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REVIEW ARTICLE

Fantastic voyage: The journey of NLRP3 inflammasome activation

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Abstract NLRP3 inflammasome, an intracellular multiprotein complex, can be activated by a range of pathogenic microbes or endogenous hazardous chemicals. Its activation results in the release of cytokines such as IL-1β and IL-18, as well as Gasdermin *D* which eventually causes pyroptosis. The activation of NLRP3 inflammasome is under strict control and regulation by numerous pathways and mechanisms. Its excessive activation can lead to a persistent inflammatory response, which is linked to the onset and progression of severe illnesses. Recent studies have revealed that the subcellular localization of NLRP3 changes significantly during the activation process. In this review, we review the current understanding of the molecular mechanism of NLRP3 inflammasome activation, focusing on the subcellular localization of NLRP3 and the associated regulatory mechanisms. We aim to provide a comprehensive understanding of the dynamic transportation, activation, and degradation processes of NLRP3.

Introduction

Inflammasomes are a series of multimeric protein complexes protecting the host from foreign pathogens and self-damages by releasing cytokines. Inflammasomes generally consist of three components: sensors, adaptors, and effectors. The sensors, referred to as pattern recognition receptors (PRRs), typically recognize exogenous or endogenous signals, and then recruit and induce the adaptor apoptosis-associated speck-like protein containing a CARD (ASC) assembly and subsequently recruit effector caspase-1 onto the inflammasomes, leading to its autoactivation and ultimately maturation, followed by cytokine release and cell death pytoptosis.[1–8] Inflammasomes are categorized into six types based on various kinds of sensors: NLRP1

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inflammasome, NLRP3 inflammasome, NLRP6 inflammasome, NLRC4 inflammasome, Absent in melanoma 2 (AIM2) inflammasome, and PYRIN inflammasome.[1] Most of them show a preference for sensing pathogen-associated molecular patterns (PAMPs) derived from exogenous invaders (such as viruses, bacteria, fungi, and parasites). For instance, NLRP1 recognizes lethal factors in *Bacillus anthracis* or double-stranded RNA in some RNA viruses.[9,10] NLRC4 identifies flagellin and type III secretion system proteins in *Salmonella* using NAIP5/6 and NAIP1/2 respectively. NLRP6, one of the major inflammasomes in the intestine, can bind directly to double-stranded RNA of the virus and gram-positive bacterial cell wall component lipoteichoic acid.[11] AIM2 inflammasome, similar to stimulator of interferon genes (STING), can be activated by double-stranded DNA from pathogens, as well as DNA from the host nucleus or mitochondria genome.[12,13] PYRIN detects bacterial toxin-induced Rho GTPases inactivation rather than PAMP directly. Of note, NLRP3 is more versatile than the aforementioned. Besides PAMPs, NLRP3 has a capacity for sensing danger-associated molecular patterns (DAMPs) released from damaged and senescent cells in the host.[14–16] Additionally, a range of metabolic crystals and protein aggregates including monosodium urate (MSU) crystals, β-amyloid, and cholesterol crystals, as well as exogenous circumstantial particles such as silica, alum, and asbestos are also recognized by NLRP3.[17,18] As a result, NLRP3 not only induces resistance to pathogenic infection but also causes a sterile inflammatory response. Aberrant or chronic NLRP3 inflammasome activation is closely related to several human diseases such as cryopyrin-associated periodic syndromes (CAPS), atherosclerosis, gout, type-II diabetes, and cancers.[19–23] In this review, we recap the dynamic subcellular localization of NLRP3 during an inflammatory response and toward inflammasome activation.

NLRP3 inflammasome activation

The NLRP3 inflammasome is the most well-studied inflammasome and is composed of the sensor NLRP3, the adapter ASC, and the effector caspase-1.[24] The structure of NLRP3 comprises three domains (Fig. 1): the N-terminal pyrin domain (PYD), the C-terminal leucine-rich repeat (LRR), and the centrally located NACHT domain. The PYD in NLRP3 can form fiber itself,[25] which also interacts and recruits ASC, facilitating the ASC filament assembly. By comparison, the role of LRR is ambiguous. It is speculated to be involved in the self-suppression of inflammasome activation since the fact that NLRP3 without LRR remains active.[26,27] The NATCH domain can be further divided into four subdomains: the nucleotide-binding domain (NBD), helical domain 1 (HD1), winged helical domain (WHD), and helical domain 2 (HD2). Among them, NBD promotes the oligomerization of NLRP3 when bound with ATP, while HD1 and WHD mediate the conformational change within NLRP3 following binding to the NIMA-related kinase 7 (NEK7), leading to the exposure of PYD and the subsequent ASC nucleation. Through the caspase activation and recruitment domain (CARD)-CARD interactions, the ASC filament in turn recruits the precursor of the effector pro-caspase-1, which is subsequently activated and undergoes autoproteolysis to form active caspase-1.[28] Active caspase-1 further enzymatically cleaves the pre-existing cytokine precursors pro-IL-1β and pro-IL-18 to produce active cytokines IL-1β and IL-18. Simultaneously, the N-terminal Gasdermin *D*, processed by active caspase-1, relocates to the plasma membrane and punches pores in it, ultimately resulting in the release of mature inflammatory cytokines and pyroptosis, a form of programmed cell death.[26,29–31]

NLRP3 activation pathways

Three patterns are now known to activate NLRP3 inflammasome, including canonical, non-canonical, and alternative pathways (Fig. 2). In non-canonical pathway, caspase-11 in mice or caspase-4/5 in humans undergo auto-activation and cleave cytokines and Gasdermin *D* to induce IL-1β and IL-18 release and pyroptosis via sensing the cytoplasmic lipopolysaccharides (LPS) captured and transported by the interferon-inducible protein IRGB10 and the guanylate binding protein GBP5 collaboratively.[32,33] The alternative pathway only exists in human monocytes, in which Toll-like receptor 4 (TLR4) recognizes extracellular LPS and induces NLRP3 activation and cytokine maturation through the caspase-8/FADD/RIPK3 signaling pathway, but neither apoptosis-associated speck formation nor pyroptosis is

