

## Review Article

# Advances in gasdermin activation, regulation, and targeted drug development

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## ABSTRACT

Proteins of the gasdermin family are executors of pyroptosis. Upon cleavage-dependent/independent activation, gasdermins form pores on the cell membrane, which damage the integrity of the cell membrane and lead to pro-inflammatory cellular content release and lytic cell death. The role of gasdermin in pyroptosis-associated diseases, such as allergic diseases, neurologic disorders, infections, and cancer, showcases the tremendous therapeutic potential of targeting gasdermin. New insights into non-pyroptotic functions continue to further our understanding of gasdermin. Herein we review current knowledge of the gasdermin family, demonstrating the mechanism of activation, downstream effects, and regulatory mechanisms. We also discuss the association with pyroptosis-associated diseases and the functions beyond pyroptosis, shedding light on the broader roles in various pathologic processes. Furthermore, we summarize the advances in the development of targeted agonists and inhibitors with strategies for developing pyroptosis-based anti-tumor therapies.

**Keywords:** gasdermin family, activation mechanisms, regulatory mechanisms, pyroptosis-associated diseases, non-pyroptotic functions, targeted drugs

## 1. INTRODUCTION

The gasdermin (GSDM) protein family, which is widely present in a variety of species (humans, mice, rats, birds [chickens [1] and ducks [2]], pufferfish [3, 4], zebrafish [5, 6], coral [7], and bacteria [8]), has a significant role in physiology and pathology. This GSDM family is comprised of six paralogous genes in humans (*GSDMA*, *GSDMB*, *GSDMC*, *GSDMD*, *GSDME* [also known as *DFNA5*], and *PJVK* [also known as *DFNB59*]). Within the GSDM family, *GSDMA/B/C/D/E* are the executor of pyroptosis, resulting in cell death and inflammation [9, 10]. In contrast, *PJVK* loses pore-forming and cell lysis functions [11], and has been shown to be associated with hearing loss [12]. Among the GSDMs, *GSDMD*, as the key executor of pyroptosis, is the most studied and has a significant role in a wide variety of diseases.

With the exception of *PJVK*, all other GSDMs share highly conserved N-terminal (NT) and C-terminal (CT) domains, which are separated by a variable linker [13]. All GSDMs, including *PJVK*, are likely to have a

role in inflammation [11] and function in physiologic and pathologic processes [13]. In the full-length (FL)-GSDM resting state the pore-forming fragment (the NT domain) is masked by the CT domain. Therefore, GSDM pore formation is autoinhibited [14]. Upon activation, GSDMs assemble into pores on the cell membrane and trigger lytic cell death. Cellular content, including inflammatory cytokines, are released into the extracellular space, which can lead to inflammation.

A comprehensive literature search of the PubMed database was performed to systematically identify relevant studies for this review with a focus on articles published between 2023 and 2025, while also considering articles from 2015–2022 to ensure a comprehensive search. Our search strategy centered on keywords, such as “gasdermin,” “pyroptosis,” “diseases,” “activation mechanism,” “regulatory mechanism,” “non-pyroptotic functions,” and “targeted drugs” combined with Boolean operators. We limited our search to articles published in English and excluded studies that were not peer-reviewed. A total of 266 articles are referenced in this review.

## 2. MECHANISMS OF GSDM ACTIVATION

Although the specific mechanisms of activation vary for each GSDM family member, the mechanisms can be broadly categorized as cleavage-dependent and -independent.

### 2.1 Cleavage-dependent activation

The CT domain folds back onto the NT domain before activation, leaving the FL-GSDM in an autoinhibited state [14]. Proteolytic cleavage at specific sites within the linker domain of GSDMs releases the pore-forming NT domain (Figure 1), which translocates to and assembles into pores on mitochondria, lysosomes, the inner leaflet of the plasma membrane, and potentially other membranous organelles [15]. Different GSDMs are activated by different proteases and the same GSDM can also be cleaved and activated by multiple proteases (Table 1).

**2.1.1 GSDMA.** GSDMA cleavage is activated upon group A *Streptococcus* (GAS) infection and is mediated by the virulence factor, SpeB. However, extracellular SpeB is not sufficient to activate GSDMA [16] and the pore-forming GAS toxin, streptolysin O, is required for SpeB to gain access to the cytosol and function. GSDMA-dependent pyroptosis in keratinocytes contributes to the immune defense against GAS, the major skin pathogen causing acute cellulitis. GSDMA-deficient mice have been reported to be more susceptible to GAS infection [16, 17].

In addition, GSDMA in non-mammals, such as amphibians, birds and reptiles, which may represent the ancestral

state of GSDMA, can also be activated by caspase-1. In contrast, caspase-1-mediated cleavage in mammalian GSDMA is disrupted due to a mutation in the linker region [37].

**2.1.2 GSDMB.** All splicing isoforms of GSDMB can be cleaved by granzyme A (GZMA), a serine protease released from neighboring cytotoxic lymphocytes that enters target cells through perforin [18, 38]. However, only cleavage of the GSDMB isoforms with exon 6 activates pyroptosis [19, 20, 38]. Thus, GSDMB differs from other GSDMs in the ability to regulate pyroptosis activity through alternative splicing. GSDMB can also be cleaved by inflammatory caspase-1 [21], which might contribute to the development of asthma.

Notably, a functional GSDMB splice variant (rs11078928) was identified in 2018, which is associated with a decreased risk of asthma. This variant deletes the entire exon 6, which abolishes the ability to induce epithelial cell pyroptosis [21].

**2.1.3 GSDMC.** Caspase-8-mediated GSDMC cleavage can be activated through poly ADP-ribose polymerase inhibitors (PARPis) [22], TNF- $\alpha$  [23], and  $\alpha$ -KG-induced DR6 receptosome [24] in cancer cells, which inhibits tumor growth and metastasis.

**2.1.4 GSDMD.** Progressive truncation studies have identified residues 1–243 of GSDMD as the minimal NT fragment capable of inducing pyroptosis [25].

Upon inflammasome activation, inflammatory caspases cleave GSDMD at Asp275 [26]. More specifically,

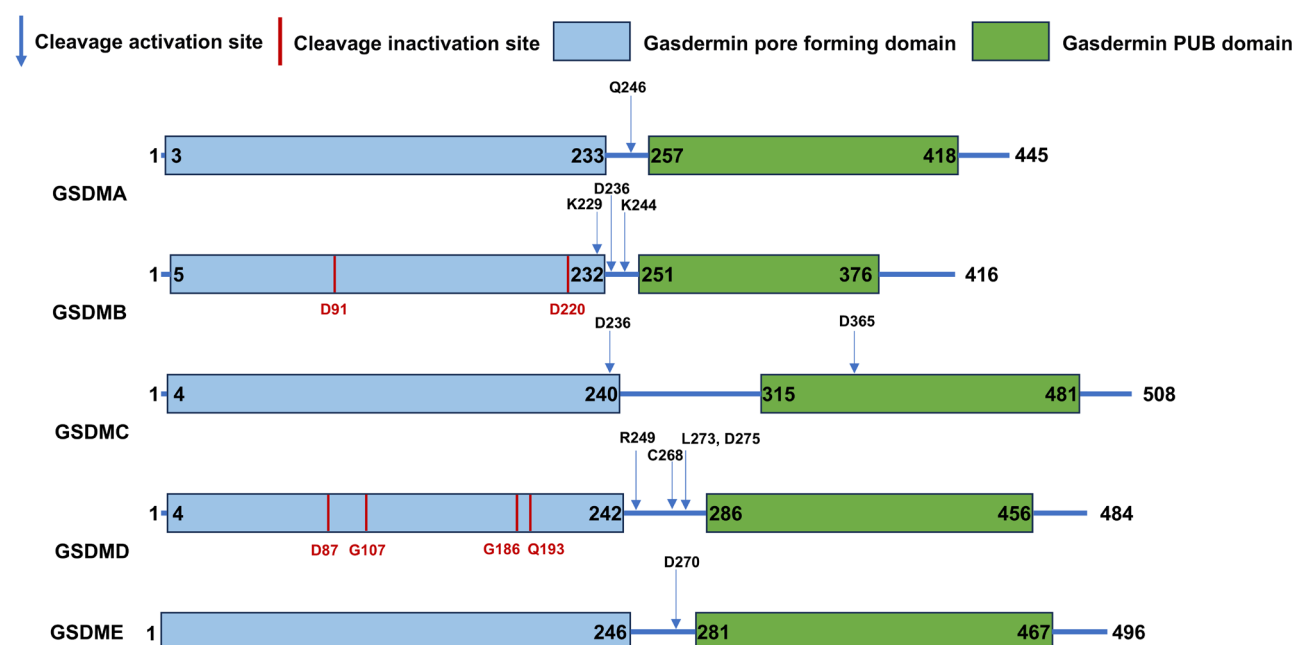


**Figure 1 | Cleavage sites of human GSDMs.**

The blue arrows indicate the sites on GSDMs where cleavage can lead to the activation of GSDMs. The red lines mark the sites where cleavage can lead to the production of smaller fragments that lose pore-forming activity. PUB: proline-rich ubiquitin-like binding domain.

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**Table 1** | Cleavage-dependent activation of human GSDMs.

| Human gene<br>(chromosomal location) | Protease            | Cleavage site               | Tetrapeptide<br>sequence | Refs.    |
|--------------------------------------|---------------------|-----------------------------|--------------------------|----------|
| GSDMA (17q21.1)                      | SpeB                | Q246                        | ILIQ                     | [16, 17] |
| GSDMB (17q21.1)                      | Granzyme A          | K244                        | CLGK                     | [18-20]  |
|                                      |                     | K229                        | GKTK                     |          |
|                                      | Caspase-1           | D236                        | EEKD                     | [21]     |
| GSDMC (8q24.21)                      | Caspase-8           | D365                        | LELD                     | [22-24]  |
|                                      | Caspase-6           | May be similar to Caspase-8 | -                        |          |
| GSDMD (8q24.3)                       | Caspase-1/4/5/11    | D275                        | FLTD                     | [25, 26] |
|                                      | Caspase-8           | -                           | -                        | [27, 28] |
|                                      | Cathepsin G         | Maybe L273 (L274 in mouse)  | -                        | [29]     |
|                                      | NS3B3 protease      | R249                        | GHKR                     | [30]     |
|                                      | Neutrophil elastase | C268                        | MMRC                     | [31]     |
| GSDME (7p15.3)                       | Granzyme B          | -                           | -                        | [32]     |
|                                      | Caspase-3           | D270                        | DMPD                     | [33-36]  |
|                                      | Mouse Caspase-7     | -                           | -                        | [3]      |

GSDMD cleavage starts with the congregation of inflammasome in canonical inflammasome-induced pyroptosis [39]. When cells are stimulated by pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs), or environmental stimuli (such as UV radiation), cytosolic inflammasome sensors recognize these signals and bind to pro-caspase-1 via the inflammasome adaptor ASC protein. These sensors include the NBD- and LRR-containing protein (NLR) family, absent in melanoma 2 (AIM2), pyrin, and CARD8 [40]. The pro-caspase-1 monomers cluster and the protease domains dimerize within the inflammasome [7]. Dimerization enables pro-caspase-1 to gain the capacity to self-cleave and form a dimeric species (p33/p10) with full enzyme activity [40]. The activated caspase-1 then cleaves GSDMD, generating a peptide containing the membrane perforation domain, meanwhile cleaving pro-IL-1 $\beta$  and pro-IL-18 [41, 42], forming mature IL-1 $\beta$  and IL-18 which are released extracellularly to recruit inflammatory cells and expand inflammation.

Caspase-4/5 (caspase-11 in mice) directly recognizes the lipid A moiety of cytosolic LPS via the CARD8 domain in non-canonical inflammasome-induced pyroptosis [43]. This process results in clustering and activation of these caspases, thereby inducing pyroptosis. Guanylate-binding proteins (GBPs) promote the recruitment and activation of caspase-4 by interacting with LPS and forming a caspase-4 activation platform on the bacterial cell surface [44]. Moreover, it is notable that IL-18, not IL-1 $\beta$ , is identified as a target of activated caspase-4/5 [45].

Of note, bacterial GSDM activation has recently been shown to be mediated by the important facilitators of

inflammasome activity (CARD domains), which protect bacteria against phages [46]. It has been suggested the CARD-dependent activation of GSDMs is evolutionarily conserved.

GSDMD-dependent pyroptosis can be activated in protein tyrosine phosphatase 1B (PTP1B)-deficient cells within hypoxic tumors. Mechanistically, PTP1B and tyrosine kinase ABL1/2 reciprocally regulate phosphorylation level of E3 ubiquitin ligase RNF213 at Tyr1275, which controls RNF213 oligomerization and promotes RZ domain activation. The activated RZ domain ubiquitylates and induces degradation of the major NF- $\kappa$ B regulator (CYLD/SPATA2), which leads to NF- $\kappa$ B activation, upregulates expression of *NLRP3*, and primes cells for pyroptosis. Hypoxia-induced endoplasmic reticulum stress provides a "second signal," triggering inflammasome assembly and GSDMD cleavage. Therefore, GSDMD-dependent pyroptosis can be activated through the PTP1B–RNF213–CYLD–SPATA2 pathway [47].

In the case of X-linked inhibitor of apoptosis protein deficiency [27] or *Yersinia* infection [28], TNFR1 and TLR signaling may drive abnormal activation of caspase-8, which cleaves GSDMD and leads to overactivation of GSDMD in macrophages. GSDMD is cleaved and activated by elastase [31] and cathepsin G in neutrophils [29], which are released into the cytoplasmic lysate during pathogen invasion. Seneca Valley virus 3C protease cleaves porcine GSDMD at Gln277 to induce pyroptosis in porcine kidney SK6 cells, which is close to the cleavage site of caspase-1 [48]. Moreover, upon infection, the Zika virus (ZIKV) protease NS2B3 also directly processes GSDMD into the activated NT fragment (1–249 aa) [30].

**2.1.5 GSDME.** GSDME is activated by caspase-3, which is a hallmark of apoptosis. The level of GSDME expression has a key role in determining whether cell death transitions from apoptosis to pyroptosis. Pyroptosis is favored over apoptosis in cells with high GSDME expression, which might cause strong pyroptosis before the onset of apoptosis. Conversely, in cells with low GSDME expression, apoptosis is likely to precede pyroptosis [33, 34]. The caspase-3-GSDME pathway can be activated by intrinsic and extrinsic apoptotic pathways. Following activation of TNF- $\alpha$ -induced extrinsic apoptotic pathways, the GSDME-NT generated through cleavage by apoptotic caspase-3 will preferentially permeabilize the mitochondria before causing plasma membrane rupture (PMR). The Cyt c released from mitochondria augments activation of intrinsic apoptotic pathway, activating the Apaf-1 apoptosome and potentiating caspase-3 activation. As a result, a self-amplifying loop for GSDME cleavage is generated [35]. More importantly, chemotherapeutic drugs (navitoclax, etoposide) and other mitochondrial outer membrane permeabilization pathway inducers (such as TNF- $\alpha$ +CHX) have been reported to induce cancer cell pyroptosis by activating the BAK/BAX-caspase-3-GSDME pathway [36], which might contribute to the tumor suppressive activity of GSDME.

In addition, GSDME has been reported to be cleaved by killer cell granzyme B (GZMB). As GZMB enters tumor cells via perforin, GZMB activates caspase-independent pyroptosis by directly cleaving GSDME at the same site as caspase 3 [32], which activates anti-tumor immunity and suppresses tumor growth. In addition, mouse caspase-7 also cleaves human GSDME in a dose-dependent manner. In contrast, human caspase-7 loses this ability due to an N234S mutation [3].

## 2.2 Cleavage-independent activation

In addition to the cleavage-dependent activation of GSDMs, cellular pyroptosis can also be triggered in a protease-independent manner. In 2016 FL-GSDM was reported to acquire the ability to autonomously induce cellular pyroptosis upon mutation in the CT domain, which disrupts autoinhibition interface and unmasks the functional NT domain [14]. Herein we summarized the advances in cleavage-independent GSDM activation.

