC transition-focus on NADPH oxidases. Biomedicines 2021;9:687. doi: 10.3390/biomedicines9060687.
64. Shi Y, Su W, Zhang L, Shi C, Zhou J, Wang P, *et al*. TGR5 regulates macrophage inflammation in nonalcoholic steatohepatitis by modulating NLRP3 inflammasome activation. Front Immunol 2020;11:609060. doi: 10.3389/fimmu.2020.609060.
65. Guo C, Xie S, Chi Z, Zhang J, Liu Y, Zhang L, *et al*. Bile acids control inflammation and metabolic disorder through inhibition of NLRP3 inflammasome. Immunity 2016;45:802–816. doi: 10.1016/j.immuni.2016.09.008.
66. Di Virgilio F, Dal Ben D, Sarti AC, Giuliani AL, Falzoni S. The P2X7 receptor in infection and inflammation. Immunity 2017;47:15–31. doi: 10.1016/j.immuni.2017.06.020.
67. Rossi C, Salvati A, Distaso M, Campani D, Raggi F, Biancalana E, *et al*. The P2X7R-NLRP3 and AIM2 inflammasome platforms mark the complexity/severity of viral or metabolic liver damage. Int J Mol Sci 2022;23:7447. doi: 10.3390/ijms23137447.
68. Rossato M, Di Vincenzo A, Pagano C, El Hadi H, Vettor R. The P2X7 receptor and NLRP3 axis in non-alcoholic fatty liver disease: A brief review. Cells 2020;9:1047. doi: 10.3390/cells9041047.
69. Zhang X, Zhang JH, Chen XY, Hu QH, Wang MX, Jin R, *et al*. Reactive oxygen species-induced TXNIP drives fructose-mediated hepatic inflammation and lipid accumulation through NLRP3 inflammasome activation. Antioxid Redox Signal 2015;22:848–870. doi: 10.1089/ars.2014.5868.
70. Lv H, Zhu C, Wei W, Lv X, Yu Q, Deng X, *et al*. Enhanced Keap1-Nrf2/Trx-1 axis by daphnetin protects against oxidative stress-driven hepatotoxicity via inhibiting ASK1/JNK and Txnip/NLRP3 inflammasome activation. Phytomedicine 2020;71:153241. doi: 10.1016/j.phymed.2020.153241.
71. Han CY, Rho HS, Kim A, Kim TH, Jang K, Jun DW, *et al*. FXR inhibits endoplasmic reticulum stress-induced NLRP3 inflammasome in hepatocytes and ameliorates liver injury. Cell Rep 2018;24:2985–2999. doi: 10.1016/j.celrep.2018.07.068.
72. Yu X, Lan P, Hou X, Han Q, Lu N, Li T, *et al*. HBV inhibits LPS-induced NLRP3 inflammasome activation and IL-1beta production via suppressing the NF-κB pathway and ROS production. J Hepatol 2017;66:693–702. doi: 10.1016/j.jhep.2016.12.018.
73. Ding X, Lei Q, Li T, Li L, Qin B. Hepatitis B core antigen can regulate NLRP3 inflammasome pathway in HepG2 cells. J Med Virol 2019;91:1528–1536. doi: 10.1002/jmv.25490.
74. Xie WH, Ding J, Xie XX, Yang XH, Wu XF, Chen ZX, *et al*. Hepatitis B virus X protein promotes liver cell pyroptosis under oxidative stress through NLRP3 inflammasome activation. Inflamm Res 2020;69:683–696. doi: 10.1007/s00011-020-01351-z.
75. He M, Chu T, Wang Z, Feng Y, Shi R, He M, *et al*. Inhibition of macrophages inflammasome activation via autophagic degradation of HMGB1 by EGCG ameliorates HBV-induced liver injury and fibrosis. Front Immunol 2023;14:1147379. doi: 10.3389/fimmu.2023.1147379.
76. Chen F, Liu Y, Li Q, Wang F. Inhibition of hepatic NLRP3 inflammasome ameliorates non-alcoholic steatohepatitis/hepatitis B – induced hepatic injury. Clin Res Hepatol Gastroenterol 2023;47:102056. doi: 10.1016/j.clinre.2022.102056.
77. Aggan HE, Mahmoud S, Deeb NE, Eleishi I, El-Shendidi A. Significance of elevated serum and hepatic NOD-like receptor pyrin domain containing 3 (NLRP3) in hepatitis C virus-related liver disease. Sci Rep 2022;12:19528. doi: 10.1038/s41598-022-22022-5.
78. Negash AA, Olson RM, Griffin S, Gale M Jr. Modulation of calcium signaling pathway by hepatitis C virus core protein stimulates NLRP3 inflammasome activation. PLoS Pathog 2019;15:e1007593. doi: 10.1371/journal.ppat.1007593.