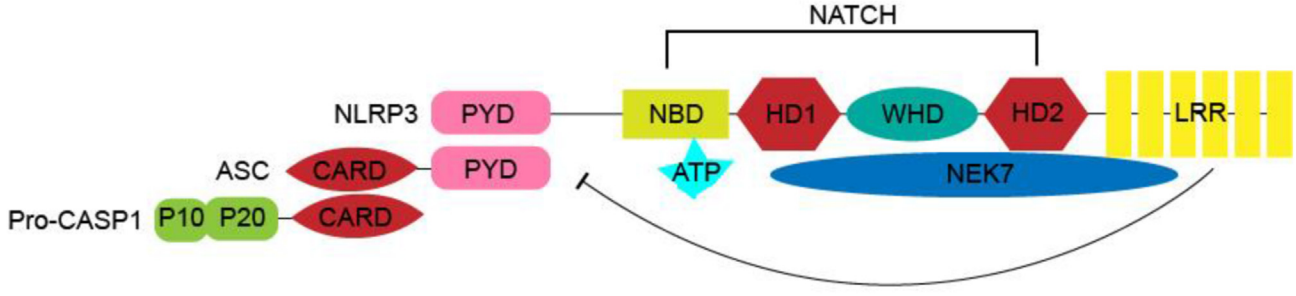

Figure 1 Domain structure of NLRP3 inflammasome complex. NLRP3 contains three main domains: PYD, NATCH, and LRR. The NATCH domain can be divided into four subdomains, labeled NBD, HD1, WHD, and HD2. Adaptor ASC contains PYD and CARD domains, while pro-caspase-1 contains caspase-1 (P20, P10) and CARD domains. The interaction between NATCH/LRR and NEK7 can induce activation and oligomerization of NLRP3, leading to the formation of an NLRP3 fiber mediated by the PYD domain, further transitions to ASC PYD fiber. Finally, the pro-caspase-1 is recruited and assembled onto the fiber, activated, and self-cleaved to produce mature caspase-1.

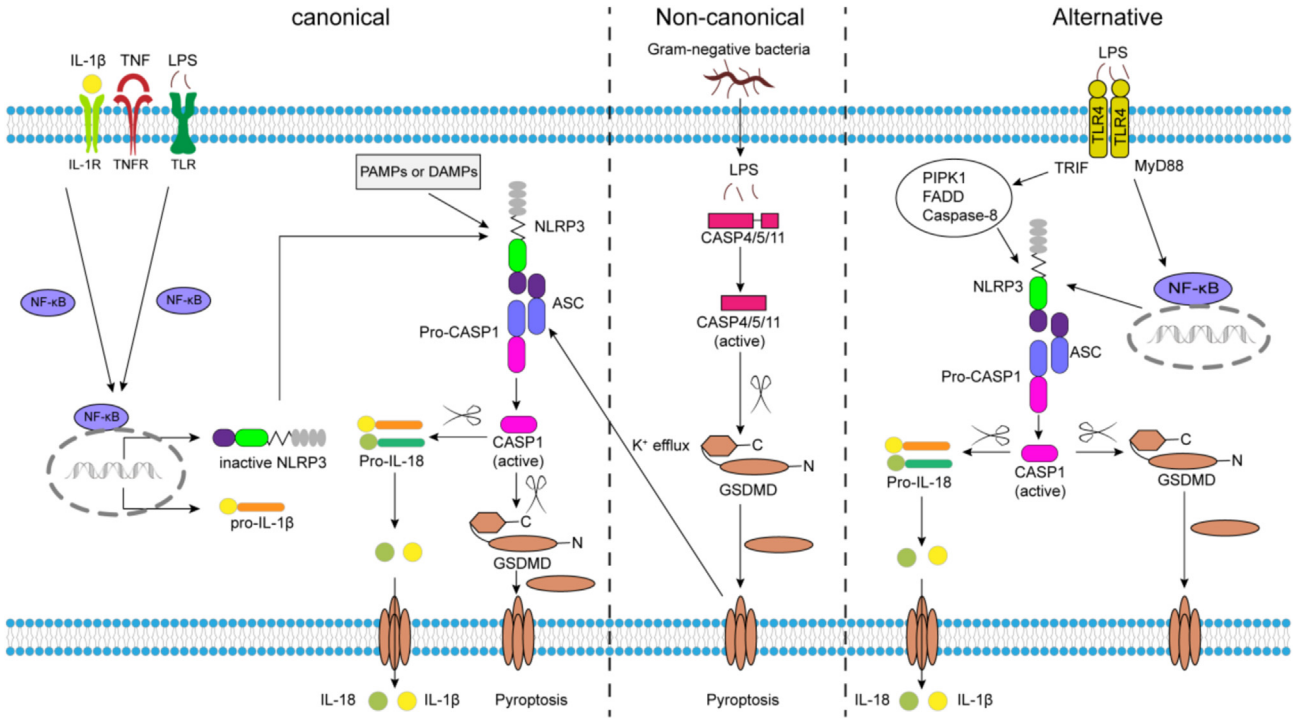

Figure 2 Canonical, non-canonical, and alternative modes of NLRP3 inflammasome signaling. Canonical NLRP3 inflammasome activation requires two steps in most cell types. During the priming step, inflammatory stimuli are sensed by TLRs, IL-1Rs, and TNFRs, which induce the expression of pro-IL-1β and NLRP3 via the NF-κB pathway. During the activation step, numerous PAMPs or DAMPs promote NLRP3 inflammasome assembly, inducing pro-caspase-1 self-cleavage and activation. Active caspase-1 cleaves the cytokine precursors pro-IL-1β and pro-IL-18 to produce active cytokines IL-1β and IL-18, respectively. It also cleaves gasdermin *D* (GSDMD) and releases its N-terminal domain, which transfers to the cell membrane and forms pores, leading to release and pyroptosis of the mature inflammatory cytokines. Non-canonical NLRP3 inflammasome activation occurs in response to cytosolic LPS sensed by caspase-4/5/11. Autocrine induction of NLRP3 inflammasome activation is triggered by K^+ efflux due to GSDMD pore formation. Alternative NLRP3 inflammasome activation only requires a single signal and occurs through TLR4 activation in monocytes by the RIP1-FADD-caspase-8 pathway, which cannot induce K^+ efflux, ASC speck formation, or pyroptosis.

