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The role of mitochondria in NLRP3 inflammasome activation

Qiuyun Liu[a], Danyan Zhang[a], Diyu Hu[a], Xiangmei Zhou[b], Yang Zhou[a,*]

[a] College of Animal Science, Southwest University, Chongqing 402460, China
[b] State Key Laboratories for Agrobiotechnology, Key Laboratory of Animal Epidemiology of the Ministry of Agriculture, National Animal Transmissible Spongiform Encephalopathy Laboratory, College of Veterinary Medicine, China Agricultural University, Beijing 100193, China

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ABSTRACT

The NLRP3 inflammasome is a multiprotein platform which is activated upon cellular infection or stress. Its activation leads to caspase-1-dependent secretion of proinflammatory cytokines like interleukin-1β (IL-1β) and IL-18, and an inflammatory form of cell death termed as pyroptosis. Recent studies have unveiled the pivotal roles of mitochondria in initiation and regulation of the NLRP3 (nucleotide-binding domain, leucine-rich-repeat containing family, pyrin domain-containing 3) inflammasome. NLRP3 activators induce mitochondrial destabilization, NLRP3 deubiquitination, linear ubiquitination of ASC, and externalization or release of mitochondria-derived molecules such as cardiolipin and mitochondrial DNA. These molecules bind to NLRP3 that is translocated on mitochondria and activate the NLRP3 inflammasome. Here we review recently described mechanisms by which mitochondria regulate NLRP3 inflammasome activation.

1. Introduction

A function of immune system is to engage an array of sensors to detect pathogens or cellular stress and stimulate release of danger signals which activate inflammatory process for host defense. Meanwhile, immunopathology and detrimental effects of inflammation should be limited. Sensors of innate immune system include pattern-recognition receptors (PRRs) which monitor extracellular and intracellular milieu via detecting pathogen-associated molecular patterns (PAMPs), microbial structures, as well as host-derived danger signals termed danger-associated molecular patterns (DAMPs), for example uric acid crystals and ATP (Schroder and Tschopp, 2010).

PRRs have several subgroups, such as toll-like receptors (TLRs), C-type lectin receptors (CLRs), nucleotide-binding oligomerization domain-like receptors (NLRs), RIG-I-like receptor (RLR) and AIM2-like receptor (ALR) (Lamkanfi and Dixit, 2014). NLRs and TLRs are evolutionarily-conserved sensors. They cooperate to mount an inflammatory response. Among NLRs, NLRP3 is the most extensively described (Subramanian et al., 2013). NLRP3 connects to caspase-1 via apoptosis associated speck-like protein containing a caspase recruitment domain (ASC) to form a macromolecular complex called the NLRP3 inflammasome in response to PAMPs or DAMPs and induces the release of proinflammatory cytokines and caspase-1-dependent pyroptosis (He et al., 2016a).

Mitochondria are dynamic double membrane-bound organelles found in eukaryotic organisms. They possess many functions besides ATP production, such as apoptosis, calcium signaling, synthesis of amino acids, lipids and haem (West et al., 2011). Emerging evidences indicate that mitochondria play a vital role in NLRP3 inflammasome activation. This review focuses on the effect of mitochondrion-derived or –resided molecules, mitochondrial destabilization and relevant signaling on NLRP3 inflammasome activation.

2. NLRP3 inflammasome activation

NLRP3 contains an N-terminal pyrin domain (PYD), a central nucleotide binding or oligomerization domain (NACHT), and a C-terminal leucine-rich repeats (LRRs) motif (Schroder and Tschopp, 2010). LRRs are associated with NLRP3 activity by regulation of NLRP3 ubiquitination (Py et al., 2013), interaction with NLRP3 inducers (Bruchard et al., 2013), autoregulation by inhibiting spontaneous oligomerization, and PAMP sensing (Kanneganti et al., 2007). Upon stimulation, NLRP3 self-oligomerization results in PYD clustering and recruitment of ASC which in turn interacts with procaspase-1. Procaspase-1 clustering leads to its autocleavage and formation of the active caspase-1 which induces the secretion of active proinflammatory cytokines such as IL-1β and IL-18 (Schroder et al., 2010). Other immune receptors are also required to engage this signaling pathway that activates NLRP3 (Fig. 1). Aberrant NLRP3 inflammasome activation is pathogenic and associated with a lot of diseases including central nervous system (CNS) diseases. Hence,

* Corresponding author.
E-mail address: yzhou@swu.edu.cn (Y. Zhou).

