Necroptosis: Fifty shades of RIPKs

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Apoptosis and necroptosis are 2 major, yet distinct, forms of regulated cell death. Whereas apoptosis requires caspase protease function, necroptosis requires activation of the receptor interacting protein kinases 1 (RIPK1) and RIPK3. Following activation, RIPK3 phosphorylates mixed-lineage kinase domain-like (MLKL), leading to cell death. Apoptosis and necroptosis are deeply intertwined such that a given death stimulus can often engage either form of cell death. Recent studies published in Cell Death and Differentiation by the Han, Oberst, and Vaux laboratories provide exciting new insights into necroptosis and how it interconnects with apoptosis. As we will discuss, their findings address key questions including: How does a cell choose between apoptosis or necroptosis? How can RIPK3 also induce apoptosis? What is the nature of the RIPK1–3 signaling cascade leading to necroptosis? Finally, data from the Oberst and Han groups strongly argue that RIPK1—previously viewed solely as pro-necroptotic—can also exert antinecrototic activity.

Introduction

Just like us, cells die in many ways; some mechanisms of cell death are regulated and involve engagement of dedicated molecular machinery whereas others are passive and occur as a result of overwhelming damage. The best understood form of regulated cell death is apoptosis, an evolutionarily conserved process that plays key roles in most areas of biology. Apoptosis requires the activation of caspase proteases that cleave hundreds of substrate proteins leading to rapid cell death.1 Recently, intense interest has focused on another form of regulated cell death called necroptosis. Morphologically, the execution phase of necroptosis resembles necrosis, in which dying cells swell prior to lysis.2 However, in stark contrast to necrosis, necroptosis is highly controlled and requires activity of the receptor interacting protein kinases 1 (RIPK1) and RIPK3.3–6 In a simplified model, active RIPK1 phosphorylates RIPK3 leading to its activation.7 In turn, RIPK3 phosphorylates the pseudokinase mixed-lineage kinase domain-like (MLKL) promoting its oligomerization and plasma membrane translocation.8–11 Through poorly understood means, oligomerized MLKL ruptures the plasma membrane thereby killing the cell. Far from existing in isolation, extensive crosstalk occurs between apoptosis and necroptosis. For example, some apoptotic stimuli can also trigger necroptosis when caspase activity is inhibited. Here we discuss recent work by the Han, Oberst, and Vaux groups that provides exciting new insight into the regulation of necroptosis.12–14 Specifically, these papers offer new understanding into how a cell decides to undergo apoptosis or necroptosis and how RIP kinases transduce the death signal, in addition to demonstrating that RIPK1—previously viewed solely as pro-necrotic—can also exert antinecrotic activity.

Pick Your Poison—RIPK3-Dependent Apoptosis or Necroptosis?

As discussed, various stimuli can trigger apoptosis and necroptosis. This crosstalk is best understood in the context of tumor necrosis factor (TNF) signaling. Binding of TNF to its receptor can trigger multiple outcomes that include prosurvival and
proinflammatory NFκB activation, apoptosis, or necroptosis. The ability of TNF to induce necroptosis requires caspase-8 inhibition, among other factors. Caspase-8 prevents TNF-induced necroptosis through cleavage of multiple prion-protective proteins that include RIPK3, RIPK1, and cylindromatosis (CYLD). RIPK3 is essential for TNF-induced necroptosis, and in most cases RIPK1 is also required. Importantly, besides their role in necroptosis, various reports have also implicated a proapoptotic role for RIPK1 and RIPK3. What dictates the ability of RIPK1 or RIPK3 to trigger either necroptosis or apoptosis? Cook and colleagues found that kinase-dead RIPK3 was necessary for necroptosis whereas dimerization was insufficient. Our laboratory has previously shown that chemically induced oligomerization of RIPK3 suffices to trigger necroptosis. Using an analogous method, Cook and co-workers show that dimerization of either RIPK1 or RIPK3 is sufficient to kill cells via necroptosis. This reductionist approach avoids potentially complicating effects of ancillary TNF signaling cascades. Importantly, the authors demonstrate that the ability of activated RIPK1 or RIPK3 to engage apoptosis or necroptosis is solely dictated by the availability of downstream target: if MLKL is lacking, RIPK1 or RIPK3 activation induces apoptosis, whereas necroptosis is induced if caspase-8 is absent (Fig. 1). As predicted from earlier studies, the kinase activity of RIPK3 is required for necroptosis but, intriguingly, is dispensable for apoptosis. Rather, the ability of kinase-inactive RIPK3 to induce apoptosis requires RIPK1, FADD-associated protein with death domain (FADD), and caspase-8. In this scenario, RIPK3 (probably dimerized) binds RIPK1, leading to recruitment of FADD and ultimately the recruitment and activation of caspase-8. Offering support for these findings in vivo, a recent study has shown that knock-in mice expressing a kinase-dead RIPK3 exhibit early embryonic lethality that can be rescued by concomitant loss of RIPK1 or caspase-8. Additionally, Cook and colleagues found that kinase-dead RIPK3 can kill cells via apoptosis, albeit to a lesser degree, in the absence of RIPK1. How this occurs is unclear, but it mirrors earlier findings from the same group showing that elevated RIPK3 levels can bypass an absolute requirement for RIPK1 during TNF-induced necroptosis. The important take-home message of this work is that the decision between apoptosis or necroptosis may not be an either/or switch mechanism, as often depicted. Undoubtedly, this situation will be more complicated in real-death situations, e.g., TNF-induced necroptosis, but these findings suggest that apoptosis and necroptosis may occur concurrently in the same cell, something that could be addressed by live-cell imaging.

