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EDITORIAL

Regulating GSDMB pore formation: to ignite or inhibit?

In the recent Nature, Wang et al. and Zhong et al. present the cryo-EM structures of Gasdermin B (GSDMB) pore and strucures of GSDMB in complex with a *Shigella* effector, IpaH7.8. The structures shed light on the structural mechanisms that govern GSDMB-mediated pyroptosis, a process that is regulated by pathogenic bacteria and alternative splicing.

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Pyroptosis is an inflammatory type of cell death closely related to host innate immunity and tumor immunotherapy. It is triggered by divergent cellular events, such as intracellular microbial infections or endogenous cellular damage, that activate various cell-intrinsic or extrinsic proteases, including pro-inflammatory or apoptotic caspases, lymphocyte-derived granzymes, neutrophil elastase, and streptococcal pyrogenic exotoxin B (SpeB) [1–5]. These proteases cleave the downstream pore-forming proteins–Gasdermins (GSDMs). Cleavage facilitates the release of the GSDM N-terminal domains, which oligomerize to form pores on the cell membrane, mediate the release of inflammatory cytokines, and ultimately cause pyroptotic cell death.

There are six GSDMs in humans, including GSDMA–E and DFNB59. GSDMB is currently the most controversial member of this family. First, it is believed that GSDMB lacks autoinhibition and exhibits clear lipid-binding capability in its full-length form [6]. Second, the pyroptotic function of GSDMB has been recently questioned. While an earlier study suggested that GSDMB induces cancer cell pyroptosis upon cleavage by Granzyme A (GZMA) from natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) [2], two recent studies have demonstrated that GSDMB is non-pyroptotic [7, 8], but plays an antibacterial role by killing intracellular bacteria [7]. Interestingly, this process can be subverted by the causative agent of Shigellosis, *S. flexneri*, which uses a type III secretion system effector IpaH7.8 to ubiquitinate GSDMB for proteasomal degradation [7]. It is worth noting that IpaH7.8 can also ubiquitinate GSDMD, but only in humans, not in mice [9]. This finding may explain why humans and non-human primates are the natural reservoirs for *Shigella*, whereas mice are not.

Recently, several studies have been published to answer the questions of whether GSDMB can induce pyroptosis, how *Shigella* IpaH7.8 targets GSDMB, and particularly, why IpaH7.8 ubiquitinates human but not mouse GSDMD [10–13]. In the latest issue of *Nature*, Wang et al. and Zhong et al. determined the structures of GSDMB-IpaH7.8 complex using cyrogenic electron microscopy (cryo-EM) and X-ray crystallography, respectively [10, 11]. Both structures contain a full-length GSDMB adopting the autoinhibited confirmation and an IpaH7.8 LRR domain interacting with the GSDMB N-terminal pore-forming domain (GSDMB-N). The IpaH7.8 LRR domain specifically recognizes an acidic motif that contains residues E15, D17, and D21 at the C terminus of helix α1 in GSDMB-N as the structural determinant. Interestingly, these three acidic residues are conserved in humans but not mouse GSDMD. Mouse GSDMD has a substitution (D17S) and an insertion (R20) in this motif, which prevent IpaH7.8 from binding and ubiquitinating mouse GSDMD. Mutation of these conserved acidic residues in GSDMB or human GSDMD disrupted their interaction with IpaH7.8, while the ubiquitination of mouse GSDMD was restored when the non-conserved residues were substituted with the corresponding residues in GSDMB or human GSDMD.