**2.2.1 GSDMA.** FL-GSDMA is activated upon phosphorylation in the CT domain. Dual depletion of amino acids and ATP allow Unc-51-like autophagy-activating kinase 1 to phosphorylate GSDMA at Ser353 during cell starvation. This phosphorylation impairs the autoinhibitory effect of the CT domain and releases the pore-forming NT domain. This starvation-induced GSDMA activation exhibits potential in suppressing gastric tumor cell growth and preventing malignancy in mouse orthotopic tumor models [49]. In the absence of GSDMA, overexpression of GSDME also induces a detectable level of pyroptosis under starvation conditions, which occurs at a later stage [49].

**2.2.2 GSDMD.** FL-GSDMD acquires pore-forming ability upon reactive oxygen species (ROS)-dependent S-palmitoylation, which might impair autoinhibition and decrease the contact between NT and CT domains [50].

**2.2.3 GSDME.** FL-GSDME mediates pyroptosis through the PARYlation-oxidation relay. Upon intensive UVC irradiation, a large amount of poly ADP-ribose (PAR) polymers are released into the cytoplasm. These polymers activate PARP5-mediated PARYlation of GSDME and lifts autoinhibition of GSDME. Then, the elevated lipid ROS signal induced by mitochondrial ROS (mtROS) and cytochrome c is directly sensed by PARYlated GSDME, leading to oxidative oligomerization and membrane translocation of FL-GSDME [51].

**2.2.4 Lower eukaryotic GSDM.** TrichoGSDM is a GSDM homologous protein encoded by *Dictyostelium filamentosum*. TrichoGSDM solely contains the membrane perforation domains and maintains a self-inhibited state as a dimer. Upon reduction of the intermolecular disulfide bonds, TrichoGSDM is activated to a monomeric state and acquires membrane-punching activity.

RCD-1-1 and RCD-1-2 are two homologous proteins encoded by the fusion lethal gene found in the filamentous fungus, *Neurospora crassa*. Upon cell fusion, RCD-1-1 and RCD-1-2 are activated through intermolecular paired interactions, assembling into heterodimers and further oligomerizing into pores, which ultimately leads to allorecognition-induced cell death [52].

## 2.3 Interplay between GSDMs

The interplay between GSDMs can be summarized in the following two modes. First, one GSDM may directly participate in activation of another GSDM protein, which has a cell-autonomous function. For example, GSDMB can directly bind to the CARD domain of caspase-4 through the NT domain (1–83 aa). GSDMB enhances the activity of caspase-4, which in turn activates GSDMD and promotes non-canonical pyroptosis [53]. However, during apoptosis, caspase-7 cleaves GSDMB at Asp91. The GSDMB-CT (92–417 aa) binds to the GSDMB-NT (1–91 aa), blocking the functional GSDMB-NT (1–83 aa) as well as GSDMB-mediated promotion of non-canonical pyroptosis [54]. Notably, GSDMB is also a substrate for caspase-4. Therefore, it has been suggested that the positive effect of GSDMB on caspase-4 can be terminated through negative feedback [53]. Second, activation of one GSDM can act as a trigger for the activation of another GSDM. For example, the K<sup>+</sup> efflux induced by GSDM pores can activate NLRP3 inflammasomes [55] and lead to canonical inflammasome-induced pyroptosis. Additionally, GSDMA/D/E-NT can permeabilize mitochondria and augment caspase-3 activation, which might in turn promote GSDME cleavage [35].

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### 2.4 Cleavage-dependent inactivation

Cleavage does not always lead to activation (Figure 1). For example, GSDMB can be cleaved by caspase-1/3/4/6/7/8/9 [38, 53, 54, 56, 57] or neutrophil elastase (NE) [38], releasing shorter NT fragments (1–91 aa or 1–220 aa) that lose pore-forming activity. Upon activation of the apoptotic pathway, both caspase-3 and -7 cleave GSDMD at Asp87, producing a non-functional p43 fragment [58]. Importantly, the simultaneous presence of p43, p30, and p20 GSDMD fragments in cells suggests that there is an enzymatic competition between caspase-1 activation and caspase-3 inactivation [59]. Moreover, GSDM can also be cleaved by a multitude of viral proteases into non-cytotoxic fragments [60–64] (Table 2).

### 3. DOWNSTREAM EFFECTS OF GSDM ACTIVATION

Upon cleavage in the linker region or modification on the autoinhibition interface, the activated pore-forming NT domain of GSDMs are released to penetrate the lipid bilayers and form pores. These GSDM pores lead to cell lysis via NINJ-1 and promote the release of cytokines, DAMPs, and other cellular contents. The GSDM pore-dependent  $\text{Ca}^{2+}$  influx triggers the assembly of F-actin-rich filopodia [65], which is preserved in pyroptotic cell corpses. Moreover, these pyroptotic cells release extracellular vesicles (EVs), which carry GSDM pores and lead to bystander cell pyroptosis [66]. In some cases, macrophages [67], dendritic cells, and neutrophils [68] are able to survive without membrane rupture and pyroptosis. These cells are known as “hyperactivated cells,” which permit the release of more inflammatory mediators, as well as other secretions (such as 11,12-EET) [69].

#### 3.1 Cellular contents released from pyroptotic cells

**3.1.1 Pore-dependent release of the interleukin-1 family.** GSDM pores mediate the “unconventional secretion” of the following pro-inflammatory cytokines: interleukin IL-1 $\alpha$ ; IL-1 $\beta$ ; IL-18; and IL-33. IL-1 $\beta$  and IL-18

require caspase-1/4/11-mediated cleavage for maturation [41, 42, 45].

GSDM pores are size- and charge-selective, which tightly control the release of mature IL-1/IL-18 in a dual mechanism [70]. GSDM pores with an inner diameter of 16–21.5 nm [19, 20, 71, 72] are large enough to allow the passage of IL-1, which is approximately 4 nm in diameter. Moreover, because the GSDM pore is highly enriched with negative charges, especially in the regions close to the cytoplasm, GSDM pore favors the passage of mature IL-1 $\beta$  and IL-18 but not the negatively charged precursors [70].

IL-33 is a crucial cytokine in type 2 immunity [73, 74]. In addition to the canonical proptotic GSDMC [74]/GSDMD pore, IL-33 is also released through the GSDMD pores with P40 (mouse) or P35 (human) fragment in airway epithelial cells exposed to allergen proteases. Notably, this process is short in duration and does not result in cell death [75].

In airway epithelial cells, the release of cytokines is controlled by switching between different cleavage forms of GSDMD. GSDMD is cleaved at D275 (human)/D276 (mouse) in typical type 1 inflammation, producing a p30 (human)/p35 (mouse) fragment that predominantly contributes to IL-1 $\beta$  release. In response to exogenous protease-active allergens stimulation, such as papain and dust mites, GSDMD is cleaved at R290 (human)/R311 (mouse). GSDMD pores with P40 fragment (1–311 aa, mouse) or P35 fragment (1–290aa, human) mediate extracellular release of IL-33, which promote type 2 inflammation [75].

**3.1.2 Pore-independent release of the interleukin-1 family.** In addition to being released through GSDM pores, the IL-1 family can also be packaged and released via GSDM-induced vesicles. GSDMD, accompanied by Cdc37/Hsp90, recruits the E3 ubiquitin ligase (NEDD4) in intestinal epithelial cells (IECs) of inflamed colonic tissues to catalyze polyubiquitination of pro-IL-1 $\beta$  upon inflammasome activation. This process serves as a signal

**Table 2** | Viral protease-dependent inactivation of GSDM.

| Virus                                              | Protease              | How to inhibit GSDM-mediated pyroptosis             | Refs.    |
|----------------------------------------------------|-----------------------|-----------------------------------------------------|----------|
| Porcine epidemic diarrhea virus (PEDV)             | 3C-like protease Nsp4 | Cleaves GSDMD to produce a 25-KD fragment           | [60]     |
| Porcine transmissible gastroenteritis virus (TGEV) |                       |                                                     |          |
| Equine arteritis virus (EAV)                       |                       |                                                     |          |
| African swine fever Virus (ASFV)                   | pS273R                | Cleaves GSDMD at G107-A108                          |          |
| Enterovirus type 71 (EV71)                         | 3C protease           | Cleaves GSDMD at Q193-G194                          | [61]     |
| Seneca Valley virus (SVV)                          | 3C protease           | Cleaves pGSDMA at Q187-G188 and hGSDMA at Q186-G187 | [62]     |
| MERS-CoV, Porcine deltacoronavirus (PDCoV)         | 3C-like protease Nsp5 | Cleaves pGSDMD (NT/FL) or hGSDMD at Q193-G194       | [63]     |
| SARS-CoV-2                                         | 3C-like protease NSP5 | Cleaves GSDMD at Q29-S30 or Q193-G194               | [63, 64] |
| Enterovirus D68 (EV-D68)                           | 3C protease           | Cleaves GSDMD at Q193-G194                          | [64]     |

for cargo loading into secretory vesicles, leading to loading of the entire GSDMD/NEDD4/IL-1 $\beta$  complex into LC3-II<sup>+</sup> EVs. These EVs are then released into the extracellular space, where pro-IL-1 $\beta$  processing occurs [76].

Pro-inflammatory cytokines, such as IL-1 $\beta$ , are also packaged and released via EVs during retinal degeneration. These EVs occur in response to the calcium influx triggered by GSDMD pores, promoting retinal inflammation. Therefore, impairing GSDMD function with RNA interference or blocking EVs release in pyroptotic cells present a therapeutic potential for alleviating inflammation in retinal degeneration [77].

**3.1.3 DAMPs.** In addition to the well-studied IL-1 family, DAMPs, also known as alarmins, are also released by damaged pyroptotic cells. These DAMPs include lactate dehydrogenase (LDH), high mobility group box-1 (HMGB1), cleaved GSDMs, ATP, cytokines, galectin-1, and cellular proteins, which may differ between cell types. Acting as danger signals, these DAMPs amplify inflammation and recruit immune cells to respond to infection or cell damage [78].

LDH, which is too large to pass through GSDM-NT pores [79], and HMGB1, which does not appear to transmit through the GSDMD pore [80] but relies on cell lysis for release, can be used to distinguish hyperactivated from pyroptotic cells. LDH is widely used as the hallmark of pyroptosis.

As an early and transient danger signal, ATP is released from macrophages upon NLRP3 inflammasome activation. The release of ATP is mediated by initial small GSDMD pores, occurring before IL-1 $\beta$  release and NINJ1-dependent cell lysis. The extracellular ATP accumulates in a non-linear manner, which induces monocyte migration toward pyroptotic cells during early pyroptosis. This controlled process might be conserved among GSDMs because ATP can permeate through various GSDM pores [81].

Moreover, galectin-1 was identified in 2021 as a *bona fide* DAMP released as a consequence of cytosolic lipopolysaccharides (LPS) sensing. Galectin-1 is released through GSDMD pores upon intracellular LPS-caspase-11-GSDMD-mediated pyroptosis and promotes systemic inflammatory responses during LPS shock [82]. Galectin-1 suppresses the phosphatase activity of CD45 during endotoxemia, which minimizes the inhibitory effects of CD45 on inflammatory pathways.

**3.1.4 Pyroptotic cell secretion-driven tissue repair.** A novel function of pyroptotic cell secretions in promoting tissue repair is uncovered using "Pyro-1," a cell model in which macrophages undergo pyroptosis without releasing IL-1 [83]. Oxylipins (e.g., PGE2) and small-molecule metabolites (such as spermidine, putrescine) are enriched in Pyro-1 supernatant, which may be beneficial for wound healing. Specifically, PGE2 promotes macrophage migration and aggregation at the wound site. These macrophages contribute to wound healing by expressing the lectin, CD301, and the cytokine, IL-27.

The effects observed in mouse macrophages have been replicated in human myeloid cells, suggesting a conserved mechanism across species.

Similarly, it has been reported that GSDMD-mediated metabolic crosstalk promotes tissue repair and GSDMD deficiency in macrophage delays tissue recovery [69]. 11,12-epoxyeicosatrienoic acid (11,12-EET), which is secreted by hyperactive macrophages in a GSDMD-dependent manner, has been identified as the main bioactive pro-healing oxylipin. Mechanistically, 11,12-EET directly enhances FGF signaling by promoting activation of the MAPK and PI3K-AKT-mTOR pathways. This enhancement is achieved through modulation of FGF liquid-liquid phase separation.

11,12-EET has emerged as a potential countermeasure against an age-related decline in tissue regeneration [69]. 11,12-EET has been shown to restore muscle viability in aging mice and to enhance both corneal epithelial repair as well as skin wound healing. Direct supplementation of 11,12-EET and knockdown of the 11,12-EET hydrolase, Ephx2, have been shown to boost the activation and proliferation of muscle stem cells, which improves muscle regeneration.

### 3.2 F-actin-rich filopodia assembly driven by GSDM pore-dependent Ca<sup>2+</sup> influx

GSDMD pore-dependent Ca<sup>2+</sup> influx triggers F-actin remodeling to generate F-actin-rich filopodia upon NLRP3 inflammasome activation. Pyroptotic filopodia enables partial preservation of cell bodies during asymmetric PMR, leaving behind a remnant corpse containing stable caches of F-actin-rich deposits [65]. The F-actin-rich filopodia of pyroptotic corpses act as a *de novo* alarmin, engaging with dendritic cells via CLEC9A and activating the antigen presentation pathways. In addition to GSDMD, diverse effectors of programmed cell lysis, including GSDMA, GSDME and MLKL, generate cell corpses decorated with filopodia, which are immunogenic.

### 3.3 Cellular lysis mediated by the GSDM pore and NINJ1

GSDMs assemble into pores upon activation, disrupting the integrity of the cell membrane and leading to the formation of large ballooning bubbles [84] and plasma membrane rupture. Cellular contents are released to the extracellular space when PMR occurs, which act as pro-inflammatory agents and exacerbate the inflammatory response.

Initially, the lysis of pyroptotic cells is completely attributed to passive osmosis-induced cell swelling caused by GSDM pores. However, recent findings have shown that GSDM pore formation is not sufficient to cause PMR and the integral plasma membrane protein, NINJ1, is a key executor of PMR [85].

**3.3.1 Structure of NINJ1 and NINJ1 oligomer.** NINJ1 resides in the cell membrane as a monomer or dimer prior to activation [85]. For monomeric NINJ1, the  $\alpha 1$  and  $\alpha 2$  helices appear to localize to the cell exterior,

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and the  $\alpha 3$  and  $\alpha 4$  helices form a transmembrane (TM) hairpin structure [86, 87]. Using the NINJ1 (K45Q) mutant to mimic the inactive NINJ1 [85], the conformation of NINJ1 dimer has also been demonstrated [88]. The dimeric NINJ1 is autoinhibited in *trans*, with inactive NINJ1 adopting a conformation with unkinked and continuous TM helix 1 (i.e.,  $\alpha 1$  and  $\alpha 2$  helices). Importantly, the face-to-face NINJ1 dimer of the 3-helix fold sequesters the hydrophilic face of NINJ1 inside the dimer core and occludes the binding site of the neighboring activated NINJ1 molecule. NINJ1 autoinhibition is seen as the mechanism that prevents unprovoked PMR. Face-to-face dimer dissociation is necessary for NINJ1 activation [88].

NINJ1 assembles into oligomers upon activation with a hydrophobic side facing the membrane and a hydrophilic side composed of  $\alpha 1$  and  $\alpha 2$ . NINJ1 subunits interact with each other by aligning the transmembrane helices in sequence and by the crossover between the  $\alpha 1$  of one subunit and the TM helices of its adjacent subunit, as if extending a hand [86, 87].

**3.3.2 Mechanism of NINJ1 activation.** The amphipathic  $\alpha 1$  and  $\alpha 2$  helices of NINJ1 insert into plasma membrane upon activation, which leads to the formation of clusters of highly branched filaments ranging from 500 nm to micrometers in size according to the amphipathic filament model [86] (Figure 2A). Adopting a kinked conformation, the  $\alpha 1$  and  $\alpha 2$  helices are separated by a kink at Leu56. The  $\alpha 2$  helix is oriented parallel to the TM  $\alpha 3$  and  $\alpha 4$ , while the  $\alpha 1$  helix protrudes at an angle of nearly 90° to the helical bundle of  $\alpha 2$ - $\alpha 4$ , bridging neighboring protomers.

These fence-like NINJ1 assemblies disrupt the hydrophobic membrane with hydrophilic surfaces, promoting membrane rupture by capping membrane edges. As a result, NINJ1 assemblies stabilize membrane lesions of variable size and morphology and ensure the release of cellular contents. NINJ1 may also form a double filament structure that opens in response to osmotic pressure in a zipper-like manner.