induced.[34] Distinct from the above two, the canonical NLRP3 activation pathway requires two signals and is divided into two steps: priming and assembly. In the priming step, PRRs on the cell surface, including TLR4, tumor necrosis factor receptor (TNFR), or IL-1 receptor (IL-1R), bind to their corresponding signal ligands LPS, TNF-α, or IL-1 family members, respectively, up-regulating expression of NLRP3 and pro-IL-1β via NF-κB pathway.[35,36] Besides, priming signals also involve either blocking degradation or relieving self-inhibition through provoking post-translational modifications (PTMs) on NLRP3, making it ready for the next assembly step.[37] The NLRP3 inflammasome assembly process has been intensively studied in the last decade.[38] Although both PAMP and DAMP signals are considered as stimuli for NLRP3 assembly, there is no evidence showing NLRP3 binds directly to any activators; hence, NLRP3 may recognize certain mediators or second signals. However, given the dynamic cellular status, no specific substance is known to serve as a mediator till now. Some researchers speculated it can be a change in cellular status. So far, many hypotheses for the activation mechanisms have been proposed, including ion homeostasis, mitochondrial damage, lysosomal rupture, endoplasmic reticulum stress (ERS), and Golgi dispersion.[34,39,40] After priming, NLRP3 is expressed and the monomers bind to the intracellular membrane and assemble into oligomers wrapping the PYD inside, which represents the inactive state[41,42]; the oligomers are captured by the dynein adapter histone deacetylase 6 (HDAC6) and then transported to the Microtubule Organizing Center (MTOC) via dynein along microtubules.[43] Within the MTOC, NLRP3 meets NEK7 to form a heterodimer, leading to conformational change within NLRP3 to reorganize the oligomer and expose the PYD.[44–46] Subsequently, NLRP3 recruits ASC via PYD–PYD interaction, leading to ASC oligomerization, which in turn confers ASC to recruit pro-caspase-1 via CARD–CARD binding. In the end, all three components were assembled to form an intact and highly active NLRP3 inflammasome.[47]

The origin of NLRP3

NLRP3 inflammasome plays a critical role in the body's first line of defense against pathogens or self-damages, which explains why NLRP3 protein is robustly expressed in peripheral blood leukocytes (including neutrophils, monocytes, lymphocytes, and dendritic cells) and non-

keratinizing squamous epithelium (such as oral, esophageal, and ectocervical mucosa).[48] While NLRP3 is constitutively expressed in these epithelial cells, its expression in neutrophils requires stimulation. As previously mentioned in the canonical pathway, endotoxic LPS binds and activates the cell surface receptor TLR4, which in turn activates the TLR downstream adapter MyD88 and subsequently induces NLRP3 expression via the NF-κB pathway.[35,49,50] The mRNA of NLRP3 is transported out of the nucleus, and translation occurs on a ribosome in the cytoplasm or ER. Before inflammasome activation, resting NLRP3 localizes to ER.[51] As an important component of the host innate immune system, NLRP3 is sensitive to alterations in many cellular pathways and involves several subcellular organelles for NLRP3 licensing, sensing signals, activation, and finally degradation. In recent years, more and more studies have revealed that the intracellular localization of NLRP3 is dynamically changing during inflammasome activation. In the following sections, we will focused on the NLRP3 inflammasome activation and regulation, and dissect the subcellular localization of NLRP3 in cells towards activation and degradation in response to stimuli, aiming for a systemic understanding of the mechanism of NLRP3 activation.

NLRP3 licensing in cytosol

Since its involvement in many immune responses, the activation of NLRP3 inflammasome must be strictly regulated. In addition to regulating its transcription level, increasing evidence shows that PTMs play crucial roles in NLRP3 inflammasome activation. Confocal microscopy imaging shows that most NLRP3 proteins localize to cytoplasmic granular structures before stimulation.[51] Indeed, PTMs usually occur in the cytosol in the NLRP3 priming step, and they are usually essential but insufficient for inflammasome assembly. NLRP3 phosphorylation at S194 site, which is catalyzed by c-Jun N-terminal kinase 1 (JNK1), facilitates NLRP3 homo-oligomerization and is essential for NLRP3 inflammasome activation.[52–54] Ubiquitin-specific peptidase 1 (USP1)-associated factor 1 (UAF1) removes the K48 polyubiquitination chain on NACHT and LRR domains to facilitate NLRP3 activation by preventing NLRP3 degradation via ubiquitination-dependent proteasome pathway.[55] Moreover, tripartite motif-containing protein 28 (TRIM28) catalyzes NLRP3 SUMOylation to inhibit NLRP3 ubiquitination and degradation through the proteasome pathway, causing the enhancement of NLRP3 inflammasome activation.[56]

PTMs on NLRP3 can occasionally play conflicting roles in regulating its activation. For instance, phosphorylation at Ser 5 site by AKT impedes inflammasome activation by inhibiting NLRP3 oligomerization. Surprisingly, Ser5 phosphorylation also prevents proteasome-mediated degradation of NLRP3 by TRIM31-mediated ubiquitination at Lys 496 during LPS priming.[57] This apparent paradox can be interpreted by the discovery that NLRP3 dephosphorylation at Ser5 site by protein phosphatase 2 A (PP2A) is able to promote inflammasome activation by strengthening the interaction between NLRP3 and ASC,[58,59] indicating that a cycle of phosphorylation in priming step and dephosphorylation in assembly step is required for proper NLRP3 activation. Similarly, both phosphorylation at Tyr32 mediated by phosphatase and tensin homolog (PTEN)[60] and acetylation of NLRP3 at Lys 21/22/24[61] enhance NLRP3 association with ASC. Studies on these licensing processes provide multiple strategies to interfere with NLRP3 inflammasome activation.

