Cell Research (2015) 25:1183-1184.

www.nature.com/cr

RESEARCH HIGHLIGHT

Gasdermin D: the long-awaited executioner of pyroptosis

Cell Research (2015) **25**:1183-1184. doi:10.1038/cr.2015.124; published online 20 October 2015

Inflammatory caspases drive a lytic form of cell death called pyroptosis in response to microbial infection and endogenous damage-associated signals. Two studies now demonstrate that cleavage of the substrate gasdermin D by inflammatory caspases necessitates eventual pyroptotic demise of a cell.

Inflammatory caspases, including caspase-1, -4, -5 and -11, are crucial mediators of inflammation and cell death. Caspase-1 is found in humans and mice. Caspase-4 and -5 are found in humans and the orthologue caspase-11 is found in mice. Inflammatory caspases form part of a dynamic multi-protein complex known as the inflammasome, which orchestrates proteolytic processing of the pro-inflammatory cytokines IL-1β and IL-18. Inflammatory caspases also induce a lytic form of cell death known as pyroptosis. Lipopolysaccharide (LPS) from Gram-negative bacteria introduced into the cytoplasm of host cells during infection potently activates caspase-11 in mice and caspase-4 and -5 in humans [1, 2]. Caspase-4, -5 and -11 directly bind LPS [3], and activation of these caspases triggers pyroptosis, NLRP3 inflammasome activity, and endotoxic shock [4]. How inflammatory caspases precisely regulate these cellular and physiological events is an unresolved question.

Research groups led by Vishva M Dixit [5] and Feng Shao [6] independently identified a key substrate for inflammatory caspases called gasdermin D, which upon its cleavage drives pyroptosis (Figure 1). Dixit and colleagues used a forward genetic approach (treatment of mice with the mutagen ENU) to screen for mutations that would impair activation of the caspase-11-dependent pathway. Through this screen, the authors found that peritoneal macrophages harvested from a mouse strain harboring a mutation in the gene encoding gasdermin D, called *Gsdmd*$^{I105N/I105N}$ (owing to an isoleucine-to-asparagine substitution mutation at position 105), did not undergo pyroptosis and release IL-1β in response to LPS transfection. On the other hand, Shao and colleagues utilized CRISPR-Cas9 technology and siRNA-mediated knockdown approaches to pinpoint gasdermin D as a culprit in mediating LPS-induced pyroptosis in mouse and human cells.

Both groups subsequently generated mouse strains with a genomic deletion of gasdermin D (*Gsdmd*$^{-/-}$) to further confirm a requirement for this protein in caspase-11-dependent pyroptosis. They found that *Gsdmd*$^{-/-}$ bone marrow-derived macrophages (BMDMs) stimulated with intracellularly-delivered LPS failed to undergo pyroptosis or secrete IL-1β into the cell culture supernatant. Dixit and colleagues also identified a lack of caspase-1 and IL-1β processing in *Gsdmd*$^{-/-}$ BMDMs in response to intracellularly-delivered LPS. Both groups deleted gasdermin D in human cell lines via CRISPR/Cas9 technology and observed that these cells were resistant to LPS-induced pyroptosis. Further, Shao and colleagues ruled out a role for gasdermin D in necroptotic and apoptotic cell death pathways. These key observations from both groups collectively establish gasdermin D as a pyroptosis-inducing factor which acts downstream of caspase-4, -5 and -11, leading to physical rupture of the cell membrane that mediates release of matured IL-1β from the cell.

To further dissect how gasdermin D might contribute to the caspase-11-dependent pathway, both groups performed a series of biochemical assays to demonstrate that recombinant caspase-11 cleaved gasdermin D between the Asp276 and Gly277 residues of mouse gasdermin D, generating an N-terminal (30-31-kDa) and a C-terminal fragment (22-kDa). Caspase-4 or -5 also induced cleavage of human gasdermin D. Expression of the N-terminal fragment of gasdermin D alone induced pyroptosis, whereas the C-terminal fragment provided autoinhibition prior to cleavage of the full-length protein. Reconstitution of a mutant gasdermin D bearing a mutation that abolishes its ability to undergo cleavage did not engage pyroptosis.

Shao and colleagues found that other members of the gasdermin family were not cleaved by inflammatory caspases, while caspase-1 also cleaved gasdermin D at the same site as caspase-11. Cleavage of gasdermin D by caspase-1 suggests that this substrate could be involved in pyroptosis mediated by caspase-11-independent canonical inflammasomes [7]. Canonical inflammasomes can be activated by a range of microbe-associated molecular patterns and damaged-associated molecular patterns [8]. Shao and colleagues, indeed, provide evidence that gasdermin D played a role in driving pyroptosis induced by the NLRP3 (LPS plus nigericin), NLRC4 (*Salmonella enterica* serovar Typhimurium or its flagellin), AIM2 (the dsDNA ligand poly(dA:dT)) or Pyrin (*Clostridium difficile* toxin B)