NINJ1 oligomerizes upon activation and encircles membrane patches like a belt according to the cookie cutter model [89] (Figure 2B). NINJ1 punches holes in the plasma membrane and leads to the release of cookie-shaped plasma membrane discs. This process leads to membrane fragmentation and loss, which ultimately results in complete PMR. The TM helices,  $\alpha 3$  and  $\alpha 4$ , are both kinked rather than straight according to the cookie cutter model, with glycine residues at the exact kink regions (Gly93/Gly95 in  $\alpha 3$  and Gly124 in  $\alpha 4$ ). Mutagenesis experiments have confirmed that the kinked conformation of these helices is crucial for NINJ1 function.

Ion influx, such as GSDMD pore-mediated  $\text{Ca}^{2+}$  influx [89], and local alterations in the physicochemical properties of the plasma membrane, such as changes in the local curvature of the lipid bilayer or changes in its

phospholipid composition [86, 89], are both plausible mechanisms underlying NINJ1 activation. Moreover, it has also been reported that NINJ1 oligomerization depends on caspase-8 and RIPK3 during heat stress [90]. Further studies are required to understand the functions of NINJ1 and to uncover the triggers that induce conformational change.

### 3.4 Bystander pyroptosis mediated by EVs

Pyroptotic cells can release EVs with a bystander killing effect [66]. These EVs carry active GSDMs in pore conformation, which transplant GSDM pores on the plasma membrane of bystander cells and drive cell death. This intercellular propagation of pyroptosis can be found *in vitro* and *in vivo* with multiple cell types susceptible to pyroptosis. This finding challenges the widely held belief that GSDMs only kill cells from within. More importantly, such dissemination of pyroptosis broadens the pathologic ramifications of pyroptosis and can lead to excessive inflammation in diseases, such as sepsis.

## 4. DISEASES MEDIATED BY GSDM PYROPTOTIC FUNCTIONS

GSDMs are emerging as critical mediators in the pathogenesis of a spectrum of diseases (Figure 3), including but not limited to allergic diseases, neurologic disorders, metabolic diseases, sepsis and infection, and cancer.

### 4.1 Allergic diseases/type 2 inflammatory diseases

The GSDM family members have been implicated to be important mediators in the pathogenesis of type 2 inflammatory diseases, including helminth infection and allergic airway inflammation (Figure 4A).

**4.1.1 Helminth infections.** Helminth infection-induced STAT6 O-GlcNAcylation activates GSDMC expression in IECs [91]. Meanwhile, intraepithelial mast cells transmit proteases, such as MCPT-1, to neighboring epithelial cells through intercellular nanotubes [74]. These proteases cleave and activate GSDMC in IECs, which leads to the release of IL-33. As a result, IL-33 promotes activation of group 2 innate lymphoid cells and Th2 cells, thereby augmenting the type 2 immune response in the intestines [74, 91].

**4.1.2 Allergic airway inflammation.** IL-33 is translocated from the nucleus to the plasma in airway epithelial cells following allergenic protease exposure and activation of stress granule assembly. Then, IL-33 is released into the extracellular space through GSDMD pores, which consist of the P40 fragment in mice or the P35 fragment in humans. The released IL-33 promotes allergic airway inflammation [75].

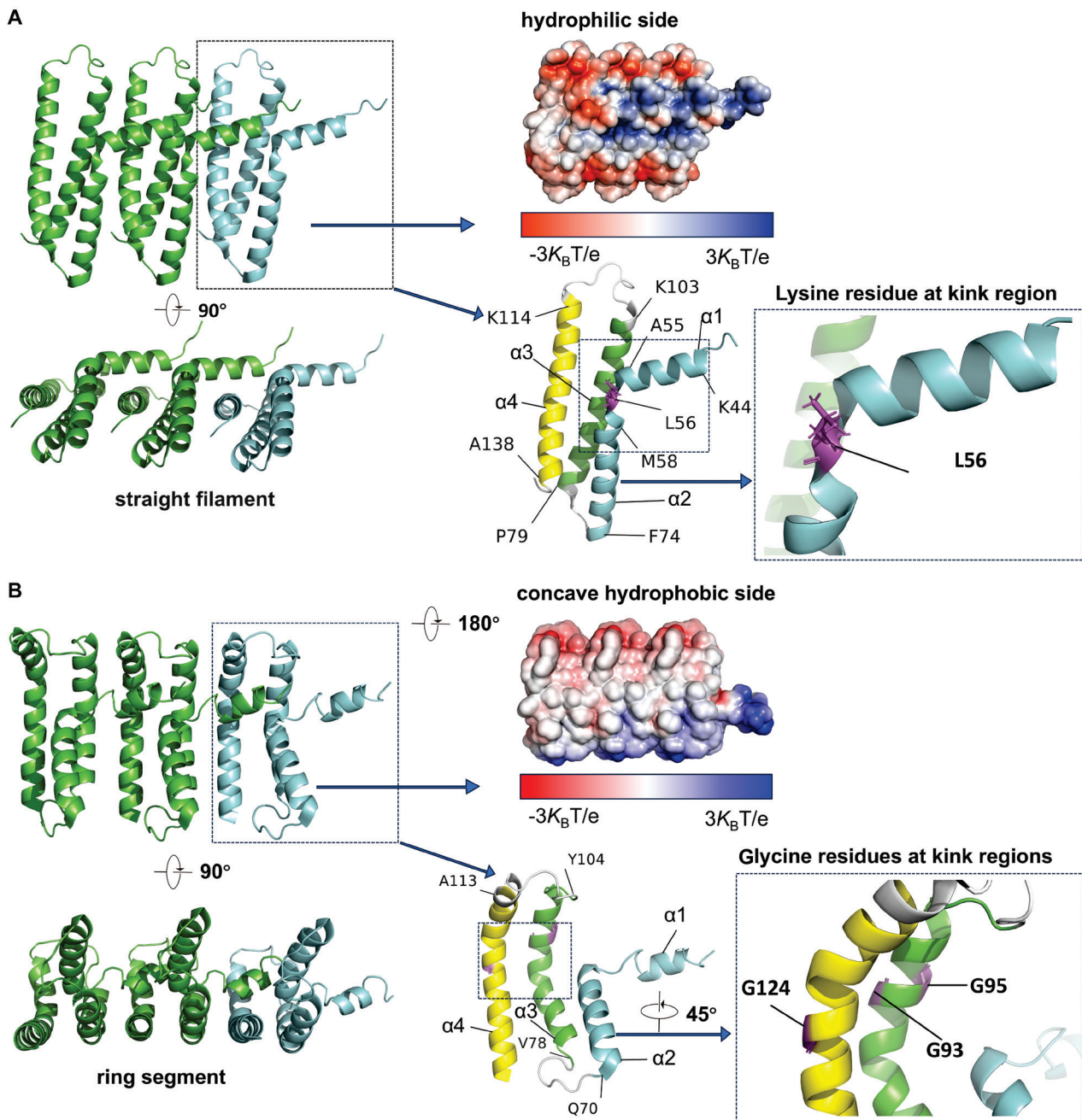


**Figure 2 | Structure of NINJ1 monomer and oligomer.**

(A) Amphipathic filament model (PDB: 8CQR): In this model the NINJ1 oligomer is a straight filament, with one face of the filament being hydrophilic and the other face being hydrophobic. NINJ1 promotes membrane rupture by capping membrane edges. The NINJ1 monomer consists the following four helices:  $\alpha 1$  (K44-A55, cyan) and  $\alpha 2$  (M58-F74, cyan), which is separated by a distinct kink at L56 (purple); and  $\alpha 3$  (P79-K103, green) and  $\alpha 4$  (K114-A138, yellow), which are both straight helices. (B) Cookie cutter model (PDB: 8UIP): In this model the NINJ1 oligomer is an irregular large ring with a concave hydrophobic side for membrane interaction. NINJ1 oligomer promotes membrane rupture by cutting and releasing membrane disks. The NINJ1 monomer also comprise the following four helices:  $\alpha 1$  and  $\alpha 2$  (N37-Q70, cyan), which are roughly 90° to each other; and  $\alpha 3$  (V78-Y104, green) and  $\alpha 4$  (A113-G140, yellow), which are both kinked rather than straight, with glycine residues (purple) at the exact kink regions.

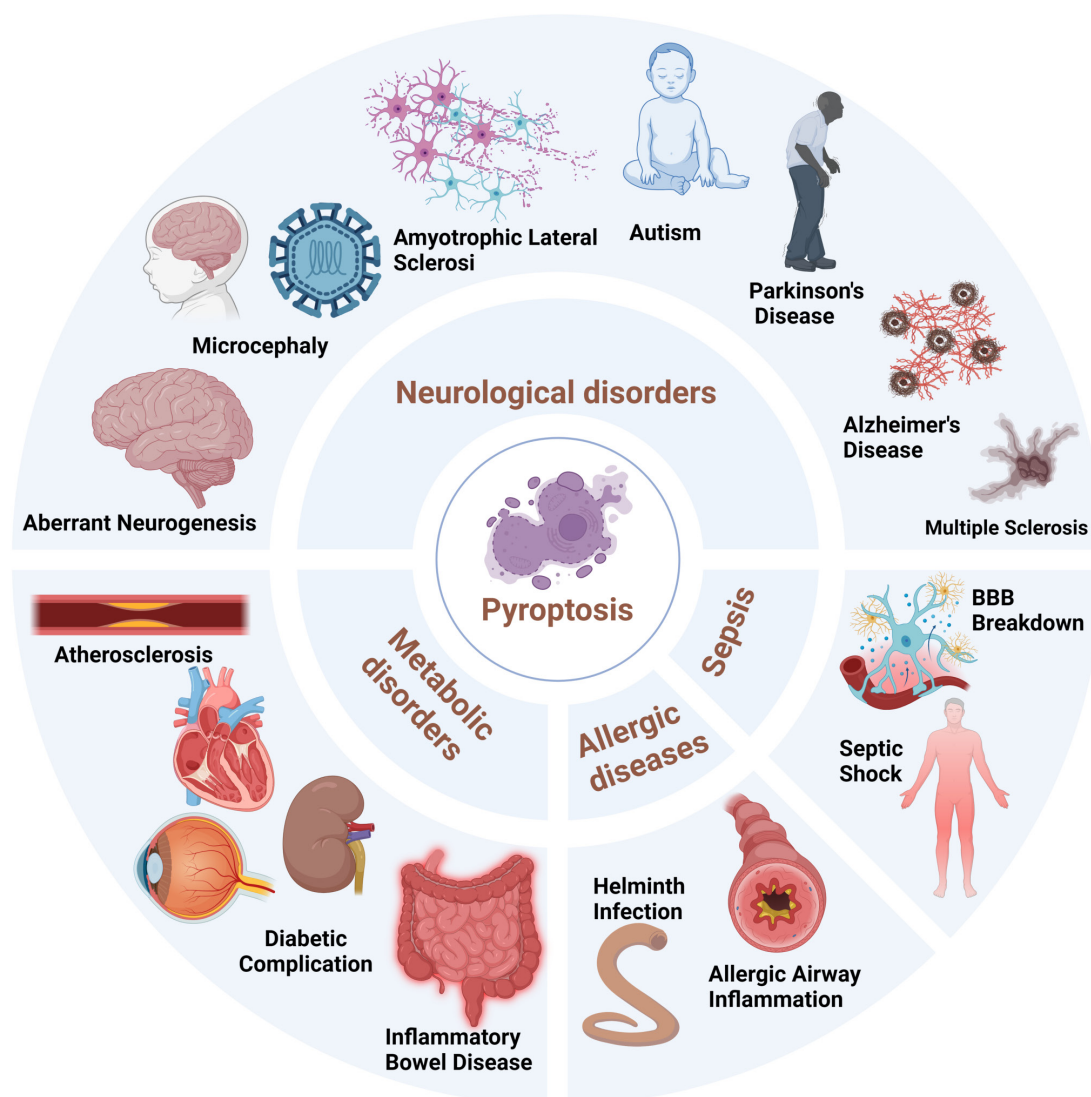


**Figure 3 | Pyroptosis-associated diseases.**

Diseases mediated by pyroptosis, including but not limited to allergic diseases, neurologic disorders, sepsis, and metabolic diseases.

## 4.2 Neurologic disorders

GSDMs also have an important role in a range of neurologic disorders, including multiple sclerosis (MS), Parkinson's disease (PD), Alzheimer's disease (AD), autism, amyotrophic lateral sclerosis (ALS), microcephaly, and aberrant neurogenesis (**Figure 4B**).

**4.2.1 MS.** GSDMD mediates the pyroptosis of peripheral myeloid cells in the experimental autoimmune encephalomyelitis (EAE) mouse model, which mimics the pathogenesis of MS. This process contributes to the establishment of an inflammatory microenvironment, which promotes T cell activation and differentiation in

peripheral lymphoid organs. Thus, GSDMD promotes infiltration of immune cells into the central nervous system (CNS), leading to neuroinflammation and exacerbating demyelination [92]. In addition, GSDMD-mediated pyroptosis is found in microglia, myelin-forming oligodendrocytes, and astrocytes, which might contribute to inflammatory demyelination [93–95]. As the NF- $\kappa$ B-NLRP3-GSDMD pathway suppressor, CLEC16A has been shown to function by promoting mitophagy in astrocytes and limiting the accumulation of dysfunctional mitochondria as well as the proinflammatory mitochondrial products, which inhibit proinflammatory pyroptosis and ameliorates CNS inflammation [94].

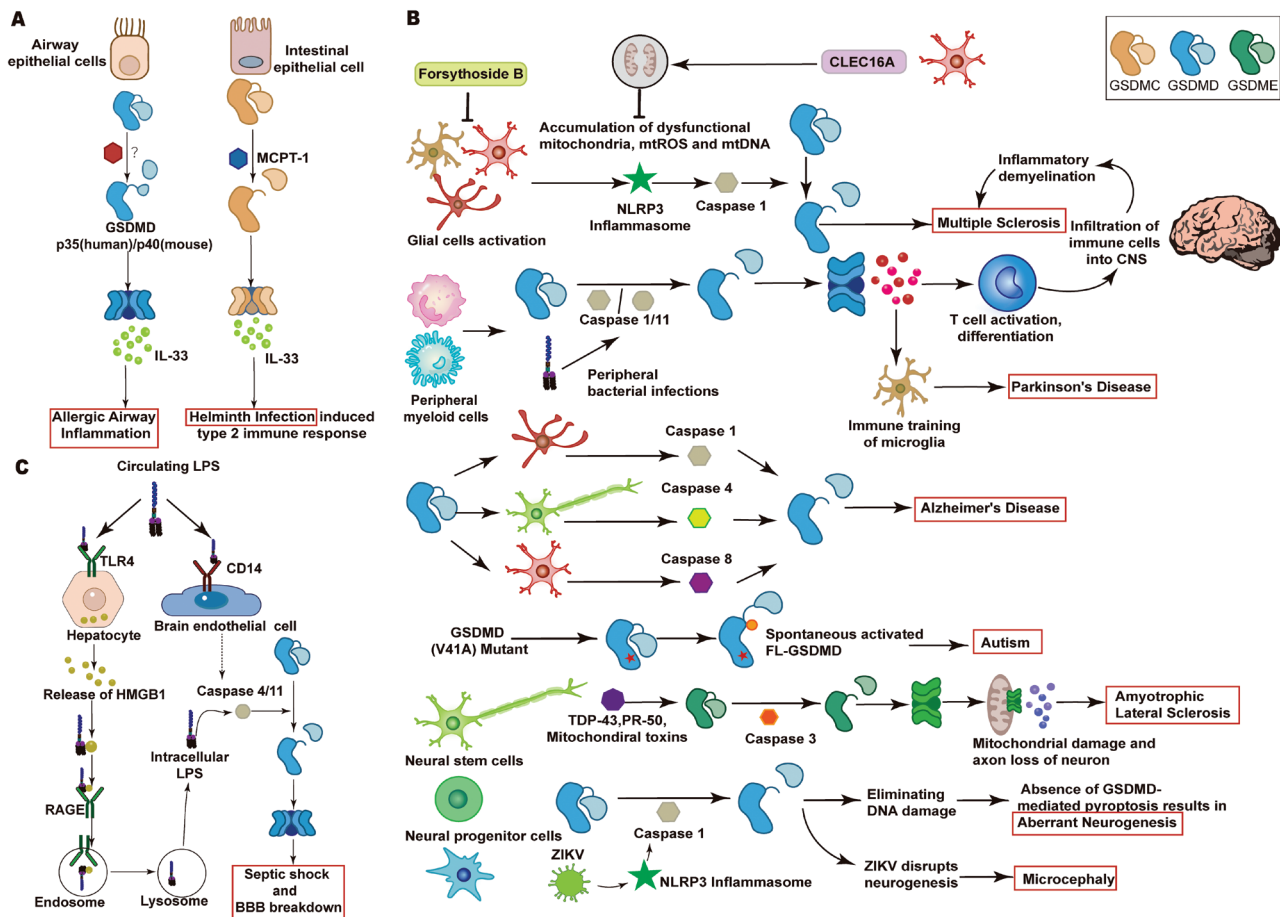


**Figure 4 | GSDMs function in the pathophysiology of allergic diseases, neurologic disorders, and sepsis.**

(A) The role of GSDM in the progression of allergic diseases, including helminth infections and allergic airway inflammation. (B) The role of GSDM in the progression of neurologic disorders, including multiple sclerosis, autism, amyotrophic lateral sclerosis, microcephaly, and aberrant neurogenesis. (C) The role of GSDM in the progression of sepsis-related BBB breakdown and septic shock.

