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The enigma of inflammasome activation by kinases

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MARK4; JNK; NLRP3; ROS; kinases; inflammasome

Inflammasomes are large multiprotein complexes composed of signal sensing platform proteins (e.g., NOD-, LRR- and pyrin domain-containing 3 [NLRP3], absent in melanoma 2 [AIM2] and NLR Family CARD Domain Containing 4 [NLRC4]), adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) and effector protein caspase-1. These complexes serve as signaling platforms that activate caspase-1, which in turn cleaves and processes inflammatory cytokines such as IL-1β and IL-18 and triggers the inflammatory response. Active caspase-1 also cleaves the pore-forming protein gasdermin D and generates the amino-terminal fragment, which oligomerizes and forms membrane pores in the plasma membrane, resulting in potassium efflux and pyroptosis. The subsequent potassium efflux leads to further NLRP3 activation.[1] By inducing the robust inflammatory response and pyroptotic cell death, the inflammasome cascade contributes to tissue inflammation and destruction in many cardiovascular diseases including atherosclerosis. Thus, better understanding of the regulation of inflammasome and identification of the molecules responsible for inflammasome activation are critical for developing effective therapies to treat diseases in which activation of inflammasome is critically involved.

Inflammasome is activated by an array of intracellular and extracellular stresses including potassium efflux, metabolic stress, reactive oxygen species (ROS), ER stress and DNA damage. These stress conditions and factors, through various pathways, activate inflammasome by inducing the transcription or the posttranslational modifications (e.g., phosphorylation and ubiquitylation) of the key components in the inflammasome complex. Increasing evidence suggests that phosphorylation of the inflammasome components[2–7] represents an important mechanism for inflammasome activation. The inflammasome activating signals induce a series of site-specific phosphorylation in different domains of inflammasome proteins (e.g., NLRP3, ASC) and alter their conformation, allowing their interaction, leading to inflammasome assembly and activation.

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In this issue of ATVB, Li *et al*. identified microtubule-affinity regulating kinase 4 (MARK4) as a crucial upstream regulator for inflammasome activation in atherosclerosis. The authors show that in human atherosclerotic lesions, MARK4 expression was increased and that it co-localized with NLRP3 in macropahges. The expression of MARK4 and NLRP3 in the atherosclerotic lesions was associated with the production of active IL-1β and IL-18 within the plaque. In Ldlr-deficient mice that were fed a high-fat and cholesterol diet, *Mark4* deficiency in bone marrow cells led to a reduction in lesion size and the levels of circulating IL-18. The authors further show that *Mark4* deficiency in macrophages inhibited cholesterol crystal-induced NLRP3 inflammasome activation. Thus, these findings support a critical role of MARK4 in inflammasome activation and atherosclerotic plaque development. Although Li *et al*. have nicely shown the key role of MARK4 in regulating NLRP3 activation and the co-localization of MARK4 and NLRP3, the exact molecular mechanism how MARK4 regulates NLRP3 during the process of atherosclerosis remains unclear. MARK4 has been shown to induce c-Jun N-terminal Kinase (JNK) activation.[8] JNK induces NLRP3 phosphorylation at S198 (S194 in mouse Nlrp3), which is critical for NLRP3 deubiquitination and the subsequent inflammasome assembly.[7] Nlrp3 activation is disrupted in *Nlrp3-S194A* knock-in mice,[7] also supporting the key role of this phosphorylation *in vivo*. Therefore, in addition to the microtubule-dependent mechanism,[9] it is an attractive hypothesis that MARP4-mediated JNK activation is also involved in NLRP3 activation.

A number of kinases have been shown to regulate NLRP3 activation. One of the most established phosphorylation sites in NLRP3 is S295 located in the NACHT domain (Figure).[10] Both protein kinase A (PKA) and protein kinase D (PKD) can phosphorylate this site, but with opposite consequences. While PKA-mediated S295 phosphorylation is inhibitory[11], PKD-mediated S295 phosphorylation is necessary for NLRP3 activation[6]. The details of this discrepancy were discussed by Sandall *et al*.[10] NLRP3 ubiquitination, which can be regulated by S295 phosphorylation, may play a role in PKA- and PKD-mediated regulation of NLRP3 activation.[10]

In addition to the serine/threonine kinases, tyrosine kinases also contribute to the regulation of NLRP3 activation[10] (Figure). Spleen tyrosine kinase (SYK), a nonreceptor protein tyrosine kinase (TK), plays a crucial role in signaling by specific immune receptors such as Toll-like receptors, C-type lectin receptors (CLRs), and NOD-like receptors (NLRs).[12, 13] SYK can phosphorylate ASC, a component of the inflammasome complex, at Y146, which is critical for ASC oligomerization and caspase 1 activation.[2–5] Proline-rich TK 2 (PYK2), another non-receptor protein TK with a role in integrin-induced cell migration, can directly phosphorylate ASC at Y146 and activate NLRP3.[14] Briton's tyrosine kinase (BTK) is another non-receptor TK, which also plays a crucial role in TLR signaling[15], and induces ASC oligomerization and caspase-1 activation in a kinase activity-dependent manner, but the phosphorylation sites of NLRP3 and ASC remain unclear[16, 17].

It is noteworthy to discuss that kinases can also regulate NLRP3 activation through ROS production. It has been reported that ROS induces inflammasome activation by regulating the interaction between thioredoxin (TRX)and TRX interacting protein (TXNIP) (Figure).[18] TRX and TXNIP bind and mutually inhibit. However, after ROS stimulation, TRX releases TXNIP, and free TXNIP associates and activate NLRP3.[19] ROS can also activate NLRP3 by

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regulating the interaction between NIMA related kinase 7 (NEK7) and NLRP3. NLRP3 agonists increase potassium efflux, and subsequently increase mitochondrial ROS (mtROS) production. mtROS induces chloride intracellular channels (CLIC) to translocate to the plasma membrane and subsequent chloride efflux, which promotes NEK7-NLRP3 association and NLRP3 activation.[20] Interestingly, this is induced by a kinase activation independent mechanism. These data suggest that ROS takes in a key role for NLRP3 activation by multiple mechanisms.

