Another face of RIPK1

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Receptor-interacting protein kinase-1 (RIPK1) sits at a signaling node controlling a number of functional pathways. These include both positive and negative control of apoptosis and necroptosis (a form of regulated necrosis). In this issue of *EMBO Reports*, Yonekawa and colleagues describe another function for RIPK1, the inhibition of autophagy via ERK-mediated phosphorylation of the transcription factor, TFEB [1]. Their findings are considered in the context of RIPK1 signaling, and how it is engaged.

See also: T Yonekawa *et al* (June 2015)

RIPK1 was discovered as a component of the signaling complexes activated by ligation of TNF receptor-1 (TNFR1) [2] and has a number of signaling functions, including activation of MAP kinases and NF- κ B [3], and regulation of both apoptosis and necroptosis [4]. Some of these functions (e.g. promotion of cell death) require the kinase activity of RIPK1, whereas others (e.g. MAPK, NF- κ B activation) do not. Active RIPK1 induces necroptosis by binding and activating RIPK3 and can also inhibit RIPK3 activation by two mechanisms: (i) RIPK1 interacts with the adapter molecule, FADD, which in turn recruits caspase-8 and c-FLIPL, a heterodimeric protease that disrupts RIPK3 oligomers [5], and (ii) kinase-inactive RIPK1 can directly disrupt RIPK3 activation, even when it proceeds independently of RIPK1 [6]. RIPK1-deficient animals die postnatally, whereas animals deficient in RIPK1, RIPK3, and either caspase-8 or FADD do not, underscoring the complex interplay of RIPK1, caspase-8, and RIPK3 [6] (Fig 1).

Remarkably, RIPK1 was identified twice, and in very different ways, in a fascinating screen conducted by Yonekawa *et al* [1]. They used a cell line that requires macroautophagy (herein, autophagy) for sustained

survival under starvation conditions *in vitro*, and searched for shRNAs that were either selected for or against under different culture conditions. When the cells were grown in complete medium, the shRNA for RIPK1 was selected *against*; that is, RIPK1 expression was favored. In striking contrast, when autophagy was suppressed (by partially silencing the autophagy protein ATG12) and cells were cultured under starvation conditions, the shRNA for RIPK1 was selected *for* (that is, RIPK1 expression was detrimental). Consistent with the latter finding, RIPK1 was shown to suppress basal autophagy, such that silencing of RIPK1 enhances autophagy under both normal and starvation conditions. One way this occurs is via the induction of ERK by RIPK1, leading to ERK-mediated phosphorylation and inhibition of the transcription factor, TFEB. TFEB induces the expression of many components of the autophagy pathway and lysosomal biogenesis.

An important function of autophagy is the recovery of biomolecules from cytosolic components to sustain survival under starvation conditions. Not surprisingly, cancers often depend upon autophagy for their growth and survival. Based on the findings of Yonekawa *et al* [1], we might therefore expect that mutations in *ripk1* would be selected in some cancers. Although deletions of *ripk1* have not been described, mutation of the *ripk1* gene does occur in cancer, albeit very rarely. In the COSMIC database, 79 mutations in *ripk1* were found in approximately 21,000 samples, of which 15 mutations were synonymous, leaving only 54 missense and nonsense mutations (<http://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=RIPK1&ln1=RIPK1&start=1&end=672&coords=AA%3AAA&sn=&ss=&hn=&sh=&id=410#>). Several of the missense mutations were in the so-called intermediate domain, which is responsible for RIPK1-mediated

MAPK activation [2]. It might prove interesting to know whether any of these affect ERK activation and autophagy suppression by RIPK1.

These considerations do not explain why the shRNA for RIPK1 was selected *against* under nutrient sufficient conditions. Neither its role in dampening TFEB activity and autophagy, nor its role in promoting apoptosis and necroptosis would explain why RIPK1 expression should be maintained. Perhaps the most likely explanation (pending identification of some other activity of RIPK1) would be its role in inhibiting two forms of cell death, as mentioned above [6]. When RIPK1 is absent, cells become sensitized to apoptosis induced by ligation of TNFR family receptors, and to necroptosis induced by TLR ligands and interferons [6]. Therefore, if such signals are present in culture and the cells express the requisite signaling molecules of the apoptosis and/or necroptosis pathways, the expression of RIPK1 will be required to sustain survival.