Moreover, Forsythoside B has been reported to ameliorate neuroinflammation by inhibiting activation of NLRP3 inflammasome in glial cells [95].

**4.2.2 PD.** GSDMD-mediated pyroptosis in peripheral myeloid cells facilitates immune training of microglia in a mouse model of PD upon first exposure to LPS. This effect makes microglia more sensitive to secondary stimuli from peripheral infections or cerebral  $\alpha$ -syn deposits. Then, neuroinflammation and neurodegeneration are exacerbated, which ultimately leads to PD-like symptoms and PD [96].

**4.2.3 AD.** Pyroptosis appears to be activated in a cell-type-specific way in AD based on research involving AD patient brains. Intact NLRP3 inflammasomes, cleaved GSDMD, and GSDMD-NT oligomers are detected in microglia, which imply the occurrence of canonical inflammasome-induced pyroptosis. While in astrocytes

and neurons, GSDMD might be activated via Caspase-8 and Caspase-4, respectively [97].

**4.2.4 Autism.** A potential association between autism and the GSDMD (V41A) mutation has been identified according to a recent genetic analysis of *de novo* and inherited variants in a large cohort of individuals with autism. This mutation is located at the NT-CT interface of GSDMD, which weakens autoinhibition of GSDMD and promotes spontaneous palmitoylation of FL-GSDMD. The spontaneous activity of FL-GSDMD is thereby enhanced [50].

**4.2.5 ALS.** GSDME is specifically enriched in neurons in patients with ALS. GSDME can be activated by mitochondrial toxins, as well as frontotemporal dementia/ASL-associated proteins (e.g., TDP-43 and PR-50). GSDME rapidly distributes to neurite-associated mitochondria upon activation, acting as the executioner of neuronal

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mitochondrial dysfunction. This process leads to local mitochondrial damage and axon loss, which might contribute to neurodegeneration and promote ALS. GSDME deficiency in ALS mouse model prolongs mouse survival and rescues spinal motor nerve loss [98].

**4.2.6 Microcephaly.** ZIKV triggers pyroptosis of neural progenitor cells via the NLRP3-caspase-1-GSDMD pathway in a ZIKV-infected neonatal mouse model. Therefore, ZIKV disrupts neurogenesis and leads to microcephaly. Knockdown of caspase-1 and inhibition with caspase-1 inhibitors can effectively alleviate neuropathy and brain atrophy [99]. Thus, blocking ZIKV-mediated pyroptosis appears to be a potential therapy to treat ZIKV-associated congenital malformations, including microcephaly.

**4.2.7 Aberrant neurogenesis.** Pyroptosis is a crucial mechanism for eliminating DNA damage in the developing cortex, which occurs during the rapid proliferation

of neural stem cells. The absence of GSDMD-mediated pyroptosis impairs DNA damage clearance and results in aberrant neurogenesis and autism-like behaviors in adult mice [100].

### 4.3. Metabolic diseases

**4.3.1 Diabetic complications.** Diabetes is a worldwide health challenge that is estimated to affect 830 million people in 2022 [101] and this number is projected to increase to 1.31 billion people by 2050 [102]. Pyroptosis has an important role in diabetic complications, including but not limited to diabetic retinopathy, diabetic nephropathy (DN), and diabetic cardiomyopathy [DCM] (Figure 5A-5C).

#### 4.3.1.1 Diabetic retinopathy

Caspase-1-GSDMD-dependent pyroptosis has emerged as a key event in human diabetic retinopathy [103]. LncRNA MIAT [103] and KCNQ1OT1 [104] promote

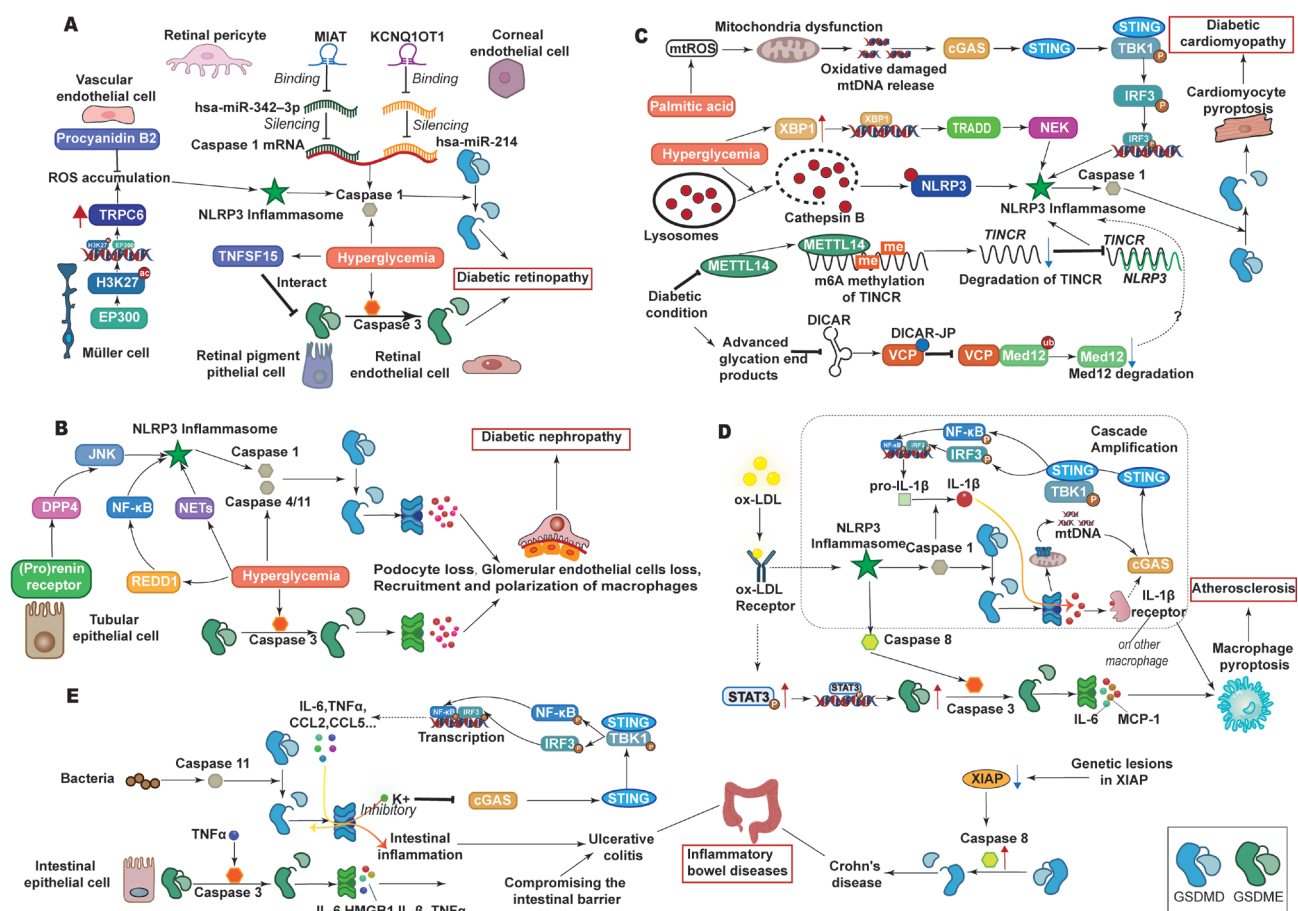


**Figure 5 | GSDMs function in the pathophysiology of metabolic diseases.**

(A-C) The role of GSDM in the progression of diabetic complications, including diabetic retinopathy (A), diabetic nephropathy (B), diabetic cardiomyopathy (C). (D) The role of GSDM in the progression of atherosclerosis. (E) The role of GSDM in the progression of inflammatory bowel diseases, including Crohn's disease and ulcerative colitis.

pyroptosis of retinal pericyte and corneal endothelium by individually binding to miR-342-3p and miR-214, antagonizing the silencing effect of these miRNA on caspase-1. Procyanidin B2 protects vascular endothelial cells from high glucose-induced pyroptosis and attenuates retinal microvascular dysfunction in diabetic mice by reducing oxidative stress and inhibiting the caspase-1/GSDMD pathway [105]. In contrast, transient receptor potential channel 6 (TRPC6) contributes to the pyroptosis of in Müller cells by inducing ROS accumulation [106].

In addition, both GSDME and caspase-3 are upregulated in the progression of diabetic retinopathy. Caspase-3 is activated in the high-glucose microenvironment, which cleaves GSDME and triggers pyroptosis in retinal endothelial and retinal pigment epithelial cells [107]. TNFSF15, which is an angiogenesis inhibitor and is also likely to be an endogenous protective regulator of pyroptosis, is also upregulated under high glucose conditions. TNFSF15 interacts with GSDME and inhibits retinal cell pyroptosis, which in turn retards diabetic retinopathy progression [107].

#### 4.3.1.2 DN

Expression of the stress response protein, REDD1, in renal cortical tissue is upregulated under hyperglycemia conditions, which in turn activates NF- $\kappa$ B signaling in podocytes, and promotes NLRP3 inflammasome activation and cytokine/chemokine production [108]. Caspase-1/11-GSDMD-dependent [109] and caspase-3-GSDME-dependent [110] pyroptosis are activated in DN. In addition to directly contribute to podocyte loss and renal injury, pyroptosis mediates the release of inflammatory factors, such as IL-1 $\beta$ , through GSDM pores, which facilitates recruitment and polarization of macrophages. Interestingly, the level of GSDME-NT protein in rat kidney cortex has been reported to increase by more times than GSDMD-NT [110], which indicates that GSDME-mediated pyroptosis may have a more important role in the pathogenesis of DN.

In addition, high glucose-induced NETs, the levels of which have been confirmed to increase in patients with DN, have also been shown to trigger dose-dependent pyroptosis of glomerular endothelial cells via cell surface charge [111]. Moreover, (pro)renin receptor expression is also reported to increase in renal tubules of DN patients. (Pro)renin receptor can bind to DPP4 and facilitate pyroptosis of tubular epithelial cells via the DPP4-JNK pathway [112].

#### 4.3.1.3 DCM

Increased cardiomyocyte pyroptosis is one of the characteristics of DCM, which is mediated by the NLRP3-caspase-1 pathway [113] with NLRP3 likely to be the key factor.

For example, high glucose levels can elevate expression of the transcription factor, XBP1, and enhance XBP1 binding to the TRADD promoter, an adapter protein. Therefore, XBP1 increases TRADD expression

in cardiomyocytes, which subsequently augments the activation of NEK7 and the NLRP3 inflammasome [114]. Additionally, excessive accumulation of free fatty acids in the diabetic heart can lead to lipotoxicity in the myocardium, which causes mitochondrial damage and release of mtDNA into the cytosol. The cGAS-STING signaling pathway is then activated, which subsequently activates NLRP3-mediated cardiac pyroptosis [115]. A Chinese herbal preparation, Fufang Zhenzhu Tiao Zhi (FTZ) formula, has been reported to block NLRP3 inflammasome activation by suppressing intercellular lipid accumulation, which shows a cardioprotective effect in DCM mice [116]. Moreover, cathepsin B, which is also upregulated in heart tissue under hyperglycemic stresses, has been reported to bind to NLRP3 and activate the NLRP3 pathway [117].

Targeting activation of the NLRP3 inflammasome appears to be a promising strategy to prevent cardiomyocyte pyroptosis and ameliorate DCM. For example, methyltransferase-like 14 (METTL14), which is downregulated in DCM, has been reported to suppress pyroptosis of cardiomyocytes in an NLRP3-dependent manner [113]. Specifically, METTL14-mediated m6A methylation of TINCR induces suppression of TINCR in cardiomyocytes, which in turn causes decreased stability of *NLRP3* and downregulation of NLRP3. In addition, a novel endogenous regulator for diabetic cardiomyopathy, CircRNA DICAR (mm9-circ-008009/hsa-circ-0131202), was reported in 2023. CircRNA DICAR functions by inhibiting pyroptosis of cardiomyocytes via DICAR-VCP-Med12 degradation [118].

**4.3.2 Atherosclerosis.** Atherosclerosis is a progressive metabolic disease characterized by abnormal lipid accumulation and accompanied by inflammation [119]. Macrophage death, including macrophage pyroptosis, is a vital factor in determining the fate of atherosclerosis. GSDMD [120] and GSDME [121] have been shown to be required for ox-LDL-induced macrophage pyroptosis and downstream proinflammatory cytokine release (Figure 5D). Inhibition or knockdown of GSDMD/GSDME effectively suppresses macrophage migration and associated inflammation, which in turn alleviates the progression of atherosclerosis *in vivo*.

**4.3.3 Inflammatory bowel disease.** Inflammatory bowel diseases, including Crohn's disease and ulcerative colitis [122], refer to chronic inflammation in and beyond the gastrointestinal tract and are closely related to pyroptosis (Figure 5E). GSDMD is activated in intestinal macrophages in a mouse model of dextran sulfate sodium-induced colitis. The GSDMD pore acts as a negative regulator of colitis, promoting K<sup>+</sup> efflux to control cGAS-dependent inflammation [123]. In contrast, GSDME-mediated pyroptosis in IECs promotes the development of colitis. By releasing proinflammatory cytokines, GSDMD promotes intestinal inflammation and compromises the intestinal barrier [124]. In addition,

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it has been reported that genetic lesions in X-linked inhibitor of apoptosis increase cleaved caspase-8 in inflamed colonic mucosae. This process promotes apoptotic and pyroptotic crosstalk, predisposing individuals to cell-death-associated inflammatory diseases, such as Crohn's disease [27].

### 4.4 Sepsis and Infection

**4.4.1 Sepsis-related blood-brain barrier (BBB) breakdown and septic shock.** Circulating LPS is perceived in sepsis caused by Gram-negative bacteria by brain endothelial cells (bECs), the most prominent and probably the first-line responders within the CNS. These cells directly sense LPS through caspase-11, which activates non-canonical inflammasome and leads to GSDMD-mediated pyroptosis (Figure 4C). Consequently, the pyroptosis of bECs results in BBB bidirectional leakage and inflammatory BBB breakdown [125]. Importantly, the absence of caspase-11-GSDMD pathway has been proven to significantly mitigate BBB leakage, microglial activation, and synaptic damage in the hippocampal CA3 region [126]. Thus, blocking caspase 11-GSDMD-IL-1 signaling appears to be a novel and effective strategy to reverse infection-induced cognitive dysfunction and treat sepsis-associated neurologic disorders.

In addition, circulating LPS is detected by hepatocytes through TLR4. Following detection, hepatocytes release large quantities of HMGB1, which is responsible for the internalization of circulating LPS into macrophages and endothelial cells in other tissues. Then, these cells sense LPS with caspase-11 and undergo GSDMD-mediated pyroptosis, which ultimately leads to infectious shock, multiple organ failure, and death (Figure 4C). If HMGB1 is neutralized with antibody, pyroptosis and lethal endotoxemia can be prevented [127]. Notably, the non-canonical inflammasome pathway dominates LPS-induced sepsis. LPS also primes NLRP3 inflammasome activation, which may amplify septic shock [128].