The ROS-mediated NLRP3 activation reminds us of the fact that the molecular mechanisms underlying inflammasome regulation by several kinases remain unclear. The phosphorylation sites on NLRP3 induced by these kinases have not been identified. It is plausible that these kinases are somehow involved in activation of NLRP3 via increasing ROS (Figure). For example, Death-Associated Protein Kinase (DAPK) can associate with NLRP3 and induce IL-1β production.[21] DAPK3 can also mediate TNFα-induced JNK, p38, and AKT activation via ROS production.[22] Therefore, it is possible that DAPK activates NLRP3 via ROS or ROS-mediated JNK activation. Another example is interleukin-1 receptor-associated kinase 1 (IRAK1). Lin *et al* have reported that IRAK-1 associates with NLRP3 and increases NLRP3 activation and pyroptosis.[23] Although they suggested that this is direct regulation by binding, it is well known that IRAK-1 can induce ROS,[24, 25] and one cannot exclude the possible indirect involvement of ROS in IRAK-1-mediated NLRP3 activation. To clarify the binding site and to specifically inhibit the complex formation with NLRP3 in the absence of ROS production would be necessary to determine whether the direct or indirect effects of ROS production is critical for NLRP3 activation.

The roles of kinases in the dynamic regulation of NLRP3 activation by various stimuli and upstream regulators remain to be understood. Especially, the role of ROS in NLRP3 activation induced by the upstream so-called direct kinases remains unclear. This study has opened a door to future investigations on these aspects, and this will help us focus on the enigma of ROS-mediated kinase signaling and its functional consequences in the biology of inflammation.

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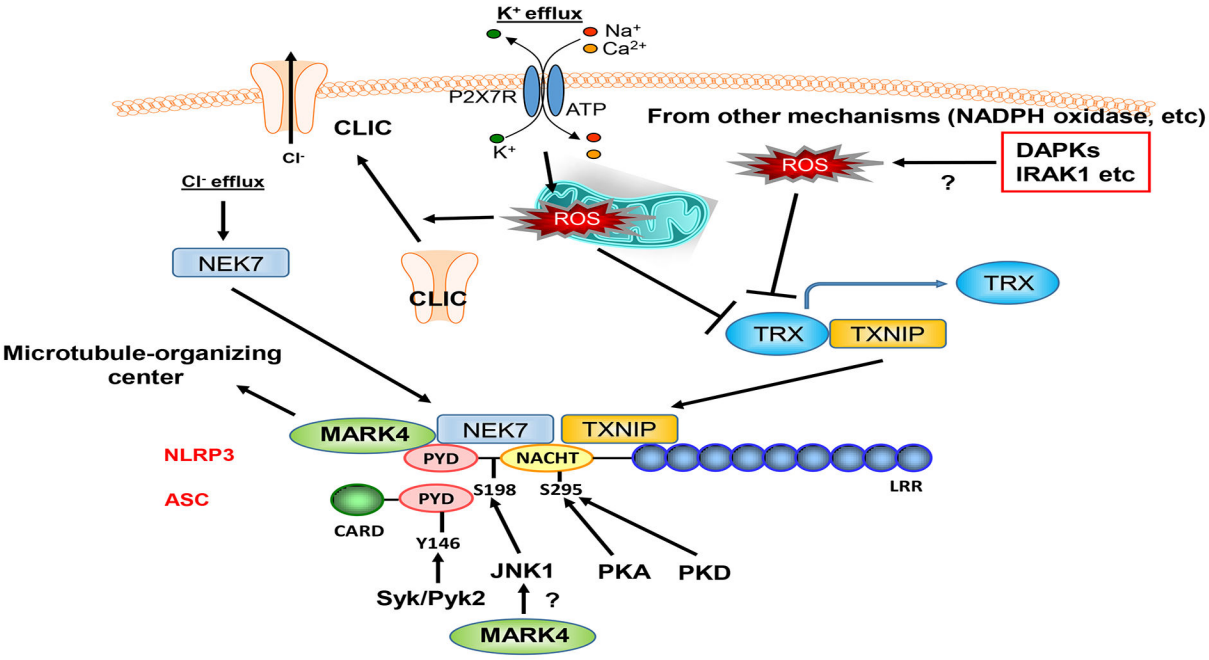

Figure. ROS, kinases, and inflammasome activation

A number of kinsess can directly phosphorylate NLRP3 and ASC and activate inflammasome activation. Furthermore, ROS can activate NLRP3 activation via promoting TXNIP and NEK7 interaction with NLRP3. It is pssible that the ROS production induced by DAPKs and IRAK1 may contribute to NLRP3 activation initiated by these kinases activation. Lastly, MARK4 can bind to NLRP3 and activate it by driveing NLRP3 to the microtubule-organizing center. Please see the details in the text. TXNIP: thioredoxin-interacting protein (TXNIP), TRX: thioreoxin, NEK7: NIMA related kinase 7, CLIC: chloride intracellular channels, DAPK: Death associated proteik kinase, IRAK1: Interleukin 1 receptor associated kinase 1, PKA: Protein kinase A, PKD: Protein kinase D, Syk: Spleen associated tyrosine kinase, Pyk2: Proline-rich tyrosine kinase 2, PYD: Pyrin domain, CARD: Caspase activation and recruitment domain, LRR: Leucine-rich repeat, P2X7R: Purinergic Receptor P2X7.