Published in final edited form as:

Science. 2014 March 21; 343(6177): 1322–1323. doi:10.1126/science.1252526.

RIP3 Takes Another Deadly Turn

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Programmed necrosis or necroptosis is a form of non-apoptotic cell death with important roles in many inflammatory conditions and related diseases. The receptor interacting protein kinase 1 (RIP1) and RIP3 form a critical axis that mediates necroptosis downstream of tumor necrosis factor (TNF) receptor, toll-like receptor 3 (TLR3), TLR4, and the T cell antigen receptor. Cells are sensitized to necroptosis when the essential apoptotic adaptors Fadd or caspase 8 is inhibited. Under such conditions, RIP1 phosphorylates and activates RIP3, which in turn phosphorylates its downstream substrate MLKL, leading to plasma membrane rupture and necrosis ⁽¹⁾. Genetic inactivation of RIP3 corrected many of the inflammatory diseases that are caused by tissue-specific Fadd or caspase 8 deficiency. Similarly, acute injury-induced inflammation, such as that triggered by drugs or ischemia-reperfusion, was ameliorated in RIP3-null mice. These findings suggest that inhibition of RIP3 kinase function is a promising therapeutic strategy for acute and chronic inflammatory diseases.

To directly test the feasibility of this therapeutic approach, Newton and colleagues created “knock-in” mice that express a kinase-inactive version of RIP3, D161N ⁽²⁾. Surprisingly, while RIP3^{-/-} mice were viable, the RIP3^{D161N/D161N} mice die at midgestation due to vascular mal-development of the yolk sac. These phenotypes are in stark contrast to the normal development of RIP3-null mice ⁽³⁾, but reminiscent of mice that lack Fadd, caspase 8 or cFLIP. While the Fadd^{-/-} and caspase 8^{-/-} mice succumbed to extensive necrosis during embryogenesis, the lethality in RIP3^{D161N/D161N} mice was caused by extensive caspase-dependent apoptosis. Consistent with a caspase-driven mechanism of cell death, RIP3^{D161N/D161N} mice that also lack caspase 8 were normal, but developed a lymphoproliferative disease akin to that caused by Fas mutations. Using a tamoxifen-inducible expression strategy, the authors elegantly showed that expression of RIP3-D161N in adult mice also led to massive apoptosis in multiple tissues and lethality. Inducible expression of RIP3-D161N in fibroblasts led to formation of an apoptosis signaling complex containing Fadd, caspase 8, RIP1 and RIP3. Although RIP1 is present in this death-inducing complex, the RIP1 kinase and necroptosis inhibitor necrostatin-1 did not rescue apoptosis induced by RIP3-D161N. Moreover, embryonic lethality of RIP3^{D161N/D161N} mice was rescued by crosses to RIP1^{-/-} mice, but not to mice expressing kinase-inactive RIP1

(D138N) or lacking the downstream necroptosis effector MLKL. These results indicate that while an overlapping set of protein adaptors are involved in necroptosis and RIP3-D161N induced apoptosis, the molecular mechanisms that drive these two cell death responses are distinct.

How can we reconcile the phenotype of RIP3^{D161N/D161N} mice when RIP3^{-/-} mice were born alive with no overt abnormalities? A straightforward explanation is that RIP3 phosphorylates and inactivates an unknown substrate that regulates assembly of the Fadd-caspase 8-RIP1-RIP3 death-inducing complex (Fig. 1). In this regard, it is noteworthy that the activities of Fadd, RIP1 and RIP3 are controlled by phosphorylation ^(4, 5). Could RIP3 directly phosphorylate any of these adaptors to control assembly of this RIP3-associated apoptosis-inducing complex?

Although inhibition of RIP3 kinase activity is a plausible cause of apoptosis in RIP3^{D161N/D161N} cells and mice, other mechanisms are also possible. For example, mice that express a single allele of D161N (i.e. RIP3^{D161N/+} or RIP3^{D161N/-}) were viable. Because gene dosage is important, the phenotypes cannot be entirely attributed to lack of kinase activity. What might be a possible alternative explanation? Previous studies show that expression of kinase domain-deleted RIP3, but not full length RIP3, led to spontaneous formation of RIP homotypic interaction motif ⁽⁶⁾ (RHIM)-dependent amyloid fibrils ⁽⁷⁾. This indicates that the kinase domain may functionally “mask” the RHIM to prevent inadvertent activation. In this scenario, the D161N mutation could alter the conformation of RIP3 such that the RHIM is now exposed for binding to RIP1 (Fig. 1). This model predicts that the kinase and RHIM domains collaborate to control scaffolding of the necroptotic and apoptotic machineries. This type of scaffolding function for kinase domains has in fact been observed with oncogenic kinases such as B-raf ⁽⁸⁾. Interestingly, RIP3-D161N was expressed at much lower level than wild type RIP3. The reduced expression of RIP3-D161N is in agreement with conformational change leading to instability of the protein. RIP3 kinase inhibitors have recently been described ⁽⁹⁾. The therapeutic efficacy of these inhibitors will depend on whether they similarly promote assembly of this apoptosis scaffold. In contrast to RIP3, mice expressing kinase inactive RIP1 were viable ⁽¹⁰⁾. As such, pharmacologic inhibition of RIP1 may prove to be a more viable option in the clinic.

Although compelling evidence indicates that RIP3 does not participate in death receptor-induced apoptosis, it is noteworthy that RIP3 was originally identified as an apoptosis inducer^(11–13). The results of Newton and colleagues show that, regardless of the mechanism, RIP3 can indeed function as an apoptosis regulator. In that sense, RIP3 biology has come a full circle. The challenge for the field will be to determine how RIP3-dependent apoptosis is induced and whether it has any unique functions in physiology.

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Figure 1.