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Editorial

RIP1's function in NF-κB activation: from master actor to onlooker

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The receptor interacting protein kinase 1 (RIP1) is a crucial component of the TNFR1 response. In this issue of *Cell Death and Differentiation*, Wong *et al.*[1] has set a cat among the pigeons by challenging the commonly accepted model in which RIP1 is essential for TNFR1-induced NF-κB activation. Their new data will force the scientific community to adapt and refine the model of NF-κB activation.

Tumor necrosis factor (TNF) is a multifunctional cytokine. Upon binding to TNFR1, it activates distinct pathways with diametrically opposed consequences: killing cells or promoting survival. Its protective effect is achieved mainly by activating the NF-κB pathway, which induces the transcription of a set of pro-survival genes. In most cells, exposure to TNF is lethal only if the NF-κB signaling pathway is inhibited. Because NF-κB has a crucial role in the pathological consequences of TNF action, the mechanism of its activation has attracted the attention of scientists over many years. The data collected so far indicate an important role for RIP1 as a signaling node that contributes to TNFR1's life and death decisions and have led to the development of a commonly accepted model (reviewed by Wertz and Dixit[2] and Skaug *et al.*[3]). According to this model, exposure to TNF results in recruitment of a complex consisting of TRADD, RIP1, TRAF2, cIAP1 and cIAP2 at the receptor, allowing cIAP1 and cIAP2 to conjugate RIP1 with K^{63}-linked polyubiquitin chains. The addition of these chains to RIP1 has two major consequences. First, it prevents RIP1 from activating cell death signaling pathways, which is dependent on FADD and caspase-8 in apoptosis and on RIP3 in necroptosis. Second, it creates a platform for the recruitment of the protein kinase TAK1 (which acts in concert with the regulatory proteins TAB2 and TAB3) and the IκB kinase complex IKKα–IKKβ–NEMO. Both TAB and NEMO were shown to dock at K^{63}-linked polyubiquitin chains, and it is believed that the close proximity of TAK1 to the IKK complex on RIP1's K^{63}-polyubiquitin chains is sufficient for TAK1 to activate IKKβ by phosphorylation. Once activated, IKKβ phosphorylates IκBα, a signal for the K^{48}-ubiquitination and proteasomal degradation of IκBα. Releasing the inhibitory effect of IκBα then permits NF-κB dimers to translocate to the nucleus and transactivate pro-survival genes. The de-ubiquitinating enzymes A20 and CYLD have been identified as negative regulators that edit RIP1 K^{63}-polyubiquitin chains, thereby limiting the duration of the NF-κB response. In the absence of cIAP1 and cIAP2, RIP1 does not get K^{63}-ubiquitinated and TNF exposure induces RIP1-dependent cell death. Therefore, in the present model, RIP1 is crucial for cell survival through activation of the NF-κB pathway.

In the current issue from *Cell Death and Differentiation*, Wong *et al.*[1] re-examined RIP1's functions downstream of the TNFR1 using *wt* and *ripk1*$^{-/-}$ primary and SV40 large T immortalized mouse embryonic fibroblasts (MEFs). When the authors treated the cells with a combination of TNF and the IAP antagonist compound A, they observed that *wt* MEFs succumbed to the treatment but *ripk1*$^{-/-}$ MEFs did not. These results are consistent with several recently published studies and confirm that RIP1 has a pro-cell death function in the absence of cIAP1 and cIAP2 activity.[4–6] More interestingly, the authors report that TNF alone has a minor impact on the viability of *ripk1*$^{-/-}$ MEFs, and that a significant difference between the survival of *wt* and *ripk1*$^{-/-}$ MEFs is observed only when NF-κB action is blocked by the translation inhibitor cycloheximide. These results are remarkable because TNF-induced activation of NF-κB had been shown to protect cells from death.[7] Therefore, although these new data confirm a pro-survival function of RIP1, they seriously question the obligate role of RIP1 in TNFR1-dependent NF-κB activation. These doubts were confirmed when Wong *et al.* demonstrated that TNF-induced IκBα degradation and recovery, as well as RelA nuclear translocation, occurs normally in both primary and transformed *ripk1*$^{-/-}$ MEFs.

The MEF results provided by Wong *et al.*[1] are at odds with previous studies that report a crucial role for RIP1 in NF-κB activation. For example, Kelliher *et al.*[8] reported that the nuclear extract from TNF-treated *Ripk1*$^{-/-}$ Abelson virus-transformed pre-B cells failed to bind to an NF-κB probe in electrophoretic mobility shift assay. Ea *et al.*[9] showed that the K^{63}-polyubiquitination on lysine 377 of RIP1 is required for NF-κB activation in human Jurkat T cells by serving as a docking site for the recruitment of TAK1 and the IKK complex. These studies might be reconciled by considering a cell-type-specific role for RIP1 in NF-κB activation, perhaps highlighting the pitfalls of not integrating cell specificity in many of the established signaling models. Differences in responses