Wang et al. then discovered that IpaH7.8 is more effective in inhibiting GSDMB than human GSDMD (Fig. 1). Firstly, IpaH7.8 directly inhibits GSDMB pore formation by restricting GSDMB β3 strand, an essential structural element for the insertion of GSDMB into the membrane. Using liposome leakage and pull-down assays, Wang et al. found that IpaH7.8-binding prevented the association of GSDMB-N with liposomes and inhibited GSDMB pore formation. Subsequent bacterial killing and cytotoxicity assays using the catalytically inactive IpaH7.8^{C357A} mutant further confirmed that IpaH7.8 significantly reduced the cytotoxicity of GSDMB-N on both *Escherichia coli* and HEK293T cells. In contrast, IpaH7.8 exhibited no significant direct inhibition of human GSDMD, likely due to its substantially weaker binding affinity for human GSDMD. Secondly, ubiquitination of GSDMB is sufficient to inhibit its pore-forming activity. Wang et al. elegantly identified three ubiquitination sites at the transmembrane region of GSDMB through in vitro ubiquitination assay. These three lysines are not structurally conserved in human GSDMD. The authors suggested that ubiquitination at these sites could affect the pore formation of GSDMB but not GSDMD, which was confirmed by liposome leakage and pulldown assays. This efficient inhibition may allow *Shigella* to escape the attack by CTLs and NK cells and quickly establish replicative niches in the host.

GSDMB was previously thought to possess a weakened autoinhibition due to the lack of a subdomain in its autoinhibitory C-terminal domain, allowing full-length GSDMB to bind acidic phospholipids [6]. However, the full-length structure of GSDMB in the GSDMB-IpaH7.8 complex revealed an even stronger auto-inhibition in GSDMB. Although the interactions in the major interface are highly conserved, the minor interface where GSDMB-N α4 lies in a gigantic hydrophobic groove formed by α9, α10 and α12 in GSDMB-C is significantly larger than that in GSDMD and GSDMA3 [14, 15]. Single-residue mutations in the minor interface are insufficient to activate GSDMB. Moreover, cleaved GSDMB exhibited slower kinetics in inducing liposome leakage, further confirming this structural analysis.

There are at least six splice variants of GSDMB in humans. Among these variants, isoform 5 contains only the C-terminal domain, while isoforms 1–4 and 6 contain conserved N- and C-terminal domains but are varied in lengths and sequences in their interdomain linkers. It is speculated that the interdomain linker may regulate the pore-forming activity of GSDMB. To test

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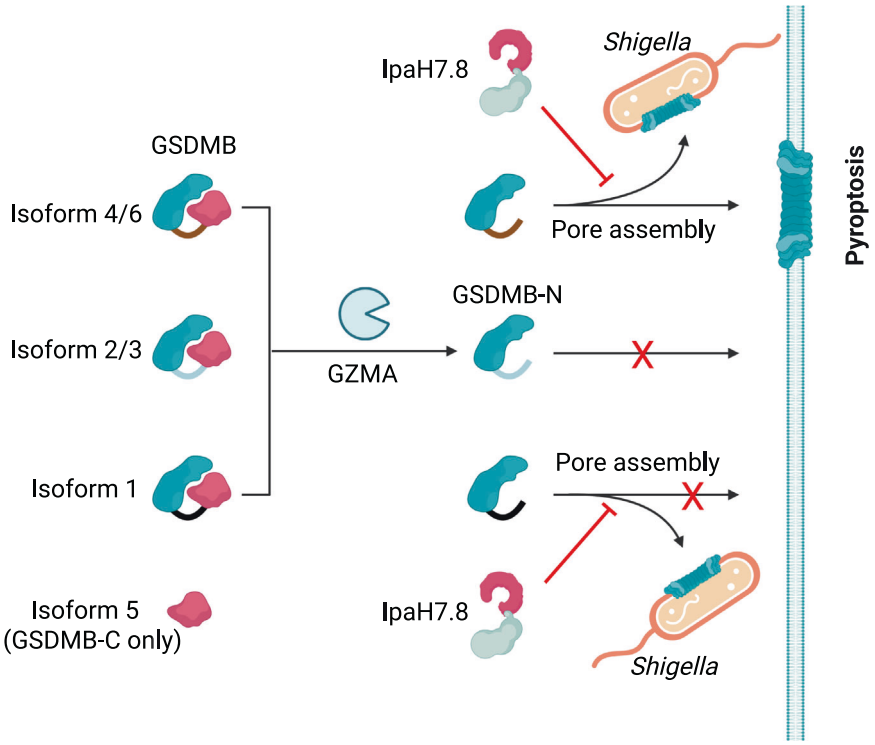