How Letting One RIP Leads to Another

The necrosome is the necroptosis-initiating TNF-induced complex that contains FADD, caspase-8, RIPK1, and RIPK3. RIPK3 activation requires interaction with RIPK1 through domains present in both proteins called RIP homotypic interaction motifs (RHIMs). These motifs allow extensive RHIM-mediated polymerization to occur, forming amyloid fibrils in the cell that consist of RIPK3–RIPK1 heterodimers and/or RIPK1 or RIPK3 homodimers. How these interactions are coordinated to activate RIPK3 and trigger necroptosis is unclear. To explore this key question, Wu and colleagues used chemically dimerizable pairs of RIPK1 and RIPK3 that allow defined hetero- and homodimerization between the different kinases. Using this method, they found that the RIPK1–RIPK3 interaction was required for necroptosis, as one would predict. However, their data show that the RIPK1–RIPK3 heterodimer lacks detectable RIPK3 activity and by itself is incapable of killing cells. Rather, it acts to seed necrosome formation by incorporating additional RIPK3 molecules that activate and kill the cell. Extending these findings, they found that RIPK3 homodimers are as effective as oligomers at inducing necroptosis. Collectively, these data argue that oligomerization of active RIPK3 dimers per se is not necessary for necroptosis; instead, the actual number of active RIPK3 dimeric units may be the key factor. With respect to this conclusion, it is important to note that the Oberst laboratory found the opposite—in their study oligomerization of RHIM-deficient RIPK3 was necessary for necroptosis whereas dimerization was insufficient.
The disparity in these findings likely relates to the different location of the dimerization domain (N- or C-terminal to RIPK3) between the 2 studies. As such, although RIPK3 dimerization is sufficient to cause necroptosis, the exact requirements for dimerization versus oligomerization in an endogenous setting are currently unclear.

**RIPK1—Killer or Savior?**

RIPK1 is widely considered to be pro-necroptotic and is often essential for necroptosis in response to different stimuli. Orozco and coworkers provide provocative new data strongly arguing that RIPK1 can also perform an antinecroptotic function. Similar to other studies, they used chemical dimerizers to dissect the role of RIPK3 dimerization versus oligomerization upon its activation during necroptosis. In itself, RIPK3 dimerization was insufficient to trigger necroptosis but led to robust RIPK3 activity and necroptosis through recruitment of other RIPK3 molecules. Importantly, caspase-8 and RIPK1 were also recruited to the RIPK3 oligomeric complex and exerted an inhibitory effect. Strikingly, either RNA knockdown or chemical inhibition of caspase-8 potentiated RIPK3-mediated necroptosis. Most surprisingly, whereas chemical inhibition of RIPK1 activity inhibited RIPK3-mediated necroptosis, knockdown of RIPK1 actually promoted it. Together with the other data presented, these findings paint a picture whereby recruitment of RIPK1 to RIPK3 complexes inhibits necroptosis by recruiting caspase-8 (indirectly via FADD), leading to destabilization of the necrosome (Fig. 2). Importantly, recent data offer in vivo evidence supporting many of these findings is already emerging. Furthering our understanding of necroptosis will provide an improved rationale for targeting this process in disease, in addition to highlighting potential therapeutic pitfalls.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**Figure 2.** Antinecroptotic functions of RIPK1. (1) Dimerization of RIPK1 with RIPK3 prevents RIPK3 kinase activity, thus allowing cell survival; 2) Dimerization or oligomerization of RIPK3 leads to its activation and necroptosis; 3) Incorporation of RIPK1 into RIPK3 oligomers recruits caspase-8, which destabilizes the necrosome allowing cell survival. RIPK, receptor interacting protein kinase.
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