79. Zhan C, Lin G, Huang Y, Wang Z, Zeng F, Wu S. A dopamine-precursor-based nanoprodrug for in-situ drug release and treatment of acute liver failure by inhibiting NLRP3 inflammasome and facilitating liver regeneration. Biomaterials 2021;268:120573. doi: 10.1016/j.biomaterials.2020.120573.
80. Wang F, Gong S, Wang T, Li L, Luo H, Wang J, *et al*. Soyasaponin II protects against acute liver failure through diminishing YB-1 phosphorylation and Nlrp3-inflammasome priming in mice. Theranostics 2020;10:2714–2726. doi: 10.7150/thno.40128.
81. Han M, Li S, Li L. Verapamil inhibits early acute liver failure through suppressing the NLRP3 inflammasome pathway. J Cell Mol Med 2021;25:5963–5975. doi: 10.1111/jcmm.16357.
82. Zhang E, Huang J, Wang K, Yu Q, Zhu C, Ren H. Pterostilbene protects against lipopolysaccharide/D-galactosamine-induced acute liver failure by upregulating the Nrf2 pathway and inhibiting NF-κB, MAPK, and NLRP3 inflammasome activation. J Med Food 2020;23:952–960. doi: 10.1089/jmf.2019.4647.
83. Zhang S, Jiang L, Hu H, Wang H, Wang X, Jiang J, *et al*. Pretreatment of exosomes derived from hUCMSCs with TNF-alpha ameliorates acute liver failure by inhibiting the activation of NLRP3 in macrophage. Life Sci 2020;246:117401. doi: 10.1016/j.lfs.2020.117401.
84. Li Z, Jiang J. The NLRP3 inflammasome mediates liver failure by activating procaspase-1 and pro-IL-1 beta and regulating downstream CD40-CD40L signaling. J Int Med Res 2021;49:3000605211036845. doi: 10.1177/03000605211036845.
85. Zhang W, Tao SS, Wang T, Li YT, Chen H, Zhan YQ, *et al*. NLRP3 is dispensable for D-galactosamine/lipopolysaccharide-induced acute liver failure. Biochem Biophys Res Commun 2020;533:1184–1190. doi: 10.1016/j.bbrc.2020.10.003.
86. Wang X, Wang L, Dong R, Huang K, Wang C, Gu J, *et al*. Luteolin ameliorates LPS-induced acute liver injury by inhibiting TXNIP-NLRP3 inflammasome in mice. Phytomedicine 2021;87:153586. doi: 10.1016/j.phymed.2021.153586.
87. Burger D, Fickentscher C, de Moerloose P, Brandt KJ. F-actin dampens NLRP3 inflammasome activity via flightless-I and LRRFIP2. Sci Rep 2016;6:29834. doi: 10.1038/srep29834.
88. Chen Q, Wang Y, Jiao F, Cao P, Shi C, Pei M, *et al*. HDAC6 inhibitor ACY1215 inhibits the activation of NLRP3 inflammasome in acute liver failure by regulating the ATM/F-actin signalling pathway. J Cell Mol Med 2021;25:7218–7228. doi: 10.1111/jcmm.16751.
89. Ye B, Chen S, Guo H, Zheng W, Lou G, Liang X, *et al*. The inhibition of bruton tyrosine kinase alleviates acute liver failure via downregulation of NLRP3 inflammasome. J Immunol 2022;209:1156–1164. doi: 10.4049/jimmunol.2001323.
90. Klück V, Jansen TLTA, Janssen M, Comarniceanu A, Efdé M, Tengesdal IW, *et al*. Dapansutrile, an oral selective NLRP3 inflammasome inhibitor, for treatment of gout flares: An open-label, dose-adaptive, proof-of-concept, phase 2a trial. Lancet Rheumatol 2020;2:e270–e280. doi: 10.1016/s2665-9913(20)30065-5.
91. Schwaid AG, Spencer KB. Strategies for targeting the NLRP3 inflammasome in the clinical and preclinical space. J Med Chem 2021;64:101–122. doi: 10.1021/acs.jmedchem.0c01307.
92. Mangan MSJ, Olhava EJ, Roush WR, Seidel HM, Glick GD, Latz E. Targeting the NLRP3 inflammasome in inflammatory diseases. Nat Rev Drug Discov 2018;17:588–606. doi: 10.1038/nrd.2018.97.
93. Tang F, Kunder R, Chu T, Hains A, Nguyen A, McBride JM, *et al*. First-in-human phase 1 trial evaluating safety, pharmacokinetics, and pharmacodynamics of NLRP3 inflammasome inhibitor, GDC-2394, in healthy volunteers. Clin Transl Sci 2023;16:1653–1666. doi: 10.1111/cts.13576.

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