**4.4.2 Infection.** Among infectious diseases, pathogens have a dual role in host cells pyroptosis (Table 3).

Pathogens impact various aspects of the pyroptosis pathway to avoid host cell death and evade host immune defenses. For example, *Yersinia pseudotuberculosis* infection can lead to a significantly decreased glucose level in macrophages, which in turn activates the glucose and energy sensor AMPK. Thus, the RIPK1-caspase-8-GSDMD pathway [131] is inhibited through AMPK-mediated RIPK1 phosphorylation [138]. A novel post-translational modification, arginine ADP-ribosylation, is mediated by the effector protein OspC3 in *Shigella flexneri* infections. OspC3 prevents activation and dimerization of caspase-4/11 by modifying caspase-4/11 at Arg314/Arg310, respectively, thereby inhibiting host cell pyroptosis [136]. During SARS-CoV-2 infection, the nucleocapsid binds to the linker region of GSDMD, hindering

caspase-1-mediated cleavage [137]. *Mycobacterium tuberculosis* (MTB) remodels the membrane lipid composition of host cells through PtpB, thereby inhibiting the insertion of GSDMD-NT into the host cell membrane and blocking pyroptosis [135].

Pathogens induce pyroptotic death of host cells and causes severe tissue damage. MTB triggers pyroptosis in macrophages through the EST12/RACK1 complex-UCHL5-NLRP3 inflammasome pathway, leading to IL-1 $\beta$  release. This process causes tissue damage, promotes MTB proliferation, and helps maintain chronic or persistent MTB infection [134]. As the major pathogen causing viral myocarditis, enterovirus Coxsackie virus B3 activates CARD8 through viral 2A and 3C protease-dependent cleavage. The CARD8-inflammasome-GSDMD-mediated pyroptosis of endothelial cell facilitates viral release, promoting Coxsackie virus B3 propagation [133]. The effector protein, YopJ, functions as a TGF- $\beta$ -activated kinase 1 (TAK1) inhibitor following *Yersinia* infection, inhibiting the phosphorylation and activation of TAK1 and the downstream I $\kappa$ B kinase  $\beta$  (IKK $\beta$ ) [28]. Upon inhibition, the FLCN-FNIP2-Rag-Ragulator complex recruits the RIPK1-caspase-8 complex to the lysosome, promoting RIPK1 phosphorylation and activating caspase-8-mediated pyroptosis [28, 131, 132]. The CARD8-caspase-1-GSDMD pathway is activated in response to HIV protease activity in HIV-infected patients. Thus, infected primary CD4<sup>+</sup> T cells and macrophages can be eliminated through cellular pyroptosis [129]. Meanwhile, ROS-driven NLRP3 inflammasome activation and downstream cellular pyroptosis also causes a sustained inflammatory response. For patients with chronic HIV-1 infection, this might contribute to the progressive reduction of CD4<sup>+</sup> T lymphocytes and be a key factor in disease progression [130]. Notably, the HIV protease is usually embedded in the Gag-Pol precursor proteins and is inactive before viral budding, whereas non-nucleoside reverse transcriptase inhibitors promote pyroptosis of infected cell by advancing HIV protease activation [129].

Moreover, SARS-CoV-2 and enterovirus D68 (EV-D68) are reported to exhibit differential regulation on the functions of GSDMs [64]. Specifically, the cleavage and inactivation of GSDMD by EV-D68 3C/SARS-CoV-2 NSP5 protease might prevent immediate cell pyroptosis and prolong the time for virus replication. The direct/indirect activation of GSDME by SARS-CoV-2 NSP3/EV-D68 2A occurs in a later stage, triggering inflammatory damage in patients.

### 4.5 Cancer

GSDMA, GSDMC, and GSDME are poorly expressed in most tumors based on protein expression profiling (PEP) of the GSDM family members, while GSDMD and GSDMB are often expressed [139]. This difference in expression may have an association with the role of GSDM in cancer cells.

**Table 3** | Pathogens play a dual role in host cell pyroptosis during infection.

| Pathogen                          | Effector protein | Effect                                                        | Mechanism                                                                                                                                                                                                                                                    | Reference      |
|-----------------------------------|------------------|---------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| HIV                               | HIV protease     | Induces host cell pyroptosis and causes severe tissue damage  | HIV protease cleaves the N-terminus of the CARD8 protein and activates the CARD8 inflammasome                                                                                                                                                                | [129, 130]     |
| <i>Yersinia</i>                   | YopJ             |                                                               | Upon inhibition of TAK1-IKK $\beta$ by the YopJ, caspase-8 is activated through the FLCN-FNIP2-Rag-Ragulator complex-RIPK1-caspase-8 pathway                                                                                                                 | [28, 131, 132] |
| Enterovirus<br>Coxsackie virus B3 | 2A-/3C- protease |                                                               | The viral 2A-/3C- protease cleaves CARD8 at Gly38, thereby activating the CARD8 inflammasome within endothelial cells                                                                                                                                        | [133]          |
| <i>Mycobacterium tuberculosis</i> | EST12            |                                                               | Upon formation of the EST12/RACK1/UCHL5 complex, NLRP3 inflammasome is activated through deubiquitination                                                                                                                                                    | [134]          |
| SARS-CoV-2 and<br>EV-D68          | NSP3/2A protease |                                                               | SARS-CoV-2 NSP3 directly cleaves GSDME at G370, thereby inducing GSDME activation. EV-D68 2A might indirectly activate caspase-3-dependent cleavage of GSDME by inhibition of protein translation                                                            | [64]           |
| SARS-CoV-2 and<br>EV-D68          | NSP5/3C protease | Suppresses host cell pyroptosis to evade host immune defenses | SARS-CoV-2 NSP5 cleaves GSDMD at both Q29 and Q193, while EV-D68 3C cleaves GSDMD at Q193                                                                                                                                                                    | [64]           |
| <i>Mycobacterium tuberculosis</i> | PtpB             |                                                               | PtpB is a bacterial phospholipid phosphatase, the activity of which is enhanced upon binding to host ubiquitin. PtpB remodels the lipid composition of the host cell membrane, which in turn affects the binding and insertion of GSDMD-NT to the membranes. | [135]          |
| <i>Shigella flexneri</i>          | OspC3            |                                                               | OspC3 mediates the arginine ADP-ribosylation of caspase-4/11 at Arg314/Arg310, thereby inhibiting caspase activation                                                                                                                                         | [136]          |
| SARS-CoV-2                        | Nucleocapsid     |                                                               | SARS-CoV-2 nucleocapsid binds the GSDMD linker region and hinders caspase-1 mediated cleavage                                                                                                                                                                | [137]          |
| <i>Yersinia</i>                   | /                |                                                               | The decreased glucose level in macrophages activates AMPK, which in turn inhibits the RIPK1-caspase-8-GSDMD pathway                                                                                                                                          | [138]          |

**4.5.1 Anti-tumor effects of GSDM.** The tumor microenvironment (TME) has a crucial role in tumor progression, metastasis, and treatment. Even within the same tumor type, the subtype with higher immune infiltration has better survival [140]. Only a small number of patients respond positively to clinical immunotherapy due to a recalcitrant immunosuppressive TME, low immunogenicity of tumor tissues, and insufficient T-cell infiltration [141, 142].

The tumor immune microenvironment can be significantly changed by inducing a small number of cancer cells to undergo pyroptosis. Cancer cell pyroptosis (CCP) can turn immunosuppressive “cold tumors” into “hot tumors” by catalyzing the release of vast quantities of pro-inflammatory cytokines and immunogens [143, 144]. CCP can also promote activation and infiltration of cytotoxic lymphocytes in tumors, which are responsible for removing tumor cells [22, 145]. GSDME-expressing tumors contain more natural killer cells and CD8+ cytotoxic T killer cells. These cells are also more active in expressing toxic proteins/cytokines, which show excellent anti-tumor activity [32].

Conversely, cytotoxic lymphocytes, which are recruited and activated by CCP, generates and releases

IFN- $\gamma$  to upregulate GSDM expression in cancer cells [22]. Cytotoxic lymphocytes also promote GSDM activation through GZMA/GZMB [18, 32], as well as other proteases activated by CCP-induced immune responses. Thus, there is positive feedback between CCP and immunotherapy.

To increase the drug sensitivity of cancer and reduce drug resistance, scientists have been working towards combining anticancer drugs, such as immunotherapy and chemotherapeutic drugs, with CCP agonists in recent years. GSDMC-mediated CCP can effectively enhance tumor sensitivity to the chemotherapeutic agent, PARPi, in various cancers. The enhanced anti-tumor immunity induced by pyroptosis in < 15% of tumor cells is sufficient to clear the entire 4T1 breast tumor [22, 145]. Thus, this combination strategy seems to hold great promise. However, it has also been reported that under hypoxia, the critical immune-checkpoint PD-L1 cooperates with p-Stat3 to translocate to the nucleus and enhance transcription of GSDMC, which switches apoptosis to pyroptosis in cancer cells and facilitates tumor necrosis [23]. As chronic tumor necrosis suppresses anti-tumor immunity and accelerates tumor growth, pyroptosis promotes tumor progression. Thus, the combination of these drugs needs to be carefully considered.

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CCP can also lead to pathologic inflammation if activated excessively. For example, during CAR-T therapy, CAR T cells rapidly activate caspase 3 in target cells through release of GZMB, which in turn activates GSDME-mediated pyroptosis of target cells. The inflammatory factors released by pyroptotic cells activate the caspase-1-GSDMD and MAPK/NF- $\kappa$ B pathways in macrophages and trigger cytokine release syndrome [146].

**4.5.2 Tumor adaptation to GSDM.** *GSDMA* is often methylated [147] and silenced [148] in gastric cancer, which is consistent with PEP. Similarly, *GSDME* is also methylated in various cancers, which inhibits *GSDME* expression and limits the use of pyroptosis to treat cancer [149]. Moreover, it has been reported that *GSDME* NT mutations can be detected in a wide variety of primary cancers and 91% of the studied cancer-related *GSDME* mutations cause LOF [32].

The DNA methyltransferase inhibitor, decitabine, is used to reverse tumor editing and to address the relatively restricted expression of *GSDME* in tumor cells, which has been proven to efficiently activate *GSDME*-mediated pyroptosis [150]. In addition, a T-cell inspired sonoporation system was reported in 2024, which applied a ultrasound-controlled perforation system (UPS)-assisted genome editing to restore *GSDME* expression. This system worked well in 4T1 tumor-bearing mouse model, enhancing low-dose x-ray-mediated pyroptosis and improving efficacy of the anti-tumor radioimmunotherapy [151].

**4.5.3 Pro-cancer effect of GSDM.** GSDMB, C, and D function in a context- and cancer-type-specific way [13] to promote tumorigenesis and cancer proliferation.

### 4.5.3.1 GSDMB

GSDMB overexpression occurs in approximately 60% of HER2 breast cancers [152]. However, the various spliceosomes of GSDMB exhibit distinct pyroptotic activity, leading to significantly different impacts on tumors [38, 153].

GSDMB2 overexpression is linked to tumorigenesis [154], invasion, metastasis, drug resistance [152], and poorer overall survival [155] in breast cancer, especially in HER2-positive cases. In genetically engineered mouse models that express GSDMB2 and HER2 oncogenes, the incidence of breast cancer is increased [154]. Moreover, upregulation of GSDMB renders HER2 cancer cells more resistant to anti-HER2 agents by promoting protective autophagy [156]. To counter this effect, a novel targeted nanomedicine using hyaluronic acid biocompatible nanocapsules was developed. This nanomedicine delivers specific anti-GSDMB antibodies to HER2 breast cancer cells, which significantly increases tumor sensitivity to trastuzumab and suppresses tumor growth [152].

In contrast, tumors with high GSDMB4 expression tend to be associated with a better clinical prognosis [38]. Thus, regulating alternative splicing of GSDMB may be a significant strategy for tumor cells to modulate

pyroptotic activity. Moreover, non-cytotoxic GSDMBs can interfere with cytotoxic GSDMBs, thereby blocking anti-tumor GSDMB pyroptosis [153].

### 4.5.3.2 GSDMC

GSDMC has been identified as a novel therapy target for pancreatic adenocarcinoma and contributes to poor prognosis by promoting proliferation, migration, and invasion of cancerocyte [157]. Overexpressed GSDMC and higher pyroptosis activity is often associated with worse prognosis and oxaliplatin resistance in pancreatic ductal adenocarcinoma patients because GSDMC is involved in regulation of the response to oxaliplatin treatment by activating the pentose phosphate pathway [158].

### 4.5.3.3 GSDMD

GSDMD is activated through the gut microbiota/NLRP3 inflammasome pathway in the early stage of colorectal tumors, promoting the development of colorectal tumors through accumulation of myeloid-derived suppressor cells. Moreover, activation of GSDMD is accompanied by the aggregation of endosomal sorting complexes required for transport (ESCRT) complex on the membrane of colorectal tumor cells, which prevents tumor cell death from pyroptosis [159].

**4.6 Non-pyroptotic functions of GSDMs.** Although it has been shown that the non-pyroptotic function of the GSDM family also has an important role in maintaining normal physiologic function and the development of diseases, the role of GSDMs beyond pyroptosis, especially the role of GSDMD, remain poorly understood. Reports on new non-pyroptotic functions of GSDMs continue to enhance our understanding of the GSDM family (Table 4).

In addition to mediating cellular pyroptosis, GSDMs also function in metabolic reprogramming [100, 160, 162], pathologic inflammation [161, 163, 175], vesicle trafficking and secretion of proinflammatory cytokines [76], colonic epithelial repair [164] and regeneration [165], intestinal immune homeostasis [166] and food tolerance [167], bone homeostasis [168], neutrophil extracellular trap formation (NETosis) [171, 172], mitochondrial homeostasis [100, 174], and atrial arrhythmogenesis [173]. More importantly, there is growing evidence that GSDMs have functions independent of pyroptosis in tumorigenesis, including roles in tumor proliferation [162], invasion, metastasis [177], and drug resistance [156, 176]. In addition, GSDMD has been shown to sensitize cancer cells to chemotherapy in a pyroptosis-independent manner [169, 170]. Thus, more research on the non-pyroptotic function of the GSDM family is still needed to enrich our understanding of the GSDM family.