The role of mitochondria for NLRP3 inflammasome activation

Upon LPS priming, NLRP3 is induced and broadly distributed throughout the cell, ready to detect danger signals. Previous research has established that NLRP3 senses mitochondrial dysfunction, such as reactive oxygen species (ROS)[51] or mitochondria DNA.[62] Damaged or ROS-generating mitochondria activate the NLRP3 inflammasome directly.[51] Cytidine monophosphate kinase 2 (CMPK2), a rate-limiting enzyme that supplies deoxyribonucleotides, can be remarkably up-regulated via the LPS-induced TLR4-MyD88-TRIF pathway[62] to produce abundant new synthetic mitochondrial DNA (mtDNA). Subsequently, the inflammasome-activating stimuli such as nigericin elicit mitochondrial damage, inducing the release of newly synthesized mtDNA and increasing ROS production. ROS then convert mtDNA to an oxidized form (ox-mtDNA), which activates NLRP3 directly via association with ox-mtDNA.[62] A recent study further explored the mechanism of the upstream ox-mtDNA-mediated NLRP3 activation.[63] The glycolytic enzyme hexokinase (HK), which typically associates with the mitochondria outer membrane, can sense the degradated bacterial peptidoglycan component N-acetylglucosamine (NAG), and its dissociation from mitochondria alone is sufficient to induce NLRP3 inflammasome activation.[64] Mitochondria play a vital role in inflammation because they determines the fate of the cell, either inducing inflammation or apoptosis.

Interestingly, besides involving in the NLRP3 activation mechanisms, studies also have shown that NLPR3 localizes to mitochondria.[65] The mitochondrial antiviral signaling (MAVS) protein, an adaptor for antiviral innate immunity on the outer membrane of mitochondria, colocalizes with NLRP3 and interacts directly with the N-terminal 21 amino acids of NLRP3. It is the mediator that recruits NLRP3 to mitochondria.[65] In addition, in response to RNA virus infection, the mitochondrial fusion protein Mitofusin 2 (MFN2) enhances the association between NLRP3 and MAVS.[66] It is also reported that mitochondrial cardiolipin directly interacts with NLRP3, which is necessary for NLRP3 inflammasome activation. Saturated long-chain fatty acid palmitate or knockdown mitochondrial enzyme cardiolipin synthase (CLS) interferes with cardiolipin synthesis, affecting NLRP3 localization to mitochondria and NLRP3 inflammasome activation.[67] In general, NLRP3 mitochondrial localization, especially induced by the dysfunctional mitochondria, is essential for inflammasome activation and cytokines release.

The role of ER in NLRP3 inflammasome activation

Interestingly, for precise description, some studies have exhibited that upon activation, NLRP3 moves to the

perinuclear space defined as mitochondria-associated ER membranes (MAM), not free mitochondria.[58] This suggests that ER participates in the regulation of NLRP3 inflammasome activation, but the underlying mechanism is still elusive. It might be related to PTMs, which license or regulate NLRP3 activation, or it might require some specific elements from the ER to activate the NLRP3 inflammasome. MAMs are important for transfering lipids and Ca^{2+} from ER to mitochondria, and research has shown that the release of calcium stored in the ER is sufficient for NLRP3 inflammasome activation.[68] Other studies have showen that the ER surface protein STING mediates downstream events of NLRP3 inflammasome assembly. There are two models explaining the role of STING in this pathway: (i) it induces lysosomal cell death followed by ATP release as well as K^+ efflux, which in turn induces NLRP3 inflammasome activation in human monocytes[69]; (ii) STING recruits NLRP3 to the ER or interacts with NLRP3 and attenuates its K48- and K63-linked polyubiquitination, facilitating the inflammasome activation against herpes simplex virus type 1 (HSV-1) infection, likewise suggesting that NLRP3 ER localization is required for NLRP3 inflammatory response.[70]

Many studies have also argued that NLRP3 inflammasome activation is independent of STING, but relies indirectly on ER stress (ERS). Infection-associated ERS initiates IRE1α-dependent mitochondrial damage and ROS, which drives NLRP3 inflammasome activation in bone marrow-derived macrophages (BMDM).[71,72] In the livers of obese mice, LPS-induced ERS activates the NLRP3 inflammasome.[73] More intriguingly, MAVS, voltage-dependent anion channel 1 (VDAC1), glycogen synthase kinase-3 beta (GSK3β), inositol 1,4,5-trisphosphate receptor type 1 (IP3R1), and Mfn2, which modulate NLRP3 localization and activation, are reported to participate in mediating or regulating MAM structure and function, supporting the notion that this ER-mitochondria connection is required for NLRP3 inflammasome activation.[74]