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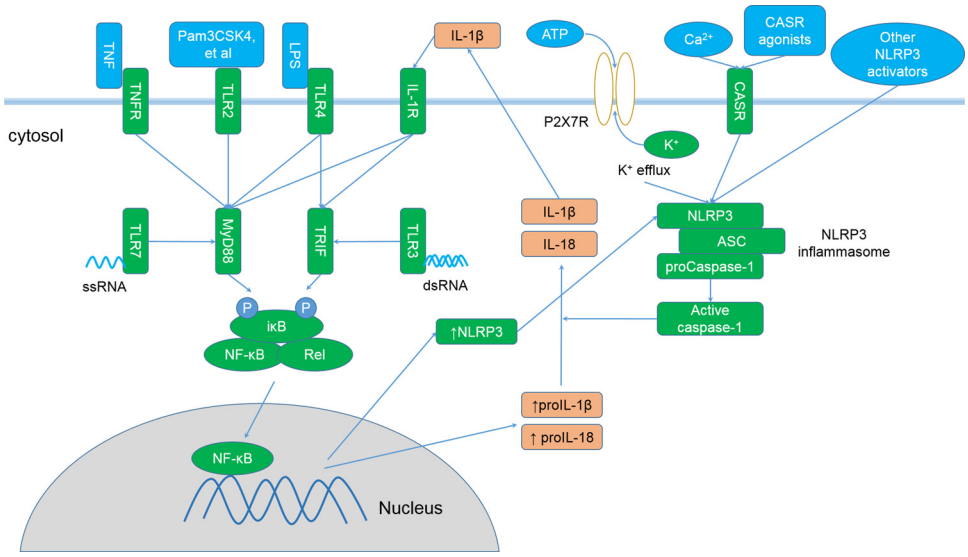

Fig. 1. NLRP3 inflammasome activation. PRRs, including TLR2/3/4/7, or cytokine receptors, including TNFR and IL-1R, activate NF-κB signaling in response to their ligands, resulting in increased expression of NLRP3, proIL-1β and proIL-18. NLRP3 inflammasome assembly is triggered by ATP which induces K^+ efflux through P2X 7-dependent pore formation, increased intracellular Ca^{2+} concentration through interaction between CASR and its agonists, or other mechanisms. Procaspase-1 clustering in the NLRP3 inflammasome enables its autocleavage and activation, and process proIL-1β and proIL-18 into the mature forms (Sutterwala et al., 2014).

NLRP3 could be considered as a new target in the prevention and treatment of CNS diseases (Liu et al., 2013).

NLRP3 inflammasome is activated upon exposure to a broad range of signals, including ATP (Pelegrin and Surprenant, 2006), nigericin (Pelegrin and Surprenant, 2007), monosodium urate (MSU) (Martinon et al., 2006), fungi (Hise et al., 2009), bacteria that produce pore-forming toxins (Gurcel et al., 2006), and viruses (Allen et al., 2009). Chronic ethanol treatment leads to TLR4-mediated impairment of blood-brain barrier, microglia infiltration and NLRP3 inflammasome activation in microglial cells (Alfonso-Loeches et al., 2016). The structural diversity within NLRP3 activators argues against direct interaction between NLRP3 and all these activators. Emerging studies support a model in which innate immune system detects endogenous indicators of cellular danger or stress rather than a simplistic self/nonself recognition model (Schroder and Tschopp, 2010).

