CELL DEATH AND IMMUNITY

Gasdermins: the hole picture emerges

Recent studies have identified gasdermin D (GSDMD) as a crucial mediator of pyroptosis downstream of inflammasome and inflammatory caspase activation, but the mechanism involved was not known. Feng Shao and colleagues now report that gasdermin proteins can bind membrane lipids and form pores in the plasma membrane, leading to lytic cell death.

Pyroptosis is an inflammatory form of lytic cell death that is important for amplifying protective immunity during infection. The inflammatory caspases (caspase 1 and caspase 11 in mice; caspase 1, caspase 4 and caspase 5 in humans) are activated by inflammasome complexes (in the case of caspase 1) or by direct binding to lipopolysaccharide (caspase 4, caspase 5 and caspase 11) and cleave GSDMD between its active amino-terminal (GSDMD-N) and autoinhibitory carboxy-terminal (GSDMD-C) domains; this leads to the release and activation of the pyroptosis-inducing GSDMD-N. It has been unclear how GSDMD-N

subsequently mediates pyroptosis, but the authors hypothesized it may directly disrupt cellular membranes. In preliminary experiments, they showed that the N-domains, but not the full-length proteins, of other gasdermin family members (specifically, GSDMA, GSDMB and GSDMC) also induce pyroptosis in human cells. By using engineered forms of GSDMD, GSDMA and GSDMA3 in which the N- and C-domains remained non-covalently bound together after cleavage they were able to examine the localization of these domains while avoiding the toxic effects of the N-domain. These studies showed that the N-domain (but not the C-domain or full-length protein) of gasdermins can bind to plasma membrane phosphoinositides and to cardiolipin, which is an important component of the inner mitochondrial membrane and some bacterial membranes.

Next, the authors examined the localization of the N-domain of gasdermins (gasdermin-N) during pyroptosis using cell fractionation. They found that full-length GSDMD localized in the cytosol; after cleavage, GSDMD-C remained in the cytosol while GSDMD-N became associated with different membrane fractions. Using a fluorescently labelled mutant form of GSDMD-N (which induces pyroptosis more slowly than the wild-type protein) they were also able to visualize GSDMD-N translocating from the cytosol to the plasma membrane during pyroptosis in HeLa cells. They further showed that the disruption of membranes by GSDMD-N correlated with its lipid-binding properties; GSDMD-N induced cell lysis when delivered to the cytosol but not when administered extracellularly, which

is consistent with the localization of phosphoinositides in the cytoplasmic leaflet of the plasma membrane. A series of experiments using liposomes with different lipid compositions confirmed that gasdermin-N can induce leakage from liposomes composed of phosphoinositides or cardiolipin, but does not affect liposomes composed of cholesterol or phosphatidylethanolamine.

Experiments using liposomes that were loaded with differently sized dextrans suggested that items with diameters of 10nm or less could pass through the pores that were presumably being induced by GSDMD-N. Indeed, using negative electron microscopy, the authors could visualize pores with diameters of 10–16nm in liposomes that had been co-cultured with GSDMD-N, with closer analysis suggesting that GSDMD-N forms a 16-mer pore complex in its target membranes. Notably, these pores are of a sufficient size to facilitate the passage of mature interleukin-1 β (IL-1 β), IL-18 and caspase 1. The authors determined the crystal structure of GSDMA3 and showed that it exists in an auto-inhibited two-domain structure; a series of mutagenesis experiments confirmed that the pore-forming activity of the gasdermin-N domain is essential for its ability to induce pyroptosis. Interestingly, in structural homology searches, gasdermin-N showed no notable similarity to other pore-forming proteins. Therefore, gasdermin-N appears to represent a new type of pore-forming protein and may insert into membranes through a novel mechanism.

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“gasdermin proteins can bind membrane lipids and form pores in the plasma membrane”

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