The Golgi apparatus involves in the transportation and oligomerization of NLRP3

The first evidence showing that NLRP3's localization to the Golgi apparatus was initiated by cholesterol biosynthesis.[75] Inflammation has been linked to cholesterol metabolism, and NLRP3 inflammasome activation is integrated with the maturation of the cholesterol master transcription factor sterol regulatory element-binding protein2 (SREBP2), which is escorted by the SREBP cleavage-activating protein (SCAP) and transported via coat protein complex II (COPII) to the Golgi. It is then processed by Site-1 protease (S1P) and Site-2 protease (S2P) on the Golgi to initiate gene expression for cholesterol biosynthesis.[75] Research revealed that stimulation promotes SCAP-SREBP2 on the ER to interact with NLRP3 NACHT domain, and then this ternary complex is translocated via COPII to the Golgi apparatus adjacent to a mitochondrial cluster.[76] Notably, NLRP3 activation events seem to rely on the activity of ER-to-Golgi translocation for SCAP-SREBP2, but not cholesterol homeostasis controlled by the transcriptional activity of SREBP2, implying that ER-to-Golgi translocation is necessary for inflammasome activation. Coincidently, NLRP3 can co-translocate from the ER to the Golgi with the activation of activating transcription factor 6 alpha (ATF6), which is processed by S1P on the Golgi to release its N-terminal fragment in response to ERS.[77] Additionally, Zhang et al found that NLRP3 activators induce the accumulation of diacylglycerol (DAG) in the Golgi, recruiting protein kinase D (PKD) to phosphorylate NLRP3 at S293 on MAM close to the Golgi, releasing NLRP3 from ER. Deficient S293 phosphorylation or PKD inactivation retains NLRP3 at MAMs adjacent to the Golgi and prevents inflammasome activation, showing that the migration of NLRP3 from the ER to the Golgi is scheduled and under strict control, and is sufficient to induce NLRP3 inflammasome assembly.[54] Nevertheless, while previous NLRP3 localization is controversial, Arumugam et al provided direct evidence that NLRP3 is transiently associated with mitochondria and subsequently recruited to the Golgi over time.[78]

Following transportation from the ER to the Golgi, NLRP3 is mainly recruited to the dispersed trans-Golgi network (dTGN), through ionic interaction between the polybasic region in the conserved KKKK motif of NLRP3 and the negatively charged phosphatidylinositol-4-phosphate (PtdIns4P) on the dTGN.[79] Similarly, the IκB kinase β (IKKβ), a kinase that mediates IκB phosphorylation and degradation for NF-κB activation, is not required for nigericin-induced dTGN, but it is necessary to bring NLRP3 close to TGN38 on dTGN.[80,81] Moreover, disruption of the interaction between NLRP3 and PtdIns4P on the dTGN blocks NLRP3 puncta formation and downstream signaling.[79] On the contrary, forcing NLRP3 relocation onto the Golgi membrane by utilizing an OSBP-tag (a well-described Golgi-binding protein that contains a pleckstrin homology domain) is capable of facilitating inflammasome activation, indicating that NLRP3 localization at dTGN is critical for inflammasome activation.[79] Recently, structural studies revealed that NLRP3 exists mainly as a monomer or dimer in the cytosol, and NLRP3 activating signals stimulate NLRP3 to oligomerize on the Golgi membrane to form an inactive double-ring cage, where the PYDs are embedded inside the cage, and the LRRs mediate the cage formation via face-to-face and back-to-back interfaces.[41,42] Importantly, only this double-ring cage on the Golgi membrane, but not the monomer or dimer in the cytosol, prompts the consequent NLRP3 inflammasome assembly. Intriguingly, the polybasic motif associated with dTGN membrane is also essential for NLRP3 to form the double-ring cage, emphasizing that NLRP3 localization onto the Golgi is critical for the inactive double-ring cage formation. However, the spatial and temporal order of these two events, namely NLRP3 cage formation and TGN dispersion, is disputed. The Chen's lab suggested that the presence of dTGN may be a prerequisite for the recruitment, aggregation, and activation of NLRP3,[79] and the Wu's lab favored that NLRP3 cage is necessary for TGN dispersion, as observed from the fact that NLRP3 cage-defective mutation impairs TGN dispersion compared to wild type.[42] Growing evidence showed that NLRP3 is assembled on the Golgi rather than on MAMs and further on MTOC. It is possible that both proposals are correct, with the Golgi membrane recruiting NLRP3 to form the inactive double-ring cage first, followed by TGN dispersion and NLRP3 translocation to MTOC, where a reassembling process occurs to form NLRP3 fiber mediated by PYDs. This

process then recruits ASC and caspase-1 for inflammasome activation. Interestingly, a very recent study demonstrated that NLRP3 activators induce PtdIns4P accumulation on endosomes, which recruit NLRP3 to endosomes for subsequent inflammasome activation.[82] This study further confirmed interactions between NLRP3 and PtdIns4P either on TGN or endosomes and revealed NLRP3 localization to Golgi and endosomes, but also raised more questions about the precise roles for Golgi and endosome in NLRP3 inflammasome activation.

NLRP3 inflammasome activation on MTOC

Although the mechanism underlying how NLRP3 exits the Golgi remains unknown, the process by which NLRP3 migrates to MTOC and assembles onto the NLRP3/ASC speck is well-established.[43] It is well documented that NLRP3 is finally delivered to MTOC and interacts with NEK7, and then assembles into an activated inflammasome. NEK7 is a centrosomal kinase that serves as a mutually exclusive switch between inflammasome response and cell division. It was first identified to interact with NLRP3 by a genome-wide CRISPR (clustered regularly interspaced short palindromic repeats) screen,[83] and afterward, He et al demonstrated that NEK7 is required for inflammasome activation,[45] and Sharif et al finally presented a cryo-EM structure of the NLRP3-NEK7 complex, conforming that NEK7 binds to the LRR domain of NLRP3 to license the NLRP3 inflammasome activation at MTOC,[44] which might be the last license NLRP3 get before activation. Interestingly, the NEK7-activated NLRP3 assembls into a flower-shaped disk with PYDs gathered together to form a filament was determined by cryo-EM recently,[46] representing a milestone in the NLRP3 inflammation field.

