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APOPTOSIS

Death by ubiquitylation

> “…p62 mediates the aggregation of ubiquitylated caspase 8…”

The extrinsic pathway of apoptosis is initiated when certain tumour necrosis factor superfamily ligands, such as APO2L (also known as TNFSF10 or TRAIL), activate cell surface death receptors. The ligand–receptor complex interacts with FAS-associated death domain protein, an adaptor protein that recruits caspase 8 — the caspase that initiates apoptosis in this pathway — to form a death-inducing signalling complex (DISC). Caspase 8 initially forms a dimer, but it must undergo multimerization (aggregation) to be fully activated. However, the exact mechanism of caspase 8 aggregation has remained unknown. Ashkenazi and colleagues now report that, to become fully active and able to commit cells to death, caspase 8 must undergo polyubiquitylation mediated by the cullin 3 (CUL3)-based E3 ubiquitin ligase, followed by ubiquitin-binding protein p62 (also known as sequestosome 1)-dependent aggregation.

The authors first observed that caspase 8 is ubiquitylated in response to APO2L and that this promotes caspase 8 aggregation rather than protein degradation. Tandem mass spectrometry of the DISC isolated from ligand-treated cells revealed the presence of the E3 subunit CUL3, the deubiquitylating enzyme A20 and p62. So, why do these proteins associate with the ligand-induced DISC and how do they influence caspase 8 activity and apoptosis?

Depletion of CUL3, or its associated subunit RING-box protein 1 (RBX1), markedly decreases the amount of ubiquitylated caspase 8 in the DISC and also decreases caspase 8 activity and apoptosis. Using ubiquitin mutants and mass spectrometry, the authors determined that caspase 8 undergoes CUL3-dependent polyubiquitylation in its C-terminal region and that this can promote caspase 8 aggregation and activation even in the absence of a ligand-induced DISC. A20 reverses the CUL3-mediated ubiquitylation of caspase 8 and the associated increase in caspase activity, which suggests that A20 is present in the DISC to serve as the deubiquitylating enzyme for caspase 8.

Finally, why is p62 associated with the DISC in cells treated with APO2L? The interaction of p62 with the DISC is dependent on CUL3-mediated ubiquitylation of caspase 8. Depletion of p62 reverses the aggregation of caspase 8 that occurs when caspase 8 is co-expressed with CUL3, and decreases caspase 8 activation. Furthermore, treatment of cells with APO2L results in the colocalization of caspase 8 and p62 to ubiquitin-rich foci in a p62-dependent manner. Together, these data suggest that p62 mediates the aggregation of ubiquitylated caspase 8 to promote its full activation and efficient apoptosis.

Thus, polyubiquitylation directly and positively influences caspase 8 in response to apoptotic stimuli and is a critical regulator of the extrinsic apoptotic pathway.

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ORIGINAL RESEARCH PAPER Jin, Z. *et al.* Cullin3-based polyubiquitination and p62-dependent aggregation of caspase-8 mediate extrinsic apoptosis signaling. *Cell* **137**, 721–735 (2009)

FURTHER READING Taylor, R. C. *et al.* Apoptosis: controlled demolition at the cellular level. *Nature Rev. Mol. Cell Biol.* **9**, 231–241 (2008)