## 5. POST-TRANSLATIONAL MODIFICATIONS OF GSDM

Post-translational modifications (PTMs) are important ways to regulate the activation and pore formation of

Table 4 | Non-pyroptotic functions of GSDMs.

|       | Cell                                       | Mechanism                                                                                                                                                                                                                         | Pathology/Physiology                                                                                                                  | Disease/Function                                        | Refs. |
|-------|--------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------|-------|
| GSDMA | B cell                                     | Unknown                                                                                                                                                                                                                           | Helps to maintain the normal BCR signaling and mitochondrial metabolism                                                               | Immune response                                         | [160] |
|       | Keratinocyte                               | GSDMA is involved in regulating HB-EGF and EGFR-Stat3/Akt signaling                                                                                                                                                               | Leads to epidermal hyperplasia and inflammation in response to PMA                                                                    | Skin disease                                            | [161] |
| GSDMB | Bladder cancer cell                        | USP24-GSDMB complex phosphorylates STAT3 at Tyr705, activating the STAT3-IgFBP3 pathway                                                                                                                                           | Promotes bladder cancer proliferation and regulates glucose metabolism (particularly enhances glycolysis)                             | Bladder cancer                                          | [162] |
|       | Airway epithelial cell                     | GSDMB exhibits RLR-like properties, which recognizes and binds viral RNA, thereby activating the MAVS-TBK1 signaling and inducing robust expression of ISGs                                                                       | Promotes airway inflammation and mucus production upon respiratory virus infection                                                    | Asthma                                                  | [163] |
|       | HER2 <sup>+</sup> /GSDMB <sup>+</sup> cell | GSDMB acts as an autophagy adaptor in response to anti-HER2 therapy, interacting with LC3B and Rab7, and eventually promoting activation of Rab7 and maturation of the autophagosome                                              | /                                                                                                                                     | HER2 <sup>+</sup> breast and gastroesophageal carcinoma | [156] |
|       | Intestinal epithelial cell                 | FL-GSDMB promotes PDGF-AA expression, which facilitates focal adhesion kinase phosphorylation                                                                                                                                     | Inhibits cell adhesion and promotes epithelial proliferation and migration, which facilitates epithelial restitution/repair           | Inflammatory bowel disease                              | [164] |
| GSDMC | Lgr5 intestinal stem cell                  | N6-adenomethylation of GSDMC transcripts protects mitochondrial homeostasis in Lgr5 cells                                                                                                                                         | Is essential for Lgr5 cell survival and colonic morphogenesis                                                                         | Maintain normal colonic epithelial regeneration         | [165] |
| GSDMD | Intestinal epithelial cell (goblet cell)   | GSDMD can be activated by commensal bacteria, triggering Ca <sup>2+</sup> influx and inducing Ca <sup>2+</sup> -dependent scinderin-mediated disassembly of cortical F-actin.                                                     | Promotes cytoskeletal remodeling and granule exocytosis, facilitating mucin secretion and mucus layer formation                       | Maintain intestinal immune homeostasis                  | [166] |
|       | Intestinal epithelial cell                 | In response to food antigens, caspase-3/7 cleave GSDMD at Asp88, producing a 13 KDa fragment that can enter nucleus to regulate the transcription of CIITA and MHCII molecules.                                                   | Induces upregulation of Tr1 cells in the small intestine (duodenum) and mediates food tolerance.                                      | Food tolerance                                          | [167] |
|       | Intestinal epithelial cell                 | In inflamed colonic tissues, full-length GSDMD recruits NEDD4 to catalyze polyubiquitination of pro-IL-1 $\beta$ , which acts as a signal for cargo loading into secretory vesicles.                                              | Promotes the pathogenesis of intestinal inflammation by guiding the release of IL-1 $\beta$ -containing small extracellular vesicles  | Inflammatory bowel disease/experimental colitis         | [76]  |
|       | Osteoclast                                 | The p20 fragment generated by RIPK1-dependent caspase-8/-3 cleavage of GSDMD can bind to PI(3)P on early endosomal membranes, thereby confining endo-lysosomal maturation                                                         | Limits lysosomal function and secretion and regulates osteoclastic bone resorption                                                    | Maintains bone homeostasis                              | [168] |
|       | Cancer cell                                | GSDMD can directly bind to eIF2 $\alpha$ , enhancing the interaction between PERK and eIF2 $\alpha$ and promoting eIF2 $\alpha$ phosphorylation. As a result, GSDMD activates the downstream ERSR and selectively activates ATF-4 | Activates apoptosis-related proteins, such as CHOP, induces cancer cell apoptosis and enhances the sensitivity of tumors to cisplatin | Sensitizes cancer cells to cisplatin                    | [169] |
|       | Colorectal cancer cell                     | Under the stimulation of chemotherapy drugs and hypoxia in the TME, GSDMD translocates into the nucleus, engaging with PARP-1 and hindering DNA repair                                                                            | Enhances apoptosis and suppresses cancer cell growth by inhibiting the DNA repair function of PARP-1                                  | Sensitizes colorectal cancer to chemotherapy            | [170] |

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Table 4 | Continued

| Cell                        | Mechanism                                                                                                                                                                                                                                                                                | Pathology/Physiology                                                                                                                                           | Disease/Function                                                                                 | Refs.      |
|-----------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|------------|
| Neutrophil                  | Upon NETosis, the GSDMD activated by NE forms pores on plasma membrane, which allows the release of NETs. Moreover, through GSDMD pore, NE can also translocate to the nucleus. Then, NE processes histones, which allows nuclear expansion and formation of NETs.                       | /                                                                                                                                                              | Induces NETosis                                                                                  | [171, 172] |
| Atrial cardiomyocyte        | Upregulated GSDMD-NITs form pores on the plasma membrane, facilitating cytokine release and increasing macrophage infiltration to the atria. Meanwhile, GSDMD-NITs also localize on the mitochondria membrane, promoting mitochondrial dysfunction and increasing the production of ROS. | Causes pro-arrhythmic Ca <sup>2+</sup> -handling abnormalities in cardiomyocytes, accelerates atrial fibrosis and Cx43 remodeling                              | Atrial fibrillation                                                                              | [173]      |
| Neural progenitor cell      | Through regulating the AMPK/PGC-1 $\alpha$ pathway, GSDMD is involved in organizing the mitochondrial electron transport chain, promoting a switch from oxidative phosphorylation to glycolysis.                                                                                         | Results in increased lactate, which acts as a signal to regulate p38MAPK/Rac1 pathway and activate NF- $\kappa$ B transcription to disrupt cortex development. | Neurologic disorders caused by genotoxic stress and neurodevelopmental disorders, such as autism | [100]      |
| GSDME Hepatocyte            | Inhibits citrullination at Drp1 Arg114, which is critical for Drp1 stability and mitochondrial function.                                                                                                                                                                                 | Affects mitochondrial respiration, intestinal nutrient absorption, systemic energy expenditure, and regulates hepatic inflammation and hepatocytes death       | Metabolic dysfunction-associated steatohepatitis                                                 | [174]      |
| Keratinocyte                | Interacts and promotes nuclear translocation of p65 and c-jun, and upregulates psoriatic inflammatory mediators, such as IL1 $\beta$ , CCL20, CXCL1, CXCL8, S100A8, and S100A9                                                                                                           | Contributes to the pathogenesis of psoriasis and promotes the progression of psoriatic skin inflammation                                                       | Psoriasis                                                                                        | [175]      |
| Tumor-associated macrophage | The non-NT fragment of mGSDME (281-512) combines with PDPK1, thereby activating the PI3K-AKT pathway                                                                                                                                                                                     | Promotes M2-like polarization and proliferation of macrophages, which facilitates resistance to anti-PD1 treatment                                             | Hepatocellular carcinoma                                                                         | [176]      |
| Colorectal cancer cell      | GSDME promotes the expression of VEGF in colorectal cancer cells                                                                                                                                                                                                                         | Engages in angiogenesis and promotes colorectal cancer growth and metastasis                                                                                   | Colorectal cancer                                                                                | [177]      |

BCR: B cell receptor, PMA: phorbol 12-myristate 13-acetate, RLR: retinoic acid-inducible gene I-like receptors, ISGs: interferon-stimulated genes, PI(3)P: phospholipid phosphatidylinositol 3-phosphate, ERSR: endoplasmic reticulum stress response, ATF4: A transcription factor, NE: neutrophil elastase, NETs: neutrophil extracellular traps, NETosis: NETs formation, VEGF: vascular endothelial growth factor.

**Table 5** | Post-translational modifications of GSDMs.

|       | Post-translational modification          | Factors                         | PTM sites                                       | Effects of the post-translational modification                                                                                                                   | Refs.      |
|-------|------------------------------------------|---------------------------------|-------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| GSDMA | Phosphorylation                          | ULK1                            | S353(M)                                         | Impairs autoinhibition and activates oligomerization of GSDMA                                                                                                    | [49]       |
|       |                                          | PLK1                            | T8(H)                                           | Impairs NTD oligomerization                                                                                                                                      | [35, 178]  |
| GSDMB | Ubiquitination                           | IpaH7.8                         | /                                               | Promotes proteasomal degradation                                                                                                                                 | [56, 179]  |
|       |                                          |                                 | K177, K190, K192(H)                             | Inhibits membrane insertion                                                                                                                                      | [19, 20]   |
| GSDME | Phosphorylation                          | AMPK                            | T6(H)                                           | Impairs NTD oligomerization                                                                                                                                      | [35]       |
|       | Deubiquitination                         | OTUD4                           | Unknown                                         | Maintains the stability of GSDME                                                                                                                                 | [180]      |
|       | Deubiquitination                         | ORFV                            | Unknown                                         |                                                                                                                                                                  | [181]      |
|       | K48-link Deubiquitination                | USP48                           | K120, K189(H)                                   |                                                                                                                                                                  | [182]      |
|       | Oxidation                                | UVC-induced lipid ROS           | Cys156, Cys180                                  | Leads to intermolecular disulfide bond-dependent oxidative oligomerization and translocation of the PARYlated FL-GSDME                                           | [51]       |
|       | PARYlation                               | PARP5                           | maybe D229, E233A                               | Alleviates the autoinhibitory state of GSDME by inducing a GSDME conformational change                                                                           |            |
|       | Succination                              | Fumarate derivatives (MMF, DMF) | C45(H)                                          | Impairs GSDME-caspase interactions and inhibits GSDME cleavage                                                                                                   | [183]      |
|       | Palmitoylation                           | ZDHHC2/7/11/15                  | C407/C408(H)                                    | Facilitates dissociation of NTD and CTD                                                                                                                          | [36]       |
| GSDMD | Palmitoylation                           | Unknown                         | Unknown                                         | May promote plasma membrane localization                                                                                                                         | [50]       |
|       | Phosphorylation                          | Unknown                         | S30, S31, T32/S45, S46, S47(H)                  | The phosphomimetic triple mutants of these sites significantly inhibit the pyroptotic activity of GSDMD                                                          | [184]      |
|       |                                          |                                 | T213(H)                                         | Impairs NTD oligomerization                                                                                                                                      |            |
|       | Dephosphorylation                        | PP1                             | T213(H)                                         | Rescues NTD oligomerization                                                                                                                                      |            |
|       | K33-Polyubiquitination                   | MIB2                            | K168                                            | K33-linked ubiquitin chains act as ligands for selective autophagy cargo receptors, which guide the USP18-mediated selective autophagic degradation of the GSDMD | [185]      |
|       | K63-linked/K43-linked Polyubiquitination | IpaH7.8                         | K55, K62, K204(H)                               | Proteasomal degradation                                                                                                                                          | [186]      |
|       | K27-polyubiquitination                   | SYVN1                           | K203, K204(H)                                   | Promotes pyroptosis                                                                                                                                              | [187]      |
|       | Oxidation                                | ROS                             | cys192(m)                                       | Promotes oligomerization and pore formation                                                                                                                      | [188, 189] |
|       |                                          | ROS                             | C38, C56, C268, C467(H) C39, C57, C265, C487(M) | Promotes cleavage by caspase-1                                                                                                                                   | [190]      |
|       | Itaconation                              | Itaconate                       | C77 (M)                                         | Impairs GSDMD-caspase interactions and inhibits GSDMD cleavage                                                                                                   | [191]      |
|       | Succination                              | Fumarate derivatives (MMF, DMF) | C191(H)/C192(M)                                 | Impairs GSDMD-caspase interactions and inhibits GSDMD cleavage                                                                                                   | [183]      |
|       | S-palmitoylation                         | ZDHHC5/9                        |                                                 | Promotes membrane localization                                                                                                                                   | [50, 192]  |
|       |                                          | ZDHHC7                          |                                                 | Contributes to overcome autoinhibition and increases caspase-1 engagement with GSDMD                                                                             | [193, 194] |

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Table 5 | Continued

| Post-translational modification      | Factors          | PTM sites | Effects of the post-translational modification                                                                                                                                                                                  | Refs. |
|--------------------------------------|------------------|-----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| Depalmitoylation                     | APT2             |           | Following translocation to the plasma membrane, APT2 subsequently depalmitoylates GSDMD-NT, which re-exposes the C192 residue and allows it to be oxidized by ROS; in contrast, may also lead to translocation to the cytoplasm | [193] |
| Cysteine-modification                | Disulfiram       |           | Inhibits NTD oligomerization                                                                                                                                                                                                    | [128] |
| Cysteine-modification/<br>alkylation | Necrosulfonamide |           | Inhibits NTD oligomerization                                                                                                                                                                                                    | [195] |
| O-GlcNAcylation                      | Unknown          | S338(M)   | Impairs GSDMD-caspase interactions and inhibits GSDMD cleavage                                                                                                                                                                  | [196] |
| SUMOylation                          | Unknown          | K177      | Contributes to GSDMD protein stabilization and promotes pyroptosis                                                                                                                                                              | [197] |
| Acetylation                          | Unknown          | K248      | Promotes k27-linked ubiquitination of GSDMD                                                                                                                                                                                     | [198] |
| Deacetylation                        | HDAC4            |           | Impairs k27-linked ubiquitination of GSDMD                                                                                                                                                                                      |       |

GSDM (Table 5). PTMs promote the formation of GSDM pores by facilitating dissociation of the NT domain (NTD)-CT domain (CTD) and promoting GSDM oligomerization and membrane translocation. PTMs inhibit the formation of GSDM pores by hindering GSDM enzymatic activation, preventing GSDM oligomerization and membrane insertion, or promoting GSDM degradation. Importantly, PTMs provide a checkpoint for pore formation and consequent pyroptosis, which uncouples biochemical GSDM cleavage from pore formation [192].

## 5.1 Different GSDM PTMs

**5.1.1 Oxidation.** The initiation of pyroptosis is usually accompanied by the production of ROS, such as mtROS [190], and ROS-derived lipid peroxidation [199]. GSDMD cleavage occurs downstream of ROS release and oxidation of GSDMD promotes caspase-1-mediated cleavage [190]. The Ragulator–Rag–mTORC1 complex, as well as other stimuli that enhance ROS production, all promote ROS-mediated oxidation of GSDMD at Cys191 (Cys192 in mice) [188, 189], which facilitates GSDMD oligomerization (excluding membrane localization) and pore formation [188, 189]. In addition, Devant & Kagan proposed that Cys191 oxidation may also contribute to the removal of another inhibitory PTM or the addition of another excitatory PTM [15]. Moreover, UVC-induced lipid ROS was reported in 2024 to promote intermolecular disulfide bond-dependent oxidative oligomerization and membrane translocation of the PARylated FL-GSDME [51].

**5.1.2 Palmitoylation.** Palmitoylation of GSDM is primarily found in GSDME. Palmitoyltransferase ZDHHC-2/7/11/15 interact with GSDME-CT and palmitoylate GSDME-CT at

C407/C408. As a result, palmitoylation promotes the separation of GSDME-CT from GSDME-NT and leads to an increase in GSDME-mediated pyroptosis [36].

Palmitoylation of GSDMD is also mediated by ZDHHC, including ZDHHC7 [193, 194] in bone marrow-derived macrophages (BMDMs) or ZDHHC5/9 [50, 192] in THP-1 (a human monocytic cell line), which activates cleaved GSDMD and intact GSDMD [50]. The modification site (Cys191) is located at the tip of the  $\beta$ -barrel [193].

Because palmitoylation enhances the binding of GSDMD-NT to phosphatidylinositol and cardiolipin, palmitoylation promotes membrane targeting and translocation of GSDMD-NT [200]. However, the palmitoyl group in FL-GSDMD may be nestled within the hydrophobic pocket, which decreases the susceptibility of FL-GSDMD to membrane binding [192]. Palmitoylation of GSDMD may also contribute to overcome autoinhibition. By increasing caspase-1 engagement with GSDMD, caspase-1 promotes GSDMD processing and activation [193].

Known as the relay model, ZDHHC and APT2 spatiotemporally control GSDMD cleaving, plasma membrane translocation, and oligomerization through the palmitoylation–depalmitoylation relay. APT2-catalyzed depalmitoylation is required for ROS-mediated oligomerization of GSDMD-NT [193].

Palmitoylated GSDMD-NT, as well as potentially palmitoylated full-length GSDMD, induces mitochondrial damage and increases ROS production [50]. The elevated ROS levels might reduce proteasomal degradation of ZDHHCs [50], leading to upregulation of palmitoyltransferases and further palmitoylation of GSDMD. Similarly, LPS stimulates upregulation of the ZDHHC enzyme in macrophages [192], promoting autopalmitylation of

ZDHHC [193] as well as LPS-dose dependent GSDMD palmitoylation. Additionally, NF- $\kappa$ B activation has been reported to be involved in LPS-induced GSDMD palmitoylation [176].

Palmitoylation has also been reported in bacteria. Bacteria GSDM (bGSDM) utilizes palmitoylation to enhance membrane interactions and induce membrane disorder, which reduces the energetic barriers of pore formation. Additionally, palmitoylation stabilizes the intermediate bGSDM assemblies and promotes full pore formation [8]. Moreover, in *Runella* bGSDM, palmitoylation at Cys3 interacts with Phe264, stabilizing the pore-forming domain in its inactive conformation. While in the *Bradyrhizobium*, this typical autoinhibitory interaction is observed between Phe253 and the Cys3 palmitoyl group [201].