The following discoveries provide more information about how and where NLRP3 binds to NEK7 in centrosomes. In 2017, Li et al showed that the catalytic domain of microtubule affinity-regulating kinase 4 (MARK4) directly interacts with NLRP3 via Pyrin-NACHT domain, driving NLRP3 transportation to MTOC. Knocking down MARK4 or interfering with the interaction between MARK4 and NLRP3 can severely affect the spatial localization of NLRP3 in cells, leading to the failure of NLRP3 arriving at MTOC and the abrogation of inflammasome activation.[84] Next, Magupalli et al showed that HDAC6-dynein, a transporting system required for pathological aggregates to transport to MTOC for degradation, is essential for NLRP3 localization at MTOC and inflammasome activation. Interestingly, NLRP3 activation is independent of the deacetylase activity of HDAC6 but relies on the ubiquitin-binding domain of HDAC6. However, it remains unknown which components in the inflammasome are ubiquitinated and bind HDAC6 to mediate the transportation.[43] Note that microtubules participate in NLRP3 transportation, they also play a role in NLRP3 inflammasome activation. The aberrant mitochondrial homeostasis causes a reduction of NAD^+ concentration, which in turn inactivates the NAD^+-dependent α-tubulin deacetylase sirtuin 2. Loss of deacetylation results in the accumulation of acetylated α-tubulin, which mediates the dynein-dependent transportation of mitochondria and subsequent NLRP3 inflammasome activation.[85]

More recent studies explored the regulatory mechanism of this process via PTMs. The centrosomal spermatogenesis-associated protein Spata2 recruits the deubiquitinase CYLD to the centrosome to deubiquitinate polo-like kinase 4 (PLK4), which subsequently binds to and phosphorylates NEK7 at S204, thus reducing the interaction between NEK7 and NLRP3, and inhibiting the activation of the NLRP3 inflammasome.[86] The Krebs cycle-derived metabolite itaconate and its derivative 4-octyl itaconic acid (4-OI) disrupt the interaction between NLRP3 and NEK7 by inducing "dicarboxypropylated" modification at the Cys 548 site of NLRP3.[87] Furthermore, casein kinase 1A1 (CSNK1A1) phosphorylates NLRP3 at the S803 site, attenuating the interaction between NLRP3 and NEK7 and promoting the ubiquitinated degradation of NLRP3.[88] More regulatory mechanisms are expected to be discovered in the future, as the final activation step is vital for the physiological and pathological roles of the NLRP3 inflammasome.

NLRP3 degradation at autophagosomes or lysosomes

The NLRP3 inflammasome is a critical mediator for innate immune response, and it is always under strict control at every step. As described above, the priming and activation are tightly orchestrated. On the other end, after pathogen clearance or unexpected activation, NLRP3 needs to be transferred from centrosomes to lysosomes for degradation to prevent excessive inflammation. Activated NLRP3 inflammasome is prone to be degraded by lysosomal pathway other than proteasomal pathway after activation, probably because enormous amounts of inflammatory components are more suitable for rapid degradation by the former.[89] Several partner proteins mediate NLRP3 into degradation. Large-scale genome-wide association studies identified a protective function of Immunity Related GTPase *M* (IRGM) against Crohn's disease and other inherited inflammatory diseases.[90,91] Mechanistically, IRGM interacts with NLRP3 as a scaffolding protein, recruiting autophagy bridging protein p62, autophagy initiation protein Beclin 1, and elongation protein autophagy-related 16-like 1 (ATG16L1), facilitating the localization and degradation of NLRP3 in autophagic vesicles.[92] Simultaneously, the autophagy-associated protein Beclin 2 can interact with NLRP3 through its CCDECD structural domain, which promotes the localization and degradation of NLRP3 in lysosomes through a ULK1/ATG9A-dependent non-classical autophagic pathway.[93]

PTMs also play essential roles in mediating NLRP3 degradation, especially before activation. Without exception, all well-known ubiquitination on NLRP3 is intended to target NLRP3 for degradation.[94] Ubiquitin-specific proteinase 5 (USP5), a deubiquitinating enzyme localized to the lysosome/autophagosome, inhibits LPS and ATP-triggered IL-1β production and attenuates alum-induced peritonitis by mediating NLRP3 into degradation. In-depth mechanistic studies revealed that USP5 acts as a scaffolding protein that recruits the E3 ubiquitin ligase membrane-associated RING—CH—type finger 7 (MARCH7), selectively promotes NLRP3 K48-linked polyubiquitination modifications, and

mediates NLRP3 entry into autophagosome to promote NLRP3 protein degradation.[95] NLRP3 has also been reported to be ubiquitinated at K380 site by K27-linked ubiquitin to block proteasomal degradation by β-transducin repeat containing E3 ubiquitin protein ligase 1 (β-TrCP1).[96] Furthermore, the K63-linked polyubiquitination of NLRP3 LRR domain mediated by ring finger protein 125 (RNF125) recruits Casitas-B-lineage lymphoma protein-b (Cbl-b) via binding its ubiquitin-associated region, which in turn ubiquitinates NLRP3 at K496 within its NBD domain and further targets NLRP3 to the proteasomal degradation.[97] Collectively, NLRP3 is degraded by the ubiquitination-proteasomal pathway to reduce its abundance and prevent activation. Usually, ubiquitination occurs in the rest state while NLRP3 is in the ER, however, degradation may occur in the priming step before inflammasome activation, or after NLRP3 finishes its role, in which the modification serves as a token for the final degradation.

Some other PTMs can also serve a similar role in NLRP3 degradation. For example, phosphorylation of NLRP3 at Tyr861 facilitates its sequestration in autophagosomal precursors phagosomes, which are then transported to lysosomes for degradation. On the other hand, protein tyrosine phosphatase non-receptor 22 (PTPN22) interacts directly with NLRP3, leading to dephosphorylation of the Tyr861 residue and sustained activation of the NLRP3 inflammasome.[98]