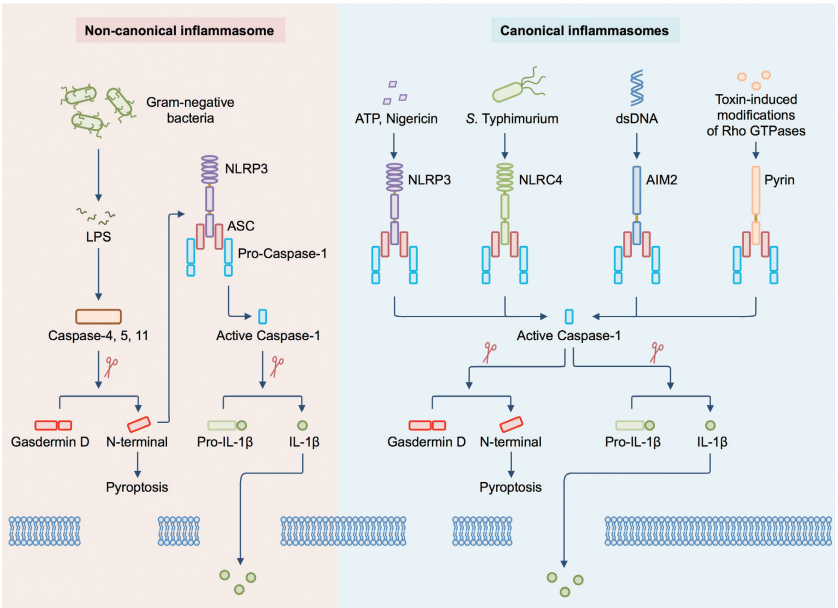

Figure 1 The role of gasdermin D in pyroptosis driven by non-canonical and canonical inflammasomes. In the non-canonical inflammasome pathway, LPS released into the cytoplasm by Gram-negative bacteria binds to caspase-4 and -5 in humans and to caspase-11 in mice. These caspases cleave the 53-kDa gasdermin D into a 31-kDa N-terminal and a 22-kDa C-terminal fragment. The N-terminal fragment initiates pyroptosis of the cell and activates the NLRP3 inflammasome to drive caspase-1-dependent maturation of the pro-inflammatory cytokine IL-1β. IL-1β is released from the cell upon membrane rupture. In the canonical inflammasome pathway, activation of the NLRP3, NLRC4, AIM2 or Pyrin inflammasome receptor triggers recruitment of the adaptor protein ASC and caspase-1 into the same inflammasome platform. In this case, caspase-1 cleaves both gasdermin D and pro-IL-1β to initiate pyroptosis and maturation of IL-1β, respectively.

inflammasome. Macrophages stimulated with canonical inflammasome activators retained the ability to undergo proteolytic processing of both caspase-1 and IL-1β in the absence of gasdermin D, placing gasdermin D downstream of canonical caspase-1 activation (Figure 1).

Dixit and colleagues also reported reduced pyroptosis in *Gsdmd*$^{-/-}$ BMDMs stimulated with canonical inflammasome activators. Unlike BMDMs lacking caspase-1, the authors found that resistance to pyroptosis in *Gsdmd*$^{-/-}$ BMDMs faded in response to prolonged inflammasome stimulation. These observations reveal that caspase-1 could be cleaving other pro-pyroptotic factors that drive canonical inflammasome cell death in the absence of gasdermin D. Finally, Dixit and colleagues injected wild-type mice or mice lacking gasdermin D or caspase-11 with LPS to induce endotoxic shock. They showed that mice lacking gasdermin D were largely protected from mortality, as were mice lacking caspase-11 [1, 2, 4, 9].

Identification of gasdermin D as a pyroptosis-inducing substrate of inflammatory caspases opens up new and exciting research questions in the field of inflammation and cell death. More than a hundred proteins are thought to be cleaved by caspase-1, including caspase-7 [10]. However, caspase-7 and many other substrates are not involved in pyroptosis. How gasdermin D precisely orchestrate cell death is an important question for future investigation. Does gasdermin D have the capacity to be inserted into the cell membrane to drive pore formation and cellular leakage seen in pyroptosis? Does the N-terminal fragment of gasdermin D cooperate with other substrates cleaved by inflammatory caspases to drive pyroptosis? The answers to these questions will bring us closer to unveiling the molecular events governing inflammasome-mediated cell death. Pyroptosis is a double-edged sword. Removal of host cells that support pathogen replication by pyroptosis is a useful tactic in the host defense against intracellular bacteria [11]. In contrast, it also depletes CD4 T cells and drives immunodeficiency during HIV-1 infection [12]. The physiological relevance of gasdermin D and pyroptosis should now be examined further in a range of diseases, including cancer and autoinflammatory conditions.

Si Ming Man[1],
Thirumala-Devi Kanneganti[1]

[1]*Department of Immunology, St. Jude Children's Research Hospital, MS #351, 262 Danny Thomas Place, Memphis, TN 38105, USA*
Correspondence: Thirumala-Devi Kanneganti
Tel: +1-901-595-3634; Fax: +1-901-595-5766
E-mail: Thirumala-Devi.Kanneganti@stjude.org

References

1 Kayagaki N, Wong MT, Stowe IB, *et al*. *Science* 2013; **341**:1246-1249.
2 Hagar JA, Powell DA, Aachoui Y, *et al*. *Science* 2013; **341**:1250-1253.
3 Shi J, Zhao Y, Wang Y, *et al*. *Nature* 2014; **514**:187-192.
4 Kayagaki N, Warming S, Lamkanfi M, *et al*. *Nature* 2011; **479**:117-121.
5 Kayagaki N, Stowe IB, Lee BL, *et al*. *Nature* 2015 Sep 16. doi:10.1038/nature15541
6 Shi J, Zhao Y, Wang K, *et al*. *Nature* 2015 Sep 16. doi:10.1038/nature15514
7 Agard NJ, Maltby D, Wells JA. *Mol Cell Proteomics* 2010; **9**:880-893.
8 Man SM, Kanneganti TD. *Immunol Rev* 2015; **265**:6-21.
9 Wang S, Miura M, Jung YK, *et al*. *Cell* 1998; **92**:501-509.
10 Lamkanfi M, Kanneganti TD, Van Damme P, *et al*. *Mol Cell Proteomics* 2008; **7**:2350-2363.
11 Miao EA, Leaf IA, Treuting PM, *et al*. *Nat Immunol* 2010; **11**:1136-1142.
12 Doitsh G, Galloway NL, Geng X, *et al*. *Nature* 2014; **505**:509-514.