**5.1.3 Succination.** GSDMD and GSDME can be succinated by the citric acid cycle intermediate, fumarate [183, 202, 203]. Succination on these GSDMs impairs GSDM-caspase interactions and inhibits GSDM cleavage. Importantly, the FDA-approved drug, DMF, has been reported to inhibit pyroptosis through succination [183], which proved the concept of pharmacologic applicability of GSDMD regulation by succination. Moreover, as an inhibitor of fumarate hydratase, FHIN1 leads to the accumulation of endogenous fumarate in cells, which mimics some of the effects of DMF and inhibits cell pyroptosis. Thus, both inhibiting fumarase activity and adding additional fumarate inhibit GSDMD-dependent cell death [183, 202].

**5.1.4 Itaconation.** Itaconate is an anti-inflammatory metabolite [204] that accumulates in cells when the Krebs cycle is disrupted [205]. Endogenous itaconate accumulates in myeloid cells in response to prolonged TLR stimulation, where itaconate covalently binds to GSDMD at Cys77 and may affect the interaction between GSDMD and caspase-1 [191]. Notably, 4-octyl itaconate (4-OI) shows protective effects on acute colitis through inhibition of GSDMD/GSDME-mediated pyroptosis [206]. This process might at least in part be attributed to 4-OI-mediated itaconation.

**5.1.5 Phosphorylation and dephosphorylation.** Phosphorylation at Thr8 in GSDMA [35, 178], Thr213 in GSDMD [184], or Thr6 in GSDME [35] impairs oligomerization of NTD through steric hindrance, which prevents GSDM pore formation and pyroptosis. In contrast, protein phosphatase 1 (PP1) rescues pyroptosis activity of GSDMD through dephosphorylation. Moreover, phosphorylation at Ser353 impairs autoinhibition of GSDMA between NT and CT, which activates FL-GSDMA oligomerization [49].

**5.1.6 Ubiquitination and deubiquitination.** Ubiquitination has a dual role in regulating GSDM-dependent pyroptosis. While the E3 ubiquitin ligase, SYVN1, acts as a positive

regulator of GSDMD-dependent pyroptosis [187], MIB2 downregulates GSDMD-dependent pyroptosis through selective autophagic degradation [185].

Furthermore, the *Shigella* ubiquitin ligase, IpaH7.8, targets human GSDMB [19, 20, 56, 179] and GSDMD [186] to prevent host cell pyroptosis. IpaH7.8 inhibits the bactericidal functions of natural killer cells and facilitates infection, which explains human susceptibility to *Shigella*. In contrast, mice exhibit resistance to *Shigella* infection due to the lack of GSDMB and GSDMD insensitivity to IpaH7.8 [19, 20, 186]. Notably, although IpaH7.8-mediated ubiquitination causes efficient degradation of GSDMB and GSDMD, for GSDMB, the ubiquitination is sufficient to inhibit membrane insertion and oligomerization of GSDMB-NT, while for GSDMD, proteasomal degradation of the ubiquitinated GSDMD is needed to inhibit GSDMD-mediated pyroptosis [186].

In contrast, the deubiquitination mediated by OTUD4 [180] and USP48 [182] stabilizes GSDME, which promotes GSDME-mediated pyroptosis in cancer cells. In addition, oncolytic parapoxvirus ovis [181] also pre-stabilizes GSDME by decreasing ubiquitination on GSDME and further triggers pyroptosis, especially in GSDME-low tumors.

**5.1.7 Other PTMs.** O-GlcNAcylation at S338 blocks the GSDMD interaction with caspase-11, thus attenuating LPS-induced endothelial cell pyroptosis [196]. SUMOylation at Lys177 contributes to GSDMD protein stabilization, thereby promoting pyroptosis. The SUMO de-conjugating enzyme, SENP3, interacts with GSDMD, diminishes GSDMD SUMOylation, promotes GSDMD degradation, and consequently blocks pyroptosis [197]. Acetylation at Lys248 promotes ubiquitination of GSDMD, enhancing GSDMD-mediated pyroptosis. In contrast, histone deacetylase 4 (HDAC4), which specifically deacetylates GSDMD, impairs GSDMD ubiquitination, thus inhibiting pyroptosis [198].

Upon intensive UVC irradiation, PARYlation mediated by PARP5 induces a conformational change in GSDME, which alleviates the autoinhibitory state and promotes oxidative oligomerization [51].

## 5.2 Interplay between different GSDMD PTMs

Because Cys191 is located within a disordered loop region in GSDMD (Figure 6A) and is exposed to the solvent [193], Cys191 is more accessible for oxidation and palmitoylation as well as other PTMs. Because these PTMs target the same residue, a cooperative or competitive relationship might exist (Figure 6B). Indeed, palmitoylation on GSDMD Cys191 has been shown to occur in a ROS-dependent manner and increased ROS levels result in significant augmentation of GSDMD palmitoylation [50]. In contrast, succination acts as a competitor to palmitoylation, which negatively regulates GSDMD Cys191 palmitoylation during pyroptosis [192]. Moreover, small molecules that target Cys191 and inhibit GSDMD-mediated pyroptosis, like NSA [195] and

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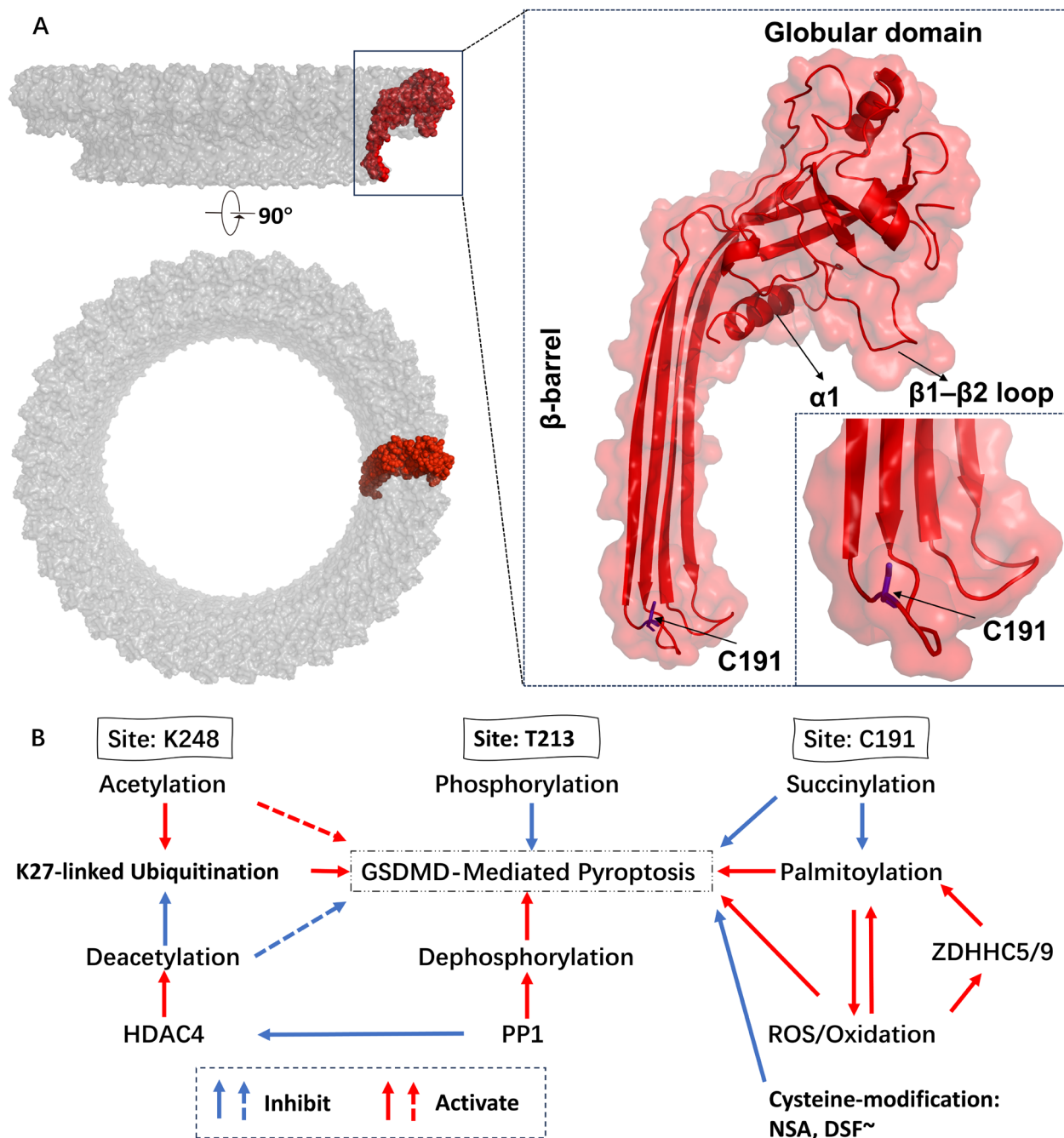


**Figure 6 | Interplay between different post-translational modifications of GSDMD.**

(A) The GSDMD-NT subunit is composed of a globular domain ("palm"), a  $\beta$ -barrel ("fingers"), an overlaid  $\alpha 1$  helix ("thumb"), and a  $\beta 1$ - $\beta 2$  loop ("wrist"). Cys191 is located at the tip of the  $\beta$ -barrel, within a disordered loop, which makes it more accessible for PTMs. (B) The regulatory network of post-translational modifications in GSDMD-mediated pyroptosis: the red arrows indicate activation; the blue arrows indicate inhibition; and the dashed lines indicate indirect effect. HDAC4: Histone deacetylase 4, PP1: protein phosphatase 1, ZDHHC5/9: zinc finger DHHC-type palmitoyltransferase 5/9, NSA: necrosulfonamide, DSF~: disulfiram.

DSF [128], might function, at least in part, through competition with GSDMD palmitoylation [50].

In addition, Lys248 undergoes acetylation and ubiquitination. Acetylation of GSDMD facilitates ubiquitination

and promotes GSDMD-mediated pyroptosis, whereas deacetylation suppresses pyroptosis. Notably, the activity of the specific deacetylase, HDAC4, is tightly regulated and enhanced by phosphorylation. PP1 $\gamma$ , which mediates

GSDMD dephosphorylation [184], counteracts the inhibitory effect of HDAC4 by inducing HDAC4 dephosphorylation and downregulates GSDMD deacetylation [198].

### 5.3 Drugs targeting on GSDM PTM

Because PTM is important for GSDM activation and oligomerization, targeting PTMs that promote pyroptosis appears to be an effective way to inhibit pyroptosis. Thus, GSDM PTMs might serve as a potential therapeutic target for infectious and inflammatory diseases.

For example, to efficiently alleviate intervertebral disc degeneration (IVDD), carbonized Mn-containing nanodots (MCDs) were developed [207]. These nanodots possess excellent enzyme-mimicking abilities and are capable of clearing ROS. Thus, these nanodots can block GSDMD oxidation and suppress pyroptosis in nucleus pulposus cells. The effectiveness of MCDs was confirmed by systematic *in vivo* evaluation in a mouse model of puncture-induced IVDD, which is at least in part attributed to block GSDMD oxidation. Moreover, by specifically inhibiting GSDMD palmitoylation, palmitate analogs, such as 2-bromopalmitate and the cell-permeable competitive peptide, CPP-W, can both effectively mitigate organ damage and extend the survival of septic mice, which suggests a promising strategy for sepsis treatment [192].

## 6. REGULATORY MECHANISM UNDERLYING GSDM PORE AND PYROPTOSIS

### 6.1 Modulating the state of GSDM pores through calcium-driven phosphoinositide metabolism

PLCG1 is a class of membrane-associated enzymes that catabolizes  $\text{PIP}_2$  into diacylglycerol (DAG) and  $\text{IP}_3$  [208]. GSDMD-NT fragments trigger macrophage pyroptosis in a PLCG1-dependent manner [199].  $\text{PIP}_2$  enrichment on the plasma membrane marks the locations where GSDMD preferentially inserts and causes calcium flares [209].

Moreover, the GSDMD pore appears to be a dynamic structure that is modulated by pore-mediated calcium influx through membrane phosphoinositide metabolism [209]. Phospholipase C (PLC) and phosphoinositide 3-kinase (PI3K) are involved in turning  $\text{PIP}_2$  into DAG and  $\text{PIP}_3$ , respectively. The bias towards  $\text{PIP}_3$  in the membrane enhances pore stability and predominantly keeps the GSDMD pores in the open state, which drives cytokine release and inflammation. In contrast, the DAG content induces the closed state (Figure 7A).

All in all, the formation and dynamics of GSDMD pores are regulated by calcium-driven phosphoinositide metabolism. Blocking  $\text{Ca}^{2+}$  influx appears to be a promising strategy to inhibit GSDMD-induced pyroptosis. The calcium antagonist,  $\text{Mg}^{2+}$ , impedes the oligomerization and membrane translocation of GSDMD-NT, which inhibits pyroptosis by preventing  $\text{Ca}^{2+}$  influx [210].  $\text{Mg}^{2+}$  rescues immortalized bone marrow-derived macrophages primed with LPS by acting as an anti-inflammatory agent and protects mice from LPS-induced

septic shock. Additionally, the  $\text{Ca}^{2+}$  chelators, EGTA and BAPTA-AM, have been reported to significantly suppress pyroptosis in HEK293T cells [210].

Furthermore, caspase-11-dependent cleavage of pan-nexin-1 leads to ATP release in non-canonical pyroptosis, which activates P2X7 and P2X7-mediated  $\text{Ca}^{2+}$  influx [58, 210, 211]. However, extracellular divalent metal cations, such as  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Ni}^{2+}$ , can bind to ATP and thereby impede the activation of P2X7 [210, 211]. The specific  $\text{Ca}^{2+}$  channel remains to be determined for canonical pyroptosis and this channel appears to be inhibited by  $\text{Mg}^{2+}$ ,  $\text{La}^{3+}$ , and  $\text{Gd}^{3+}$  [210, 212].

### 6.2 Membrane repair mechanism

Whether GSDM triggers pyroptosis depends on the balance between membrane pore formation and membrane repair [146]. Herein we demonstrated two mechanisms involved in repairing GSDMD pores.

**6.2.1 ASM-mediated membrane repair.** GSDMD and caspase-7 are cleaved and activated by caspase-1 upon activation of inflammasomes. GSDMD pore-mediated  $\text{Ca}^{2+}$  influx triggers lysosomal exocytosis that delivers pro-acid sphingomyelinase (ASM) to the cell surface. The activated caspase-7 passes through the GSDMD pore to encounter ASM, which cleaves and activates ASM. Activated ASM leads to the accumulation of ceramide, which enhances endocytosis and promotes membrane repair (Figure 7B). In this way, ASM is engaged in fixing the holes punched by GSDMD on the plasma membrane [213, 214].

**6.2.2 ESCRT-mediated membrane repair.** Upon GSDM pore formation, pore-mediated calcium influx triggers the initiation of membrane repair. The ESCRT machinery is recruited to the damaged membrane areas, which removes GSDM pores through ESCRT-induced ectosomes [215, 216] and negatively regulates cellular pyroptosis [217] (Figure 7B). In contrast, by specifically targeting and inhibiting the ESCRT-dependent cell membrane repair, the  $\text{Ca}^{2+}$  chelator, BAPTA-AM, has been reported to boost GSDM-mediated tumor pyroptosis [216].

During arrhythmogenesis, atrial cardiomyocytes counteract the effect of NLRP3-GSDMD inflammatory signaling by upregulating ESCRT-mediated membrane repair. Atrial cardiomyocytes escape from pyroptosis and remain viable, which maintains contractile and electrophysiologic properties [173].

