Nature Reviews Molecular Cell Biology | AOP, published online 1 August 2012; doi:10.1038/nrm3415

CELL SIGNALLING

A necrosome build-up

Receptor-interacting protein 1 (RIP1; also known as RIPK1) can control several crucial cellular functions, including cell survival downstream of nuclear factor-κB (NF-κB) signalling, apoptosis and programmed necrosis. Here, Wu and colleagues report that RIP1 interacts with RIP3 to form an amyloid-like fibrillar complex that is key in the initiation of programmed necrosis.

Previous studies had shown that RIP1 and RIP3 form a complex (known as the necrosome) in response to tumour necrosis factor-α (TNFα) signalling in the absence of functional caspase 8. The conserved RIP homotypic interaction motifs (RHIMs) were previously shown to be essential for necrosome formation, but the structure of the resulting complex was unknown. When Wu and colleagues overexpressed and co-purified the RHIM of RIP1 and His-tagged RIP3, they observed the formation of large macromolecular complexes. Electron microscopy imaging then showed that such complexes form filamentous structures. Moreover, overexpressed and purified full-length RIP1 and RIP3 proteins also formed similar fibrils.

Interestingly, fluorescence measurements of amyloid-specific dyes such as thioflavin T (ThT), circular dichroism, infrared spectroscopy, X-ray diffraction and solid-state nuclear magnetic resonance (NMR) studies indicated that the RIP1–RIP3 fibrillar complexes form an amyloid-like structure. Similarly to other amyloids, RIP1–RIP3 complexes had cross-β quaternary structures, with β-sheets lying parallel to the fibre axis and extended β-strands positioned perpendicular to this axis.

The authors next characterized the amyloid core of the RIP1–RIP3 complex, showing that it consists of 16 residues of the RIP1 RHIM and six residues of the RIP3 RHIM, and that mutation of these residues impaired fibril formation. Although these conserved residues could also promote the oligomerization of RIP1 or RIP3 to form homotypic fibrils, the authors suggested that the formation of RIP1–RIP3 heterotypic fibrils might be energetically favourable.

Finally, the authors stained amyloid-like RIP1-RIP3 complexes with ThT in RIP1- and RIP3-expressing cells that had entered programmed necrosis. Intriguingly, amyloid staining colocalized with RIP3-containing puncta structures, which have previously been suggested to be specific to necrotic cells. Moreover, ThT, which is known to inhibit amyloid oligomerization, promoted cell survival even in the presence of pro-necrotic stimuli. A similar pro-survival effect was observed in cells expressing RIP1 or RIP3 mutants that displayed defective oligomerization *in vitro*.

Together, these findings support the emerging concept that amyloid-like structures have physiological functions in addition to their well-known role in disease, and suggest that the gradual but irreversible oligomerization of signalling proteins into fibrils might be crucial for cellular functions. This notion is corroborated by the fact that proteins involved in pathogen-sensing and pro-inflammatory processes also contain RHIM domains.

Maria Papatriantafyllou

ORIGINAL RESEARCH PAPER Li, J. *et al.* The RIP1/RIP3 necrosome forms a functional amyloid signaling complex required for programmed necrosis. *Cell* **150**, 339–350 (2012)