RESEARCH HIGHLIGHT

A BAF-fling connection to RIPK1

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RIPK1 is a multifaceted kinase with roles in diverse cellular processes, including cell death, innate immunity, and inflammatory signaling. RIPK1 can also facilitate inflammatory diseases, like amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD), independent of its cell death functions; in a recent *Cell Research* paper, Li et al. show that this results from RIPK1 acting as a transcriptional coregulator, forming a nuclear complex with the BAF complex through direct interactions with BRG1 and SMARCC2, mediating phosphorylation of SMARCC2, and promoting chromatin remodeling and transcription of proinflammatory genes.

Receptor interacting serine/threonine protein kinase 1 (RIPK1) is canonically linked to programmed cell death (PCD) pathways, such as pyroptosis, apoptosis, necroptosis, and PANoptosis.[1] It has an N-terminal kinase domain important for autophosphorylation, a C-terminal death domain important for interacting with TNFR1, and an intermediate RHIM-containing domain important for interacting with other RHIM domain-containing cell death molecules.[2] The interaction of cytosolic RIPK1 with other cell death molecules (caspase-8, TRADD, and FADD) drives apoptosis, while caspase-8 inhibition triggers the assembly of a necrosome complex, containing RIPK1 and RIPK3, and switches the cell death to MLKL-mediated necroptosis.[1] Posttranslational modifications also affect PCD, as inhibition of RIPK1 ubiquitination leads to apoptosis or necroptosis.[1] Beyond apoptosis and necroptosis, RIPK1 has also been recently reported in PANoptosis.[3] PANoptosis is a unique inflammatory cell death pathway that integrates components from other cell death pathways and is implicated in driving innate immune responses and inflammation. It cannot be individually accounted for by pyroptosis, apoptosis, or necroptosis alone. PANoptosis is regulated by PANoptosomes, multifaceted macromolecular complexes, two of which have been molecularly characterized. The Z-DNA-binding protein 1 (ZBP1)- and absent in melanoma 2 (AIM2)-PANoptosomes both have been shown to include RIPK1 as a key molecular facet which drives cell death; moreover, in the context of TAK1 inhibition resulting from *Yersinia* infection or genetic mutations in TAK1, RIPK1 is a key modulator of PANoptosis.[3] NF-κB signaling mediated by the scaffolding function of RIPK1 and RIPK1 kinase activity-dependent cell death are both important for the innate immune response.[1]

RIPK1 promotes the transcription of proinflammatory cytokines in murine models of amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD), and in human ALS and AD.[4,5] Furthermore, RIPK1 inhibitors are in clinical trials for many human diseases, showcasing the clinical relevance of RIPK1 as a drug target.[2] However, the molecular mechanisms of RIPK1's cell death-independent functions are not extensively studied. Therefore, it is critical to understand the mechanisms by which RIPK1 exerts inflammatory effects.

A recent study using murine embryonic fibroblasts and human cell lines showed that activated RIPK1 in TNF-stimulated cells localized in the nucleus and once there, nuclear RIPK1 directly bound BRG1/BRM-associated factor (BAF) chromatin-remodeling complex proteins, SMARCC2 and BRG1 (also known as SMARCA4) (Fig. 1).[6] Furthermore, transcription factors recruited the RIPK1/BAF complex to H3K4me1 and H3K27ac-marked active enhancer and promoter regions, and RIPK1 phosphorylated SMARCC2 at serine 306. This phosphorylation event was crucial for modulation of the chromatin accessibility and ultimately allowed for transcriptional activation of downstream inflammatory genes such as *Il6*, *Cxcl1-3*, and *Il1a*.[6] Treatment with the RIPK1 kinase inhibitor R-7-Cl-O-necrostatin-1 (Nec-1s) reversed all effects; the RIPK1/BAF complex recruitment was dampened, and SMARCC2 phosphorylation was suppressed, as was proinflammatory gene transcription.[6] This illustrates the specific and critical role of RIPK1 kinase activity in transcriptional control of proinflammatory genes in response to TNF stimulation. Whether and how the nuclear RIPK1/BAF complex regulates other damage- and pathogen-induced antimicrobial immune factors, beyond the context of TNF stimulation, remains to be determined.

RIPK1's functions in transcriptional regulation can also have disease implications. A subset of ALS patients carry a gene encoding an uncleavable RIPK1 variant (D324V or D324H); these patients have increased transcription of proinflammatory cytokines.[7,8] Cell lines carrying non-cleavable versions of RIPK1, which mimic these uncleavable human RIPK1 genetic variants, have elevated nuclear RIPK1 activation and elevated RIPK1/BAF-mediated chromatin remodeling activity.[6] This demonstrates the proinflammatory role of RIPK1/BAF in ALS patients with genetic variants (D324V and D324H).