Targeting the NLRP3 inflammasome activation pathway

The importance of NLRP3 inflammasome activation in human diseases has drawn significant attention to targeting the NLRP3 signaling pathway. Understanding the NLRP3 inflammasome activation pathway has led to the identification of several NLRP3 inhibitors for research or clinical trials (summarized in Table 1). The diarylsulfonylurea compound MCC950, which binds to walker B motif of the NATCH domain and inhibits ATPase activity, is the most potent and specific inhibitor for NLRP3 inflammasome activation.[41,99] MCC950 is widely used in lab research. OLT1177, a β-sulfonyl nitrile molecule reported to covalently modify the NATCH domain, is under clinical trial to treat acute gout and heart failure.[100] It blocks the interaction between NLRP3 and ASC. Oridonin, a traditional Chinese herbal medicine used to treat inflammatory illnesses, has been shown to specifically inhibit the NLRP3 inflammasome. Mechanistically, oridonin forms an irreversible covalent bond with NLRP3 Cys 279. As Cys279 is in the NBD subdomain but not on the interface between NLRP3 and NEK7, oridonin binding may affect the NATCH domain confirmation to prevent the interaction between NLRP3 and NEK7. It has sound therapeutic effects in animal models of peritonitis, gout, and type 2 diabetes.[101] Tranilast is an analog of a tryptophan metabolite, which directly binds to the NLRP3 NACHT domain and prevents NLRP3 oligomerization via blocking direct NLRP3-NLRP3 interaction. Tranilast has strong therapeutic and preventative effects in mouse models of T2D, CAPS, and gout. Based on the high safety level of tranilast in the clinical trial, it shows significant value for treating NLRP3-driven disorders.[102] Given the ingenuity in dynamic subcellular localization for NLRP3 inflammasome activation, pharmacological inhibition of NLRP3 translocation between organelles can also mitigate NLRP3-mediated inflammatory disorders. Indeed, several inhibitors have been developed in this direction. Betulin and fatostatin, both of which inhibit SCAP-SREBP2 interaction to prevent ER-to-Golgi translocation, significantly decrease ATP-induced IL-1β or IL-18 secretion in a dose-dependent manner.[76] Tubastatin A, a specific pharmacological inhibitor of HDAC6, decreases NLRP3 inflammatory response in iBMDM and SH-SY5Y cells.[43,103] Consistently, microtubule-disrupting drugs colchicine and nocodazole are competent to reduce the interaction between MARK4 and NLRP3, thus down-regulating NLRP3 inflammasome activation.[84] In general, a comprehensive understanding of the dynamic NLRP3 journey in cells will continue to shed new light on inhibiting NLRP3 inflammasome activation.

Table 1 NLRP3 inflammasome inhibitors.

Inhibitor	IC_{50}	Inhibition mechanism	Specificity	Clinical status
MCC950	8 nM	Bonds to walker B motif of NATCH domain and inhibits ATPase activity	NLRP3	Phase II
OLT1177	1—100 nM (mouse) 1 uM (human)	Blocks the interaction between NLRP3 and ASC	NLRP3	Phase II
Oridonin	0.5 uM	Prevents the interaction between NLRP3 and NEK7	NLRP3	—
Tranilast	25 uM	Blocks direct NLRP3-NLRP3 interaction	NLRP3	Approved
Betulin	5 uM	Inhibits SCAP-SREBP2 ER-to-Golgi translocation	SCAP	—
Fatostation	5 uM	Inhibits SCAP-SREBP2 ER-to-Golgi translocation	SCAP	—
Tubastatin A	50 uM	A specific inhibitor of HDAC6	HDAC6	—
Colchicine	10 uM	Reduces the interaction between MARK4 and NLRP3	Microtubule	—
Nocodazole	10 uM	Reduces the interaction between MARK4 and NLRP3	Microtubule	—

In addition to canonical inflammatory diseases, accumulating evidence shows that the NLRP3 inflammasome is tightly related to heart disease and neurological disease.[104] Indeed, treatments targeting NLRP3 gain benefits in both pre-clinical and clinical trials of these diseases. For example, MCC950 and oridonin treatment alleviate cardiac fibrosis in a mouse model of acute myocardial infarction (MI),[105] and OLT1177 has been used to improve symptom severity in patients with systolic heart failure.[106] Furthermore, MCC950 has been reported to mitigate the severity of traumatic brain injury,[107] and Tranilast is potent in inhibiting inflammation in the acute phase of traumatic spinal cord injury in experimental animal models.[102] Thus, targeting NLRP3 inflammasome activation not only benefits autoimmune diseases but also shows encouraging progress in cardiovascular diseases and central nervous system (CNS) diseases.

Discussion

NLRP3 is a cytosolic pattern recognition receptor that senses and eliminates pathogenic invaders and damaged cells through assembling inflammasome with ASC and pro-caspase-1, leading to caspase-1 activation as well as subsequent cytokine release and pyroptosis. Three models for NLRP3 activation have been described, namely canonical, non-canonical, and alternative pathways. The canonical pathway, which includes a priming step and an activation step, has been well characterized, although many unknowns remain to be discovered. Our current knowledge has shown that NLRP3 activation is very complicated and is fine regulated by many factors or pathways. Dysregulation of NLRP3 activation causes a range of diseases including CAPS, type-II diabetes, gout, atherosclerosis, and cancers. Over the past two decades, studies on the modulation of NLRP3 activation mainly focused on PTMs of NLRP3, particularly ubiquitination and phosphorylation. However, increasing evidence emerging in recent five years indicates that NLRP3 translocation among organelles, referring to the journey of NLRP3 in cells, is crucial for NLRP3 inflammasome activation.

The NLRP3 journey in immune cells from priming to activation and degradation is schematically presented in Figure 3. The priming signals from outside or inside of the cell induce transcription of NLRP3, and NLRP3 mRNA comes out of the nucleus and translates into the cytosol or ER. Before activation signals arrive, NLRP3 is in the resting state and exists mainly as a monomer or dimer in the cytoplasm. PTMs usually occur in the priming step and are usually essential but insufficient for inflammasome assembly. PTMs, especially phosphorylation and ubiquitination, alter the conformation of NLRP3, which may impact the oligomerization and assembly of the inflammasome. In response to exogenous or endogenous stimuli which exert specific stresses on multiple organelles and lead to organelle dysfunction, NLRP3 identifies or binds to specific substances released by these organelles, followed by locating