It is widely believed that NLRP3 inflammasome activation requires two signals: signal I induces NF-κB-dependent expression of both proIL-1β and NLRP3 in response to activation of transcription-modulating PRRs, such as TLRs, or of proinflammatory cytokine receptors, which is called priming, and signal II triggers the assembly and activation of the NLRP3 inflammasome (Bauernfeind et al., 2009; Prochnicki and Latz, 2017; Yu and Lee, 2016). For example, ethanol (signal I) activates the NLRP3 inflammasome in glial cells by inducing increased NLRP3 expression and TLR4-dependent proIL-1β production along with mitochondrial reactive oxygen species (mROS) formation (signal II) to trigger caspase-1 maturation and IL-1β secretion (Alfonso-Loeches et al., 2014; Montesinos et al., 2016). Basal level of proIL-1β is very low in unstimulated cells (Zhou et al., 2016). Priming with TLR2 ligand Pam3CSK4, TLR4 ligand LPS or TLR7 ligand R848 induces increased production of proIL-1β, as well as caspase-1 maturation and active IL-1β secretion in ATP-stimulated cells (Chuang et al., 2011; Scheibel et al., 2010), while stimulation with ATP without priming fails to activate the NLRP3 inflammasome (Juliana et al., 2012). Priming with different concentrations of LPS leads to caspase-1 maturation in ATP-treated macrophages in a dose-dependent manner (Bauernfeind et al., 2009). However, this two-step model is not always applicable. Lin and colleagues suggested that the early phase of NLRP3 inflammasome activation, rapid NLRP3 inflammasome activation, is independent of priming, which is directly controlled by TLR signaling via IL-1 receptor-associated kinase 1 (IRAK-1), while the late phase is priming-dependent (Lin et al., 2014).

Mechanisms by which the NLRP3 inflammasome is activated are controversial and several models have been proposed, including K^+ efflux (Munoz-Planillo et al., 2013; Petrilli et al., 2007), ROS (Cruz et al., 2007; Dostert et al., 2008; Zhou et al., 2011), release of cathepsin B from damaged lysosomes (Hornung et al., 2008), and Ca^{2+} mobilization (Murakami et al., 2012). Among these models, ROS has been demonstrated to play a role in the priming step, but not in the activation step (Bauernfeind et al., 2011). IL-1β secretion and caspase-1 cleavage induced by cathepsin B-deficient bone marrow-derived macrophages (BMDMs) is comparable to that of WT BMDMs in response to hemozoin, MSU or alum (Dostert et al., 2009).

3. Mitochondria-associated endoplasmic reticulum (ER) membranes (MAMs): location of NLRP3 inflammasome assembly?

Mitochondria physically interacts with ER via mitochondrial outer membrane protein Mitofusin 2 (Mfn2), leading to formation of a distinct membrane compartment between these organelles, known as MAM (de Brito and Scorrano, 2008). MAM plays key roles in material transfer and signal transduction, including Ca^{2+} signaling. Recent studies have shown the essential involvement of MAM in initiation and regulation of the innate immune system (Hayashi et al., 2009; Misawa et al., 2017).

In inactivated form most of the NLRP3 protein resides on ER. Upon stimulation with NLRP3 inducers, NLRP3 and ASC colocalize with MAMs in the perinuclear space (Lee et al., 2016; Zhang et al., 2017; Zhou et al., 2011). N-terminal residues 2–7 of NLRP3 are necessary for its mitochondrial location (Subramanian et al., 2013). Reports of subcellular location of resting ASC vary significantly. Misawa and colleagues found that endogenous ASC is located in the mitochondria in BMDMs. Dynein-dependent transport of mitochondria along microtubules contributed to the approximation of ASC on mitochondria to NLRP3 on ER in the perinuclear region in response to NLRP3 inducers (Misawa et al., 2013). In contrast, Zhou and colleagues reported most ASC was cytosolic, and no or very little colocalization of ASC and mitochondria was observed in resting THP-1 cells (Zhou et al., 2011). Sagulenko and colleagues' study showed that ASC was predominantly nuclear in the absence of a stimulus in BMDMs (Sagulenko et al., 2013).

However, Zhang and colleagues' study showed that following stimulation with various NLRP3 inflammasome activators, mitochondria primarily cluster around the Golgi apparatus. Diacylglycerol (DAG) rapidly accumulates in Golgi, and recruits protein kinase D (PKD). PKD at the Golgi contributes to ASC oligomerization, phosphorylation of NLRP3 at Ser293, release of NLRP3 from MAMs, and resultant assembly of the fully mature NLRP3 inflammasome in the cytosol. PKD inactivation by the inhibitor or *PKD1-PKD3* (two members of PKD family) double-KO causes retention of NLRP3 at MAMs adjacent to Golgi and