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Inflammation NODs to Antagonists of RIP2-XIAP Interaction

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While innate immunity is crucial for host defense, dysregulated signaling activation leads to pathological inflammation. In this issue of *Molecular Cell*, Goncharov et al. (2018) present a strategy to combat inflammatory diseases by disrupting RIP2-XIAP interaction in NOD2-mediated signaling.

Innate immunity comprises pathways that respond to pathogen- or danger-associated molecular patterns. Despite diverse signal sensing and transduction mechanisms, these pathways crosstalk and their outcomes converge, manifesting as cytokine production and/or cell death. One pathway in this intricate immune network is mediated by nucleotide-binding oligomerization domain (NOD)-containing protein 2 (NOD2), an intracellular pattern recognition receptor (PRR) that also comprises an N-terminal tandem caspase recruitment domain (tCARD) and a C-terminal leucine-rich repeat (LRR) domain. NOD2—more specifically its LRR domain—recognizes the bacterial cell wall component muramyl dipeptide (MDP) (Figure 1). Although regulated activation of the NOD2 pathway stimulates an inflammatory response and host defense, unchecked signaling results in inflammatory disorders such as Crohn's disease, sarcoidosis, and Blau syndrome. Therefore, therapeutics targeting the NOD2 signaling pathway represent promising treatments to pathological inflammation. With understanding on the antagonism of an essential protein-protein interaction in the pathway, Goncharov et al. (2018) achieve a significant advance on developing blockade of NOD2 inflammatory signaling.

Downstream of NOD2 is receptor-interacting serine/threonine-protein kinase 2 (RIP2), which is composed of an N-terminal kinase domain (KD) and a C-terminal CARD. MDP-activated NOD2 recruits RIP2, which in turn associates with a number of ubiquitin ligases, including X-linked inhibitor of apoptosis protein (XIAP), cellular IAPs (c-IAP1/2), Pellino3, and linear ubiquitin assembly complex (LUBAC), which polyubiquitinate RIP2. This leads to activation of mitogen-activated protein kinases (MAPKs), the transcription factor NF-κB, and proinflammatory cytokines (Krieg et al., 2009; Damgaard et al., 2012; Figure 1). Although human genetic data support the non-redundancy of XIAP in NOD2-induced inflammatory responses, Goncharov et al. (2018) demonstrate its indispensable role in RIP2 polyubiquitination and signaling using newly developed XIAP-selective antagonists, as well as XIAP knockout cells, and identify critical RIP2 ubiquitination sites at K410 and K538 using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

XIAP contains three N-terminal baculoviral IAP repeats (BIR1–BIR3) followed by a ubiquitin-associated domain (UBA) and a C-terminal RING domain. Using surface plasmon resonance (SPR), Goncharov et al. (2018) revealed a direct interaction between RIP2 KD and XIAP BIR2. Consistently, only BIR2-targeting XIAP-selective antagonists disrupt the RIP2-XIAP interaction, leading to failure of XIAP recruitment and RIP2 polyubiquitination, and abrogation of downstream activation of MAPKs and NF-κB. Consequently, proinflammatory cytokines, including interleukin-12 (IL-12), keratinocyte chemoattractant (KC), and RANTES, are markedly downregulated. Of note, the chosen XIAP BIR2-selective antagonists do not negatively affect cell viability, and thus, the decreased cytokine production is not due to cell death.

Previously, a variety of compounds have been shown to inhibit RIP2 kinase activity. Counterintuitively, however, no correlation was observed between RIP2 kinase inhibition and attenuation of NOD2-mediated signaling, suggesting that RIP2 kinase activity and autophosphorylation are not required for the pathway. Indeed, the RIP2 K47A and D146N mutations, which kill catalytic activity, do not significantly affect the ability of RIP2 to activate NOD2 signaling (Goncharov et al., 2018). A novel role for the RIP2 KD—to facilitate binding to XIAP through its BIR2 domain, as evidenced by pull-down, immunoprecipitation, and SPR assays—thus comes into the picture. Supporting this assertion, RIP2 inhibitors that block NOD2-induced inflammatory responses also compromise the RIP2-XIAP interaction. Furthermore, a type I kinase inhibitor (GSK583), which usually binds to active kinases, did not significantly block signaling mediated by the K47A inactive RIP2 mutant. In contrast, a type II inhibitor (ponatinib), which usually targets inactive kinase conformations, efficiently reduced signaling by WT, D146N, and K47A RIP2.

This scaffolding function of RIP2 is reminiscent of IL-1 receptor-associated kinase 1 (IRAK1) in Toll-like receptor and IL-1 receptor signaling in innate immunity. It has been reported that the kinase activity of IRAK1 is dispensable for the signal transduction (Knop and Martin, 1999). A second analogy between RIP2 and IRAK1 is that they both recruit ubiquitin ligases for K63-linked polyubiquitination—XIAP for RIP2 and TNF receptor-associated factor 6 (TRAF6) for IRAK1—which is required for activating inflammatory signaling (Conze et al., 2008). From this perspective, the scaffolding function of kinases, beyond their traditional phosphorylation role, emerges to be a common, and likely frequently used, molecular mechanism.