Fig. 1 GSDMB pore formation is regulated by alternative splicing and bacterial effector IpaH7.8. In humans, there exist six splicing transcripts for GSDMB, with isoforms 1–4 and 6 containing conserved N- and C-terminal domains that differ in their interdomain linker. Isoform 5, on the other hand, only contains the C-terminal domain. GSDMB isoforms 4 and 6, which possess the canonical interdomain linker, are capable of inducing pyroptotic cell death and killing intracellular bacteria. Meanwhile, GSDMB isoform 1 has an alternative interdomain linker with a four-amino-acid insertion and displays reduced pore-forming activity that is insufficient to induce pyroptosis but is enough to kill bacteria. GSDMB isoforms 2 and 3 lack the entire canonical sequence in the interdomain linker and exhibit no pore-forming activity. Additionally, the Shigella effector IpaH7.8 efficiently inhibits the pore-forming activity of GSDMB by binding to GSDMB-N and through the ubiquitination of GSDMB transmembrane region. (The figure was created in Biorender.).

this hypothesis, Wang et al. expressed the N-terminal domains of GSDMB isoforms in HEK293T cells and observed that isoforms 4 and 6, which contain the canonical sequence encoded by exon 6 in their interdomain linker, exhibited significant pyroptotic activity. In contrast, isoforms 1, 2, and 3 did not induce cell death (Fig. 1).

To gain a better understanding of how the interdomain linker regulates GSDMB pore-forming activity, Wang et al. and Zhong et al. conducted cryo-EM studies to determine the structures of the GSDMB pore form by isoforms 1 and 6, which were composed of 24–26 and 26–30 subunits, respectively. Despite differences in their stoichiometry, the mechanism for pore assembly in GSDMB is highly conserved with that in GSDMD and GSDMA3 [16, 17]. Detailed analysis of the GSDMB pore structure reveals that the canonical sequence in the interdomain linker plays a crucial role in both the oligomerization and lipid binding. The first half of the canonical linker (Region I) is involved in the interaction between subunits. Mutations in this region abolish the pore-forming activity of GSDMB. Whereas the second half of the canonical linker (Region II) contains three basic residues that directly interact with negatively charged phospholipids on the membrane, forming an additional lipid-binding site. This lipid-binding site is conserved in GSDMB isoforms 4 and 6, which exhibit strong pore-forming activity. However, in GSDMB isoform 1, a four-amino-acid insertion in the interdomain linker disrupts the interaction with the membrane, resulting in reduced pore-forming activity in vitro. Strikingly, this attenuated activity of isoform 1 is sufficient to kill bacteria but fails to trigger pyroptosis in cells because of the membrane repair efforts by ESCRT III. Isoforms 2 and 3 lack the entire canonical sequence, leading to the complete loss of their pore-forming activity. The additional lipid-binding site is conserved in human GSDMD. Mutation of the structurally conserved basic resides markedly reduced the activity of GSDMD.

Overall, the structures of GSDMB-IpaH7.8 complex and GSDMB pore provide valuable insights into the mechanisms that govern GSDMB-mediated pyroptosis, which is regulated by pathogenic bacteria and alternative splicing. GSDMB is expressed in diverse organs and tumor types, including melanoma, breast cancer, and colon cancer [2, 18]. High levels of GSDMB have been linked to increased cell proliferation, resistance to cell death, and a more aggressive tumor phenotype, suggesting a pro-tumor function of nonpyroptotic GSDMB [8]. However, in the context of GSDMB-mediated cancer cell pyroptosis triggered by NK and T-cells, GSDMB plays an antitumor role through its pyroptotic function [2]. Studies by Wang et al. and Zhong et al. emphasize the significance of GSDMB isoforms with distinct pyroptotic activities. Further research is urgently needed to investigate the physiological relevance of GSDMB isoform distribution and redundancy in cancer. Moreover, recent studies have identified S-palmitoylation as a novel mechanism that regulates GSDM pore formation [19, 20]. However, it is still unclear whether this modification occurs in all GSDMB isoforms and how it impacts GSDMB pore formation.

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DATA AVAILABILITY

This article does not present any new primary data.

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COMPETING INTERESTS

The author declares no competing interests.

ADDITIONAL INFORMATION

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