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between cell types could also explain why Wong *et al.*[1] observed that cells harvested from E18 *Ripk1*$^{-/-}$ liver and thymus responded to TNF-induced NF-κB activation, whereas cells isolated from lung did not. Nevertheless, this hypothesis does not explain the differences in TNF-induced IL-6 production in *Ripk1*$^{-/-}$ MEFs reported by Wong *et al.*[1] and by Lee *et al.*[10,11]

Redundancy between cIAP1 and cIAP2 and between TRAF2 and TRAF5 has been reported, and suppression of both cIAPs or both TRAFs is often required to detect defects in canonical NF-κB activation.[12,13] RIP2 shares strong structural homology with RIP1, but is recruited to receptor complexes via a caspase recruitment domain (CARD) rather than through a death domain (DD) such as RIP1. RIP2 binds IAP and TRAF proteins, and cIAP-mediated K^{63}-polyubiquitinated RIP2 serves as a scaffold for recruitment of the TAK1 and IKK complexes and for the downstream activation of NF-κB.[14,15] Therefore, an interesting possibility could be that RIP2 compensates for loss of RIP1 function in a cell-specific manner. This could conceivably occur through the recruitment of adaptor proteins containing a CARD domain (e.g., cIAP1 or cIAP2) or through promiscuity of the homotypic interaction between DD superstructures.

The negative effect of A20 and CYLD in TNF-mediated NF-κB signaling was explained, at least in part, by their ability to remove the K^{63}-polyubiquitin chains on RIP1.[3,16] However, as these new results demonstrate that RIP1 is not essential for TNF-induced NF-κB activation, the action of A20 and CYLD might involve other mechanisms. One possibility is that A20 and CYLD deubiquitinate other K^{63}-ubiquitinated substrates that bridge TAK1 to the IKKs. Interestingly, A20 has been reported to negatively regulate RIP2-dependent NF-κB activation by editing RIP2 K^{63}-polyubiquitin chains.[17] Other targets of A20 and/or CYLD are the TRAF and possibly the cIAP proteins.[16,18] Their recruitment to TNFR1 is essential for NF-κB activation, and upon TNF stimulation all of them are conjugated with polyubiquitin chains. NEMO and TAK1 could also directly recruit each other: they are indeed both conjugated with K^{63}-linked chains and edited by CYLD.[16] In a recent study, Tokunaga *et al.*[19] described a new type of ubiquitin chains conjugated to NEMO by the E3 ubiquitin ligase complex LUBAC. These modifications consist of head to tail-linked linear ubiquitin chains. Interestingly, the authors showed by pull-down experiments that TAB2 and TAB3 proteins bind to linear polyubiquitin chains.[19] These last results have been questioned by another recent study showing that NEMO and TAB2 bind specifically and exclusively to linear and to K^{63}-linked chains, respectively, and that CYLD can hydrolyze both types of chains with equal efficiency.[20] Nevertheless, Xu *et al.*[21] demonstrated, using a ubiquitin replacement technology, that lysine 63 of ubiquitin is dispensable for TNF-mediated NF-κB activation. Together, those results indicate that chains other than K^{63}-linked forms can serve as scaffolds for recruitment of the TAK1 and IKK complexes. Moreover, linear chains most probably have an important physiological role in TNFR1 signaling because loss of HOIL-1 and HOIP, the two components of LUBAC, reduces TNF-induced NF-κB activation.[19] The discovery of linear polyubiquitin chains is a recent development and it will not be surprising if other substrates conjugated with those chains are reported in the coming years.

Activation of IKK is the key step in stimulation of the transcription factor NF-κB. The initial model, and the above discussion, focused on the ability of TAK1 to activate IKKβ by phosphorylation. However, other members of the MAP3K family have been shown to activate IKK. In particular, MEKK3 directly phosphorylates IKKβ and *mekk3*$^{-/-}$ MEFs cannot activate NF-κB in response to TNF.[22] Although MEKK3 was shown to bind RIP1, it will be important to test whether MEKK3 requires RIP1 to activate NF-κB upon TNF stimulation.

In conclusion, the results presented by Wong *et al.*[1] in this issue of *Cell Death and Differentiation* challenge earlier models of canonical NF-κB activation. These data, together with results recently published by other groups, highlight the need for more work to elucidate how the signal is transmitted from TNFR1 for the activation of NF-κB dimers. In particular, identification of the proteins recruited to the TNFR1 complex in *Rip1*$^{-/-}$ MEFS will provide information that can be used to reshape the model. The changing insight into the role of RIP1 as reported by Wong *et al.* exemplifies what Thomas Kuhn described in his landmark book *The Structure of Scientific Revolutions* (1962)[23]; scientific knowledge steadily progresses through cycles of constructing paradigms, which then become widely accepted and repeated. But then, at a certain point, the models become questioned by novel (and old) facts that do not fit anymore. In RIP1 research, time has come for challenging the established models and for building novel paradigms.

Conflict of interest

The authors declare no conflict of interest.

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