Overall, while historically RIPK1 has been known to be critical for cell death, more recent evidence shows that RIPK1 is involved in cell death- and NF-κB-independent direct transcriptional regulation in the nuclei of cells. We lack a complete understanding of the function of nuclear RIPK1; the mechanism and the domain responsible for its localization remain unknown. Characterization of RIPK1 has shown its ability to localize in the nucleus both with and without TNF stimulation; furthermore, nuclear RIPK1 has been shown to be ubiquitinated in cells stimulated with TNF.[9] It is suggested that this modified RIPK1 interacts with nuclear RIPK3 to initiate RIPK3 activation.[9] Additionally, necroptotic molecules RIPK3 and MLKL have been shown to localize in both the cytoplasm and nucleus, and RIPK3, RIPK1, and MLKL localize in the nucleus when NLRP3 inflammasomes are induced;[9] all these proteins can act as components of PANoptosomes. Together, these findings suggest that some aspects of PCD may, in part, take place in the nucleus. Furthering this line of evidence is the fact that nuclear RIPK1 was found to be involved in peroxide-induced PARP1-mediated

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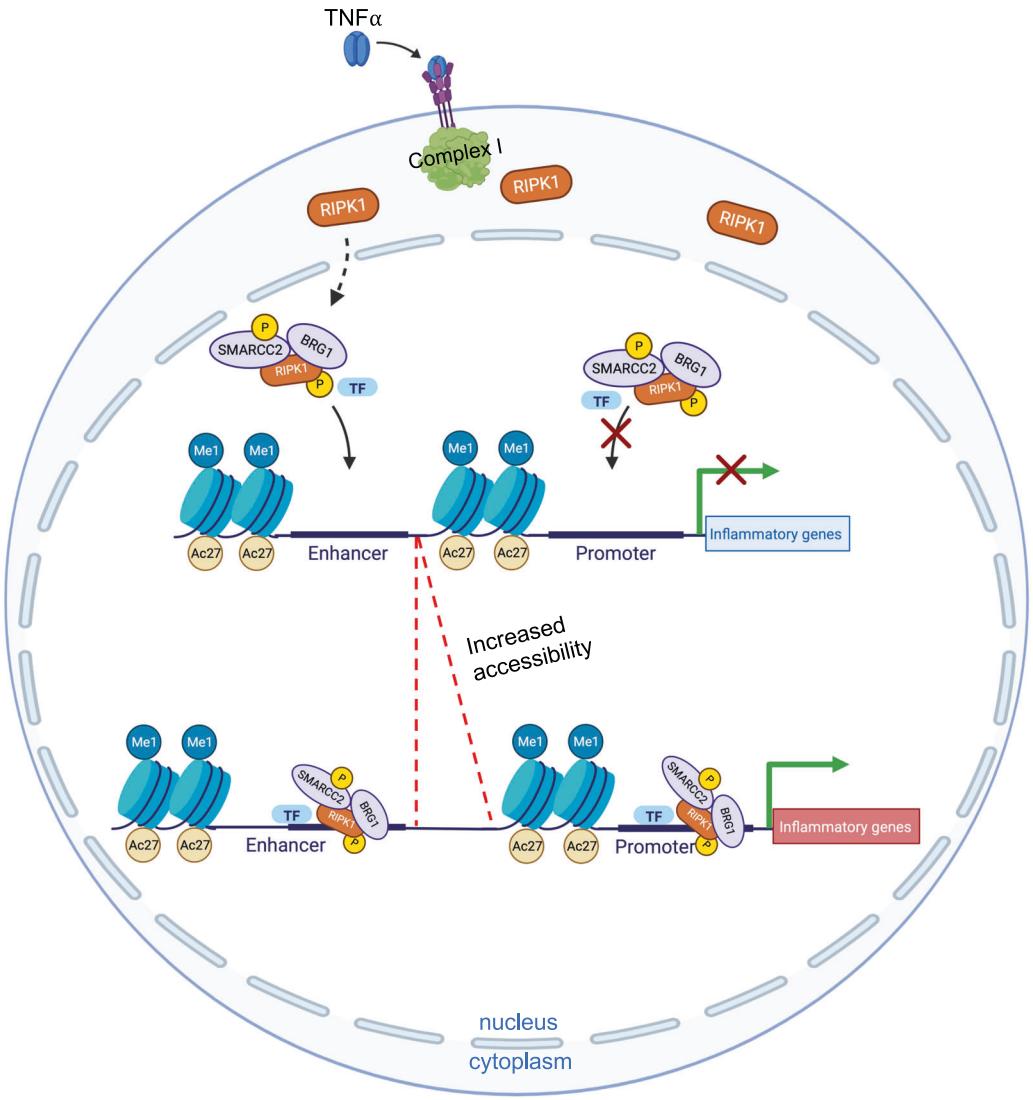

Fig. 1 Effect of TNF stimulation on RIPK1/BAF complex formation and inflammatory gene transcription. As shown by Li et al., TNF stimulation promotes the association of nuclear RIPK1 with BAF complex proteins, specifically SMARCC2 and BRG1. The phosphorylation of SMARCC2 by RIPK1 is necessary for RIPK1/BAF-mediated chromatin remodeling, specifically in H3K4me1 and H3K27ac-marked active enhancer and promoter regions. The resulting increased accessibility allows for transcription factor (TF) binding and transcription of inflammatory genes such as *Il6*, *Cxcl1-3*, and *Il1a*. The figure was created with Biorender (accessed 25 May 2022).

necrosis.[10] Fully understanding the nuclear ubiquitination status of RIPK1, as well as a more comprehensive understanding of where cell death molecular platforms may form, would help provide additional context to nuclear RIPK1's functions. Moreover, given the role of RIPK1/BAF in modulating inflammatory responses in a subset of ALS patients with RIPK1 variants, there is likely a connection between RIPK1 and other human diseases associated with mutations in the BAF chromatin complex. Improved understanding of the multifaceted roles of RIPK1 will continue to inform therapeutic strategies for inflammatory diseases.

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ADDITIONAL INFORMATION

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