Formation of membrane pores by gasdermin-N causes pyroptosis

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Pyroptosis, a lytic, highly inflammatory form of programmed cell death, plays an important role in the defense against microbial infections and danger signals. However, excessive pyroptosis can be detrimental to host defense. Pyroptosis is uniquely dependent on inflammatory caspases such as caspase-1, murine caspase-11, and its human homologs caspases-4 and -5. Caspase-1 is a central component of canonical NLRP3, NLRC4, AIM2, and pyrin inflammasomes, and its activation leads to proteolytic processing and secretion of proinflammatory cytokines IL-1 β and IL-18 as well as to pyroptosis (Man and Kanneganti, 2016). Intracellular lipopolysaccharide (LPS) from gram-negative bacteria can directly bind caspases-4, -5, or -11, and the activation of these caspases causes pyroptosis and non-canonical inflammasome activation (Hagar et al., 2013; Kayagaki et al., 2013; Shi et al., 2014). In 2015, two independent studies published in *Nature* by the Shao and Dixit groups identified gasdermin D (GSDMD) as a substrate for inflammatory caspases and showed that it was essential for inflammatory caspases-dependent pyroptosis and IL-1 β secretion (Kayagaki et al., 2015; Shi et al., 2015). Both groups demonstrated that inflammatory caspases cleaved GSDMD to generate N-terminal (~31 kDa) and C-terminal (~22 kDa) fragments. Ectopic expression of the N-terminal GSDMD fragment alone, but not of full-length or C-terminal GSDMD, induced pyroptosis, suggesting that C-terminal GSDMD has an autoinhibitory effect on the intrinsic pyroptosis-inducing activity of N-terminal GSDMD. Further, Shao and colleagues reported that other members

of the gasdermin family, such as GSDMA, GSDMB, GSDMC, DFNA5, and DFNB59, were not cleaved by inflammatory caspases. The identification of GSDMD as a substrate of inflammatory caspases is an important step forward in our understanding of the mechanism of pyroptosis. Thus, despite the crucial role of N-terminal GSDMD in pyroptosis, the precise regulation of N-terminal GSDMD induced pyroptosis and the functions of other members of the gasdermin family remain to be elucidated.

An exciting study recently published in *Nature* by the Wang and Shao groups reveals the molecular mechanism of gasdermin-N domain-induced pyroptosis (Ding et al., 2016). This study showed that gasdermin-N domains of human GSDMD, GSDMA, GSDMB, GSDMC, and DFNA5 and of mouse GSDMA3 were cytotoxic to mammalian cells. Moreover, the overexpression of GSDMD gasdermin-N domain (GSDMD-N) also killed bacteria. In contrast, the expression of full-length or C-terminal domain of the gasdermin family induced minimal cytotoxicity. One possible mechanism by which the gasdermin-N domains induce cytotoxicity is by disrupting membrane integrity, which leads to cell death. To confirm this possibility, the researchers purified full-length gasdermins, gasdermin-N domains, and gasdermin-C domains. By using the liposome co-sedimentation assay, they showed that only the gasdermin-N domains specifically bind to the membrane lipids phosphoinositide and cardiolipin. They further demonstrated that gasdermin-N domains translocated from the cytosol to the membrane and had a punctate distribution during pyroptosis. This led to the development of swelling bubbles in cells, followed by cell rupture. Notably, the induction of pyroptosis by gasdermin-N domains was observed only

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when they were delivered cytosolically but not when added extracellularly. This finding is consistent with the localization of phosphoinositides in the cytoplasmic leaflet, which indicates that lipid binding is a crucial step for the pyroptosis induced by gasdermin-N domains. The liposome-leakage assay showed that gasdermin-N domains caused leakage of phosphoinositide- or cardiolipin-containing liposomes. Further analysis showed that the threshold diameter of the leaked products was approximately 10 nm, suggesting that gasdermin-N domains can form regular pores in membranes. Indeed, the researchers found that membrane binding by gasdermin-N induced its oligomerization. Negative-stain electron microscopy revealed that incubation with gasdermin-N induced the formation of multiple pores on cardiolipin-, phosphoinositide-, or natural lipid-containing liposomes. Detailed analysis revealed that gasdermin-N forms 16-mer symmetric pores with inner diameters of 10–16 nm, which are wide enough for the passage of mature IL-1 β (4.5 nm) and caspase-1 (7.5 nm) (Figure 1).

Consistent with the above findings of pore-forming activity of gasdermin-N domains, a very recent study by the Dueber and Dixit groups showed that the human GSDMD-N domain P30 exhibits pore-forming activity in membranes during pyroptosis (Aglietti et al., 2016). The liposome-floating assay revealed that GSDMD P30 strongly colocalized with mitochondrial- or plasma membrane-like liposomes. Electron microscopy analysis revealed ring-like structures with darkly stained centers in liposomes incubated with GSDMD cleavage agents. Collectively, these two breakthrough findings support that the GSDMD-N domain forms pores in the membranes, which leads to pyroptosis. Whether membrane pore formation induced plasma membrane permeability is a fundamental question in cell death field, particularly for pyroptosis and necroptosis. This is the

first evidence to show that the membrane-pores composed of gasdermin-N domains are required for pyroptosis.

How is autoinhibition of the gasdermin family regulated? The Wang and Shao groups determined the 1.90 Å crystal structure of GSDMA3, which revealed that it is made up of the gasdermin-N and gasdermin-C domains (Ding et al., 2016). Structural homology analysis indicated that the gasdermin-N domain represents a new type of pore-forming protein (PFP). Crystal structure data showed that autoinhibition of the gasdermin family arises due to extensive interaction between the two domains. This finding was further supported by the observation that mutants with weak interactions had constitutive activation of GSDMA3. Finally, structure-guided point-mutation analysis identified key residues critical for the activity of gasdermin-N, which further confirmed that the membrane binding, oligomerization, and pore-forming activity of gasdermin-N are essential for pyroptosis.

Bacterial pore-forming toxins (PFTs) are the largest and best-characterized class of PFPs. Bacteria use PFTs to either kill other bacteria or to facilitate colonization and dissemination in host cells (Peraro and van der Goot, 2016). Most PFPs kill host cells from the outside. The identification of the gasdermin-N domain as a novel type of PFP raises exciting questions about the role of the gasdermin family in cell death, inflammation, and disease. How does gasdermin-N assemble to form pores in plasma membranes? How does gasdermin-N contribute to the membrane rupture of intracellular organelles? The cleavage mechanism for other gasdermin members, such as GSDMA, GSDMB, GSDMC, and DFNA5, continues to remain enigmatic. Future studies need to examine the role(s) of gasdermin-dependent cell death in infectious diseases, inflammatory diseases, and cancer. Uncovering the functional mechanism by which the

Figure 1 Membrane pore-forming activity of gasdermin-N domains during pyroptosis.

gasdermin family induces cell death can aid the development of novel therapeutic strategies to prevent the progression of infections and excessive immune responses.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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