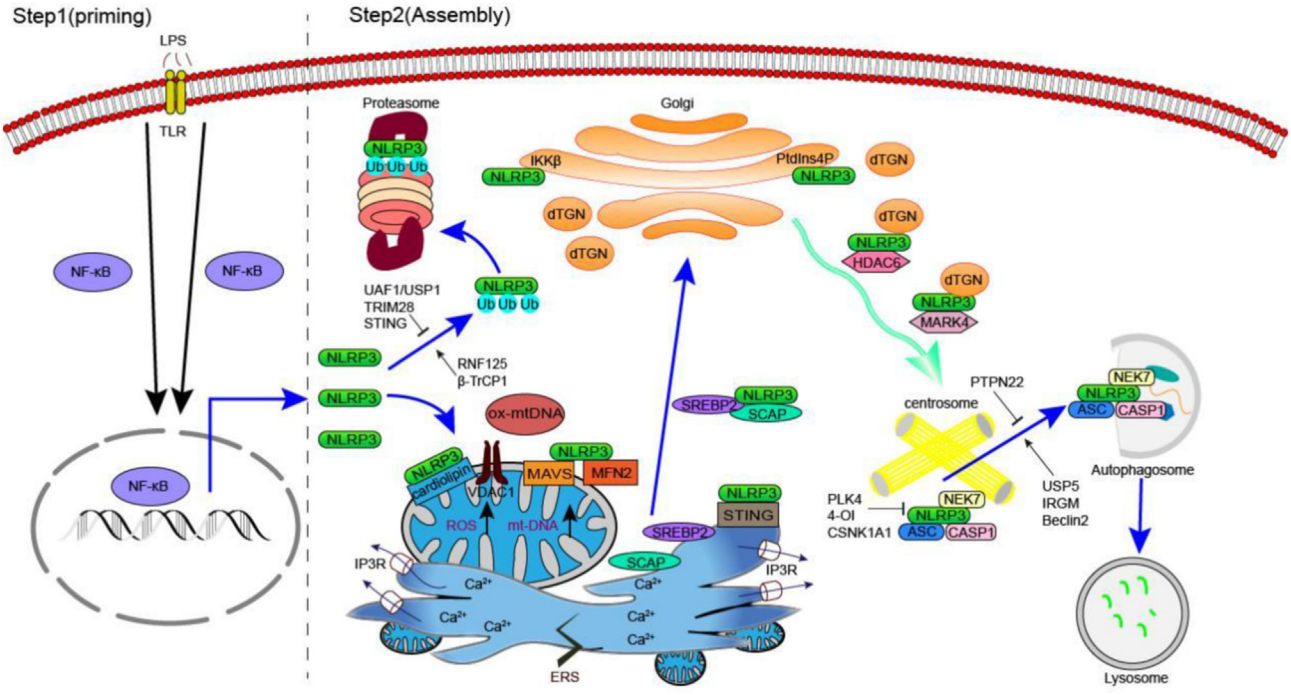

Figure 3 Spatiotemporal journey of NLRP3 for inflammasome activation. LPS induces the expression of NLRP3 via the NF-κB pathway, and then NLRP3 exists mainly as a monomer or dimer in the cytoplasm. When NLRP3 senses mitochondrial dysfunction or ERS, it is recruited to mitochondria and ER by binding to cardiolipin, MAVS, STING and other molecules. Next, NLRP3 associates with SCAP-SREBP2 to form a ternary complex that translocates to the Golgi where NLRP3 incorporates with dTGN through the interaction between polybasic region and PtdIns4P. Finally, NLRP3 is delivered to MTOC by the HDAC6-dynein transport system or MARK4 along the microtubules, which leads to the interaction with NEK7 to facilitate NLRP3 inflammasome assembly. After the removal of the danger signals, the NLRP3 inflammasomes on MTOC is captured by autophagosomes and sent to lysosomes for degradation.

onto them, such as mitochondria and ER. It is initially attracted to mitochondria by proteins such as MAVS, or by cardiolipin, or it might bind to ER through STING. Afterward, by binding with SCAP-SREBP2, NLRP3 on MAMs hitches a ride on COPII to Golgi, where NLRP3 incorporates with dTGN through interaction between the polybasic motif and PtdIns4P. This polybasic motif is necessary for NLRP3 to form the inactive double-ring oligomer, and the association with TGN membrane is also required for this assembly. After leaving dTGN, NLRP3 is captured and delivered to the MTOC by the HDAC6-dynein transport system or MARK4 along the microtubules. NEK7 meets NLRP3 at the MTOC, driving the conformation shift of the NLRP3 cage (may have other factors involved) for NLRP3 PYD exposure and fiber formation. NLRP3 PYD fiber then transits to ASC PYD fiber, which recruits pro-caspase-1 to form a large-scale multimeric inflammasome that promotes the cytokine maturation and release. Since NEK7 is an indispensable regulator of NLRP3, some metabolites and signal pathways affect NLRP3 inflammasome activation by disrupting the interaction between NEK7 and NLRP3. Several inhibitors targeting NEK7-NLRP3 interaction have been explored to treat inflammatory diseases. After the removal of the danger signals, NLRP3 inflammasomes on MTOC are captured by autophagosomes and sent to lysosomes for degradation, impeding excessive inflammation. Through removing NLRP3 inflammatory components, autophagy also involves in suppressing inflammasome activation, thus providing negative feedback loops that are essential for maintaining the balance between defensive inflammatory response and excessive inflammation.

The engagement of multiple organelles in NLRP3 activation is necessary for the proper assembly and function of the inflammasome. It raises an obvious and intriguing question: Why does the NLRP3 inflammasome activation necessitate the engagement of so many organelles? It is possible that the double-ring cage, the essential intermediate form of NLRP3, must be formed on the Golgi, which is the assembly plant of nascent proteins. MTOC, in addition to organizing the generation of microtubules, assists ASC filament nucleation and acts as a hub to promote the fusion of inflammasome-containing autophagosomes with lysosomes for inflammasome degradation. The precise control of NLRP3 subcellular localization is critical for inflammasome activation. Dysregulation in any of these steps could lead to the development of inflammatory diseases, such as autoimmune diseases. Therefore, further exploration of dynamic localization and regulation of NLRP3 will lead to a more comprehensive understanding of NLRP3 activation and its implications in human diseases.

Conflict of interests

The authors declare no conflict of interests.

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