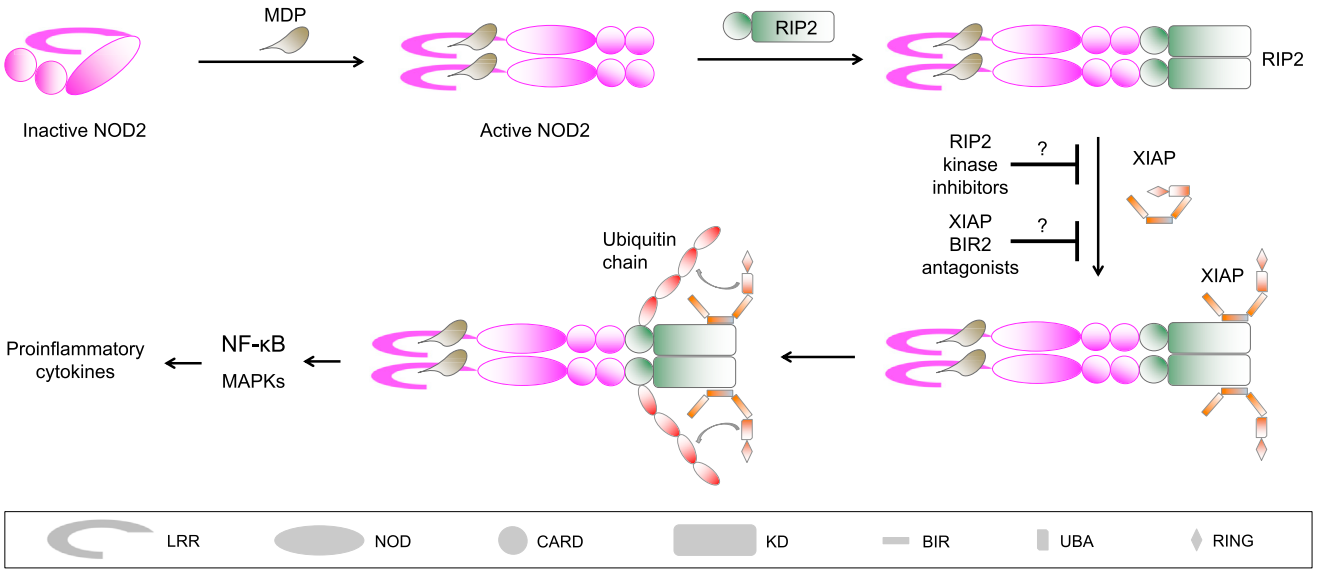

Figure 1. Mechanism of NOD2 Signaling Blockade by RIP2 Kinase Inhibitors and XIAP BIR2 Antagonists
Binding of bacterial cell wall component MDP to the LRR of NOD2 triggers NOD2 oligomerization and recruitment of RIP2 via CARD-CARD homotypic interaction. RIP2 KD then dimerizes and associates with the BIR2 domain of the ubiquitin ligase XIAP, causing K63-linked polyubiquitination of RIP2, downstream activation of MAPKs and NF-κB, and production of proinflammatory cytokines. XIAP BIR2-selective antagonists and some RIP2 kinase inhibitors disrupt RIP2-XIAP association to restrain NOD2 signal transduction. Specific domains of the proteins are labeled below the pathway.

Goncharov et al. (2018) thus redefine the mechanism of action of RIP2 kinase inhibitors that deter NOD2 signaling. The ability of a RIP2 kinase inhibitor to block inflammation depends on whether it disrupts RIP2-XIAP interaction as opposed to whether it disables RIP2 kinase activity. Therefore, we may need to redesign our therapeutic strategies to take into account whether inhibition of kinase activity or protein-protein interaction is expected to be a more effective approach.

In summary, Goncharov et al. (2018) uncovered a mechanistic detail of NOD2 signaling, where RIP2 KD and XIAP BIR2 must interact to elicit downstream responses. Previous studies suggest that MDP-induced NOD2 oligomerization and RIP2 dimerization are required for eliciting active NOD2 signaling (Mo et al., 2012; Pellegrini et al., 2017). Combining these discoveries, we can postulate a model for NOD2 signal transduction, in which the recruitment of RIP2 to the oligomeric NOD2 platform promotes RIP2 KD dimerization, and the dimerization creates a binding site for XIAP BIR2 domain (Figure 1). Interference with the RIP2-XIAP interaction by small molecules binding to either RIP2 or XIAP illustrates a straightforward approach to therapeutic interventions against NOD2-mediated inflammation. It is worth mentioning that a combination therapy with suboptimal doses of a BIR2-targeting XIAP antagonist (XB2m54) and a RIP2 inhibitor (GSK583) outmatches treatment with either individual drug alone (Goncharov et al., 2018). Therefore, in the context of the numerous inflammatory pathways and intertwined communication systems, the cure to inflammatory disorders may rely on mechanistic understanding of the individual pathways and their synergistic modulation.

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