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EDITOR'S CORNER

A novel role of ATG9A and RB1CC1/FIP200 in mediating cell-death checkpoints to repress TNF cytotoxicity

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ABSTRACT

TNF (tumor necrosis factor) is an important cytokine that regulates immune responses in response to microbial infection. Two fates can be induced by TNF sensing, including activation of NFKB/NF-κB and cell death, which are mainly regulated by the formation of TNFRSF1A/TNFR1 (TNF receptor superfamily member 1A) complex I and complex II, respectively. Abnormal TNF-induced cell death leads to detrimental outcomes, underlying several human inflammatory diseases. The actions of "protective brakes", or so-called specific "cell death checkpoints", are important to prevent TNF cytotoxicity. A recent study published in *Science* characterizes novel functions of ATG9A, RB1CC1/FIP200 and TAX1BP1 as components of a previously undiscovered TNF-induced cell death checkpoint, independent of its roles in canonical macroautophagy/autophagy. Notably, this ATG9A-controlled cell-death checkpoint contributes to the prevention of inflammatory skin disease, demonstrating its crucial role in serving as a safeguard against the threat of TNF cytotoxicity.

KEYWORDS

Autophagy; inflammation; skin disease; tumor necrosis factor; ULK1 kinase complex

RB1CC1 is an essential component of the ULK1 kinase complex, which is responsible for integrating upstream signals (such as from MTOR and AMPK) and initiating autophagosome formation [1]. ATG9A is a transmembrane lipid scramblase that supports membrane expansion of the phagophore to form a complete autophagosome [2,3]. The contribution of the two proteins in canonical autophagy is well-characterized. Here, we highlight a novel study performed by Huyghe *et al.* [4] that illustrates the roles of ATG9A and RB1CC1 in preventing TNF-induced cell death.

Through a genome-wide CRISPR-Cas9-based screen to identify the essential genes capable of switching TNF responses between survival and death, the authors became interested in *Atg9a* and *Rb1cc1*. Although the two products of these genes participate in different complexes with distinct functions in canonical autophagy [5], they regulate the same cell-death checkpoint; simultaneous deletion of the two genes in mouse embryonic fibroblasts does not have additive effects compared to either single deletion.

As mentioned above, two complexes are involved in determining distinct cell fate upon TNF signaling [6]. The formation of complex I leads to NFKB/NF-κB activation and therefore regulates transcription of inflammatory and pro-survival genes, whereas the assembly of complex II has the potential to result in cell death. Furthermore, two subtypes of complex II have been reported: IIa, the complex that assembles simply by sensing TNF and whose activity is repressed by the NFKB-dependent upregulation of pro-survival genes; and IIb, the complex II that forms only upon RIPK1 kinase activation [7]. By a series of experiments knocking out *Atg9a* and *Rb1cc1* in mouse embryonic fibroblasts, Huyghe *et al.* demonstrate that the protective role of ATG9A and RB1CC1 is independent of RIPK1 kinase and of NFKB activity, indicating that ATG9A and RB1CC1 regulate a novel cell death checkpoint. It was previously reported that Met1 (M1)-ubiquitination contributes to RIPK1 kinase-independent apoptosis [8]. The authors showed M1-ubiquitin chains act as a third player in the ATG9A-RB1CC1-controlled checkpoint by analyzing the effect of ATG9A and RB1CC1 depletion in cells that are incapable of generating M1-ubiquitin chains [9].

Of note, using CRISPER-Cas9, the authors inactivated various *ATG* [2] genes in five distinct functional complexes of the autophagy machinery (the ULK1 kinase complex, the PtdIns3K complex, the ATG9 trafficking system, the Atg8-family Ubl conjugation system and the ATG12 Ubl conjugation system) [10], and find no obvious similarity between the genes sharing a cytoprotective role. For example, the ATG2A, ATG2B, ATG13, ATG101, PIK3C3/VPS34 and ATG14 gene products function in the same checkpoint as ATG9A and RB1CC1, whereas ATG9B, ULK1/ULK2, BECN1 and BECN2 do not. Moreover, the checkpoint does not rely on the activation of ATG12 and Atg8-family conjugation systems, suggesting that a type of unconventional autophagy is involved in this process.

To identify the detailed mechanism for how ATG9A regulates the assembly and movement of complex IIa, the authors immunoprecipitated CASP8, which is activated by complex II [11], in various compartments from mechanically lysed cells to monitor the cytosolic and vesicular localization of the complex. Using detergent to break the cellular vesicles and release their content, and bafilomycin A_1 treatment to prevent lysosomal degradation, Huyghe *et al.* found that the role of ATG9A in preventing TNF cytotoxicity is to promote complex IIa encapsulation by a phagophore before fusion of the

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autophagosome with a lysosome. The encapsulation of complex IIa removes it from the cytosol, preventing its capacity to transmit the lethal signal and therefore promotes cell survival.

Furthermore, the authors found that TAX1BP1, a well-characterized selective autophagy receptor [12], recognizes M1-ubiquitinated RIPK1 in complex IIa through its ubiquitin-binding domains (UBZ1 and UBZ2). In addition, the N-terminal SKICH domain of TAX1BP1 is responsible for binding to RB1CC1 and further recruiting the autophagic initiation machinery to induce the encapsulation of the complex.

In addition to characterizing the molecular mechanism, Huyghe *et al.* also identified the physiological role of the ATG9A cell-death checkpoint in inflammatory skin diseases. Mice with a keratinocyte-specific deletion of *Atg9a* develop strong inflammatory symptoms, including epidermal thickness, inflammatory lesions, swollen lymph nodes, and increased amounts of IL6. Deletion of *Tnfrsf1a*/*Tnfr1* abolishes the inflammation phenotype caused by ATG9A deficiency, demonstrating that aberrant TNF-driven cell death drives the skin disease. Interestingly, mice harboring a similar deletion of *Atg16l1* do not present obvious inflammation, supporting the conclusion that also in mice ATG9A prevents TNF cytotoxicity via an unconventional form of autophagy [13].

Overall, the work from the Bertrand lab provides novel insight into the roles of ATG9A, RB1CC1 and TAX1BP1 as part of a novel cell death checkpoint in preventing TNF cytotoxicity. This is the fourth checkpoint that has been revealed to counteract TNF-induced cell death. In the checkpoint highlighted here, the early autophagy machinery is required to encapsulate complex IIa to prevent apoptosis. Moreover, the elucidation of how TAX1BP1 bridges complex IIa and RB1CC1 further adds to our understanding of the involvement of ATG proteins in the TNF-induced cell-death checkpoint and sheds light on the understanding of inflammatory skin disease pathogenesis.

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