Dissecting the RIPK3/MLKL necroptotic molecular switch

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Necroptosis is an inflammatory form of programmed cell death that is implicated in various human pathologies[1]. Signalling proceeds via a regulated kinase cascade involving Receptor Interacting Protein Kinases RIPK1 and RIPK3. RIPK3 phosphorylates the pseudokinase domain of Mixed Lineage Kinase-domain Like protein, MLKL, flipping a molecular switch that triggers MLKL activation, oligomerisation and translocation to the plasma membrane which it disrupts, causing cell death.

Our structural understanding of the MLKL molecular switch has, so far, been incomplete[2-4]. Furthermore, the interspecies incompatibility of the mouse and human necroptotic pathways has called into question whether structural and biochemical studies performed on the mouse pathway are representative of the human system[5]. Here we sought to address these key gaps in the literature with the first published crystal structures of human RIPK3; in complex with a Type I kinase inhibitor and in complex with MLKL[6]. We found that hRIPK3 exhibited a classic active kinase conformation when in complex with a Type I inhibitor but was in the kinase inactive conformation when in complex with MLKL. MLKL, which is unphosphorylated in the complex, is also in a kinase inactive-like conformation. Therefore, we propose the structure represents a basal pre-associated human RIPK3 and MLKL complex, which dissociates upon MLKL phosphorylation[4, 6]. Comparison with the previously published mouse MLKL:RIPK3 complex[7] helped explain the incompatibility between mouse and human necroptotic signalling, and supports the idea that MLKL and RIPK3 have co-evolved within species as a signalling cassette in response to varying selective pressures.