But now we have a conundrum. If RIPK1 expression is favored during nutrient replete cell culture because it prevents cell death, how can its loss under starvation conditions (with partially silenced ATG12) be favored without invoking the same counter selection seen under nutrient conditions? That is, why do the starved cells not die when RIPK1 levels are decreased?

Part of the answer may lie in incomplete silencing by shRNA; different results might be obtained with ablation of the *ripk1* gene. The levels of RIPK1 required to inhibit cell death may be quite different from those required to dampen autophagy, and the efficacy of silencing under each condition was not fully explored by Yonekawa and colleagues [1]. Another, perhaps more interesting possibility arises when we consider the roles of autophagy in the inhibition of cell death. Cells that lack autophagy are known to die if

Figure 1. Signaling by RIPK1.

RIPK1 can be activated either directly by ligation of the TNF receptor, or indirectly by ligation of TLRs (via TRIF) or interferon receptors. The latter occurs through RIPK3, which can bind and activate RIPK1. RIPK1 recruits and activates the FADD-caspase-8-FLIP complex, which in turn inhibits RIPK1 function. This interaction can also lead to apoptosis under some conditions. In the absence of this FADD-caspase-8-FLIP function, RIPK1 can activate RIPK3 (after TNFR ligation), leading to MLKL activation and necroptosis. Yonekawa and colleagues now show that RIPK1 also dampens autophagy by inhibiting, via ERK, the function of the transcription factor, TFEB [1]. In turn, autophagy affects cell death. Intriguing questions remain as to how RIPK1 is activated to mediate this autophagy inhibitory activity in a constitutive manner.

starved of nutrients. It is not useful, however, to conclude that “autophagy inhibits cell death” even if it is superficially true; anything that sustains cell survival “inhibits cell death.” In the case of autophagy, however, it turns out that there is more at play than this simple tautology. Several studies, including the one under discussion, have shown that the turnover of some molecules involved in inhibiting or promoting specific cell death pathways are under the control of autophagy. For example, the phosphatase FAP-1, which antagonizes CD95-induced apoptosis, is reduced in cells with increased autophagic flux, rendering these cells more susceptible to cell death by ligation of CD95 [7]. In contrast, autophagy-mediated degradation of the pro-apoptotic protein PUMA, which promotes apoptosis via the mitochon-

drial pathway, renders cells with high autophagic flux more resistant to signals that kill cells via this pathway [8]. PUMA is expressed under starvation conditions [9], and therefore, increased autophagic flux promoted by silencing of RIPK1 may act to counter the pro-apoptotic effects of PUMA expression. Indeed, Yonekawa *et al* [1] show that under starvation conditions, silencing of RIPK1 renders cells relatively resistant to TRAIL, which promotes apoptosis via the mitochondrial pathway in many cell types.

Loss of RIPK1 sensitizes cells not only to apoptosis via TNFR death receptors but also to some ligands that promote necroptosis independently of RIPK1. Is it possible that increased autophagic flux may counter necroptosis upon loss of RIPK1? If so, this might help to explain why under nutrient

deficient conditions, loss of RIPK1 is favored: the resulting increase in autophagy may counter any increase in cell death. Under nutrient replete conditions, any increase in basal autophagy (as is observed) may be insufficient to do so. Although we do not have sufficient information to test this idea and its potential relevance to the conundrum poised above, we can speculate further. If interferon production—which can engage the necroptosis pathway in the absence of RIPK1 [6]—is affected by the level of autophagic flux, then under the starvation conditions that favor loss of RIPK1, the ensuing increase in autophagic flux may antagonize interferon production and its cell death effects. Indeed, a connection between autophagy and interferon production has been observed [10], and thus, elevation of autophagic flux under these conditions might limit any cell death caused by the loss of RIPK1. If so, neutralization of interferon signaling under nutrient replete conditions might similarly favor the loss of RIPK1. It might be worth finding out.

References

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