Cardiomyocyte death is determined by competition between cardiac injury-induced plasma membrane rupture and membrane repair machinery during ischemia/reperfusion. Mechanistically, ALG-2-interacting protein X (Alix) senses calcium entry and initiates ESCRT assembly in cardiomyocytes upon membrane rupture. This process triggers the recruitment of vacuolar protein sorting 4a (Vps4a) to the injury sites, which promotes plasma membrane blebs shedding and membrane resealing. Vps4a-mediated membrane repair helps maintain plasma

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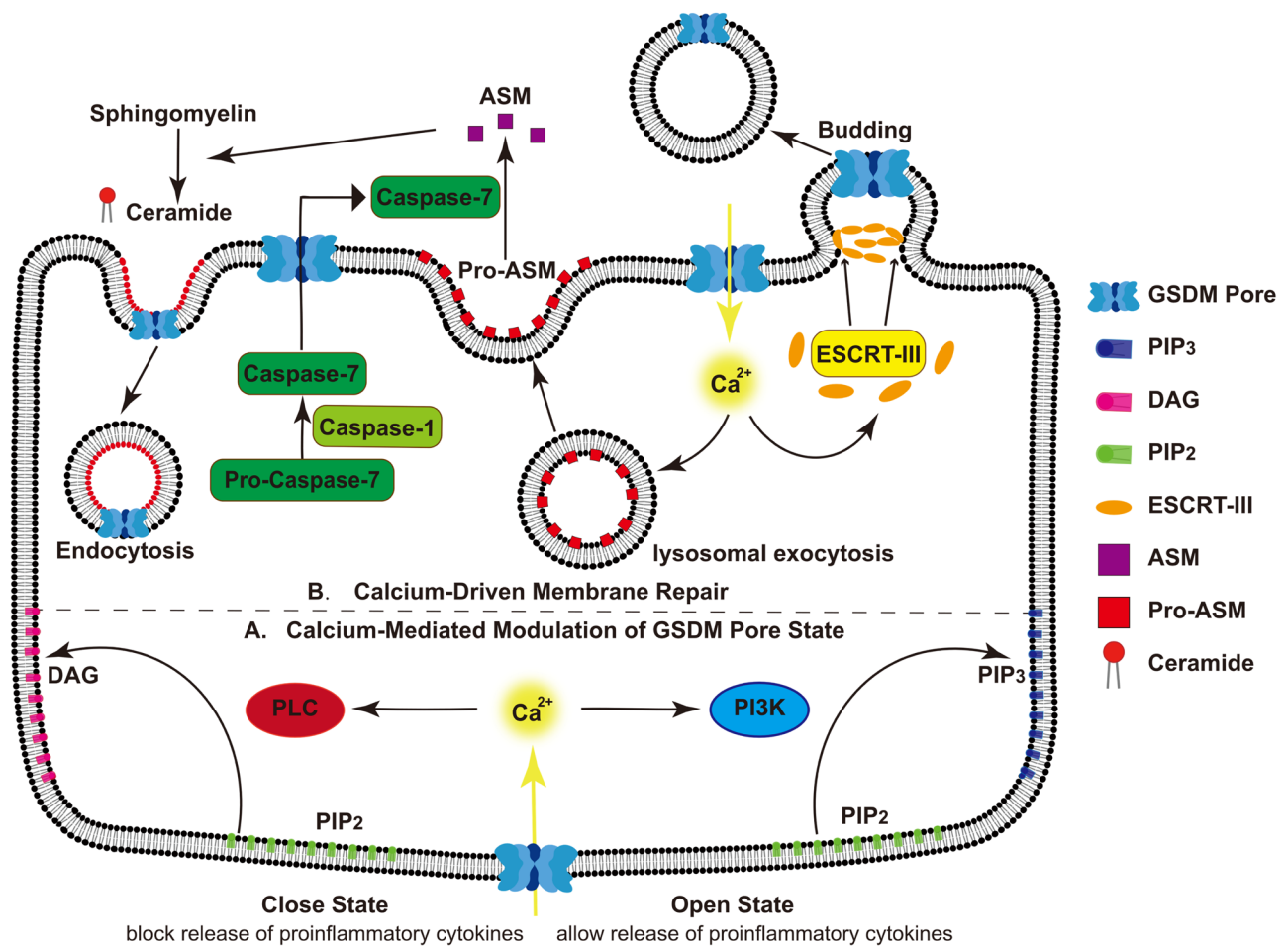


**Figure 7 | Regulatory mechanism of the GSDM pore.**

(A) Calcium-mediated modulation of the GSDM pore state: the dynamics of GSDM pores is regulated by calcium-driven phosphoinositide metabolism. The bias towards PIP<sub>3</sub> in the membrane keeps GSDM pores in the open state. In contrast, the content of DAG induces the closed state. (B) Calcium-driven membrane repair: upon GSDM pore formation, the  $Ca^{2+}$  influx activates both lysosomal-exocytosis-mediated delivery of pro-ASM and recruitment of the ESCRT machinery, in turn repairing membrane pores through ASM-dependent endocytosis and ESCRT-III-dependent exocytosis. ASM: acid sphingomyelinase, PI3K: phosphatidylinositol 3-kinase, PLC: phospholipase C.

membrane integrity of cardiomyocytes, antagonizing cardiomyocyte pyroptosis as well as other programmed cell death [218].

More importantly, ESCRT-mediated membrane repair may enhance tumor cell resistance to T cell attack. ESCRT-mediated membrane repair limits granzyme entry into the cytosol by repairing perforin holes, which impairs granzyme-mediated apoptosis and pyroptosis [219]. Conversely, the killing effect of T cells may be enhanced by inhibiting recruitment of the ESCRT machinery and delaying plasma membrane repair in tumor cells. Moreover, this approach can also rescue granzyme-induced GSDM activation as well as downstream pyroptosis [146]. In addition, the ESCRT system may be involved in repairing endosomal and lysosomal membranes, which reduces exposure of the phagocytosed bacteria

to the cytoplasm and to some extent, diminishes the activation of inflammasome [215].

Neither ESCRT- nor ASM-mediated membrane repair can be accomplished instantaneously. However, modulating the formation of GSDM pores through palmitoylation may provide the necessary time window for membrane repair, which in turn potentially attenuates or reverses pyroptosis-induced inflammatory responses [194].

### 6.3 Other pyroptosis regulators

**6.3.1 TRIM21.** As a positive regulator of GSDMD-dependent pyroptosis, TRIM21 extends the half-life of GSDMD by engaging with GSDMD and forming a stable complex. Notably, the effect of TRIM21 on GSDMD is independent of its E3 ligase activity and the

PRY-SPRY domain of TRIM21 alone is sufficient to promote pyroptosis [220].

**6.3.2  $\alpha$ -mangostin.** Reduction of the endoplasmic reticulum (ER) membrane protein, reticulon 4 (RTN4), leads to significant ER membrane curvature remodeling and ER-plasma membrane fusion, which results in formation of the “bubble” structure of pyroptotic cells.  $\alpha$ -mangostin acts as a “molecular glue,” recruiting the E3 ligase, UBR5, to degrade RTN4 and inducing potent cellular pyroptosis [221]. Interestingly, RTN4 is highly expressed in osteosarcomas, which makes RTN4 a potential anti-tumor therapeutic target [221].

**6.3.3 Myosin 6 (MYO6).** MYO6 is a unique actin-based motor protein. MYO6 functions as an adaptor, bridging GSDME-NT with the endocytic adaptor, AP2, thereby mediating endocytosis of GSDME-NT. Of note, the methionine-derived metabolite, spermidine, directly interacts with MYO6, which enhances MYO6 selectivity for GSDME-NT. In contrast, blockage of the methionine-spermidine metabolic axis reduces the interaction between AP2M1 and GSDME-NT, dampening GSDME-NT endocytosis and promoting GSDME-mediated pyroptosis [222].

Clinically, higher levels of tumor spermidine and expression of the methionine-to-spermidine metabolism-related gene signature have been shown to be associated with poor prognosis. Moreover, methionine restriction therapy appears to boost efficacy of cetuximab by enhancing cetuximab-mediated antibody-dependent cell-mediated cytotoxicity (ADCC) and promoting ADCC-induced pyroptosis [222]. SLC7A5 is also likely to be a potential target for enhancing tumor pyroptosis as the major methionine transporter of tumor cells.

## 7. ADVANCES IN GSDM-BASED THERAPEUTICS

### 7.1 Discovery and application of GSDMD agonists

A potential oral drug for diabetic patients, 6,7-dichloro-2-methylsulfonyl-3-N-tert-butylaminoquinoline (DMB) [223], was reported in September 2024 to be a selective agonist of GSDMD [139] (Table 6). This small molecule agonist functions effectively in humans and mice, activating pore formation of GSDMD without the need for cleavage and promoting pyroptosis. Mechanistically, DMB works by specifically targeting and covalently modifying the Cys191 residue of GSDMD. This effect contributes to overcoming GSDMD autoinhibition and facilitates GSDMD binding to the cell membrane, a process that mimics the effects of palmitoylation.

DMB was administered to mice bearing EMT6/CT26/B16 tumors to test the effects of DMB in cancer treatment [139]. The dose used was chosen based on pre-clinical studies of DMB for diabetes. At this dose, DMB treatment was shown to be sufficient to trigger pyroptotic death of tumor cells without causing measurable

toxicity. In addition, DMB induces immunogenic cell death that provides protective anti-tumor immunity and DMB-treated tumor cells can act as a tumor vaccine. Although DMB works well inside small tumors, combined treatment with a PD-1 monoclonal antibody is needed to treat large tumors. There is a strong correlation between tumor size and DMB responsiveness. DMB shows potential for clinical use as an effective post-treatment strategy to reduce tumor burden for patients with advanced-stage cancer.

### 7.2 Discovery and application of GSDME inhibitors

Methylcobalamin (MeCbl), which is known as a coenzyme form of vitamin B12, has recently been reported to be a specific and direct inhibitor of GSDME [231] (Table 6). By adopting the base-off conformation, MeCbl approaches GSDME via the  $\alpha$ -face of corrin ring and coordinates with Cys180 in GSDME. MeCbl blocks the caspase-3/GzmB-GSDME interaction and prevents GSDME from cleavage. More importantly, MeCbl appears to be a promising drug against GSDME-initiated acute liver failure, as evidenced by the protective effects in a mouse model of cholestatic liver failure or cisplatin-/concanavalin A-induced liver injury.

### 7.3 Development and application of GSDMD inhibitors

**7.3.1 Disulfiram.** The FDA-approved drug, disulfiram (DSF), is primarily used for treating alcohol addiction, and was recently shown to specifically inhibit GSDMD pore formation without affecting caspase-1 activation or GSDMD cleavage [128] (Table 6). Mechanistically, the diethyldithiocarbamate (DTC) moiety of DSF covalently binds to GSDMD at Cys191, which specifically inhibits NTD oligomerization. The cellular efficacy of DTC is greatly enhanced by complexation with copper gluconate [Cu (II)], forming the bis (N, N-diethyldithiocarbamate) copper (II) complex (CuET). Specifically, the  $IC_{50}$  of DSF for inhibiting pyroptosis decreased 24-fold with Cu (II), from  $10.3 \pm 0.5 \mu M$  to  $0.41 \pm 0.02 \mu M$ .

In addition to LPS-induced sepsis [128], DSF and CuET have been reported to have potential in clinical use for multiple inflammatory disease (Table 7), including systemic sclerosis [237], developmental sevoflurane neurotoxicity [238], peritoneal fibrosis [239], systemic lupus erythematosus [240], LPS-induced acute respiratory distress syndrome [241], ovarian carcinoma [242], acute kidney injury [243], and neonatal-onset multisystem inflammatory disease [244].

**7.3.2 Dimethyl fumarate.** Dimethyl fumarate (DMF) is an FDA-approved drug (Tecfidera) for treating MS [252]. DMF converts Cys191 in GSDMD and Cys45 in GSDME to S-(2-succinyl)-cysteines, preventing the interaction with caspases and blocking activation [183] (Table 6). Moreover, DMF has an inhibitory effect on the onset of EAE and familial Mediterranean fever, and improves

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**Table 6** | GSDMD-targeted agonists and inhibitors.

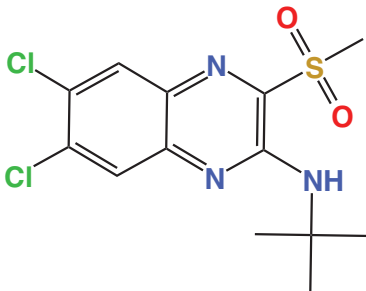
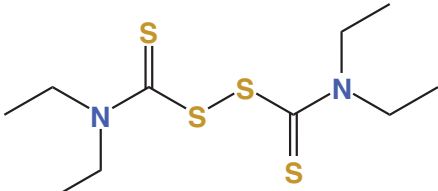
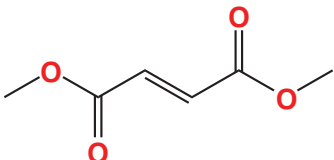
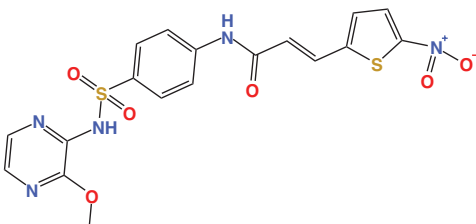
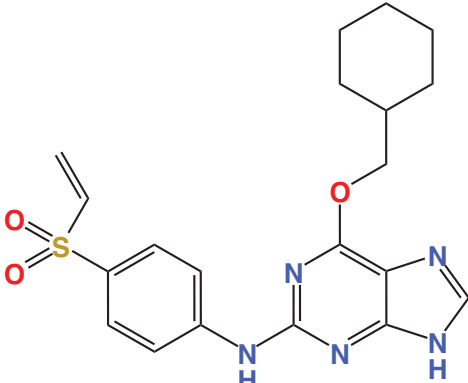
| Drug                | Feature                                                                             | Mechanism                                            | Refs.                                                                                                                          |
|---------------------|-------------------------------------------------------------------------------------|------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| Agonist of GSDMD    |                                                                                     |                                                      |                                                                                                                                |
| DMB                 |    | A glucagon-like peptide-1 receptor agonist           | Covalently modifies Cys191 of GSDMD, contributes to overcoming autoinhibition, and facilitates membrane binding of GSDMD [139] |
| Inhibitors of GSDMD |                                                                                     |                                                      |                                                                                                                                |
| DSF                 |    | An FDA-approved drug for treating alcohol addiction  | The DTC moiety of DSF covalently binds to GSDMD at Cys191 and inhibits NTD oligomerization [128]                               |
| DMF                 |  | An FDA-approved drug for treating multiple sclerosis | Converts Cys191 in GSDMD and Cys45 in GSDME to S-(2-succinyl)-cysteines, and prevents the interaction with caspases [183]      |
| NSA                 |  | The first specific inhibitor of GSDMD                | Binds to Cys191 of GSDMD and inhibits oligomerization of GSDMD dimers [195]                                                    |
| NU6300              |  | A covalent cyclin-dependent kinase 2 inhibitor       | Covalently interacts with Cys191 of GSDMD, blocks GSDMD cleavage, and impairs the palmitoylation of GSDMD [224]                |

Table 6 | Continued

| Drug      | Feature                                         | Mechanism                                                                                             | Refs. |
|-----------|-------------------------------------------------|-------------------------------------------------------------------------------------------------------|-------|
| LDC7559   | A drug targeting GSDMD-mediated NET formation   | Binds to GSDMD and blocks the activity of the GSDMD-NT domain?                                        | [171] |
| GI-Y1     | A drug with cardioprotective effect             | Targets Arg7 of GSDMD, impairs lipid-binding, and pore-forming activity of GSDMD-NT                   | [225] |
| GI-Y2     | A drug with vascular protective effect          | Targets Arg10 of GSDMD, and impairs lipid-binding and pore-forming activity of GSDMD-NT               | [226] |
| C202-2729 | /                                               | Binds to GSDMD-NT, blocks GSDMD activation, and prevents membrane translocation of GSDMD-NT           | [227] |
| SAE       | The major active component of Danhong injection | Shows a strong affinity to mouse GSDMD Cys192, and blocks GSDMD-NT oligomerization and pore formation | [228] |

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Table 6 | Continued

| Drug                                    | Feature                                                                                                       | Mechanism                                                                                                                                                                                                                                               | Refs. |
|-----------------------------------------|---------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| CA                                      | /                                                                                                             | Forms hydrogen bonds with residues located at or near the $\alpha 1$ helix and $\beta 1$ - $\beta 2$ loop of GSDMD-NT, and blocks GSDMD activation                                                                                                      | [229] |
| SCR-1481B1                              | A drug possessing vasoinhibitory properties and exhibiting anti-tumor effects                                 | Binds to the “drug pocket” within the oligomeric interface I $\beta 2$ and $\alpha 3$ regions of GSDMD-NT, forms three hydrogen bonds with Cys56, Asn58, and Glu143 and one halogen bond interaction with Lys145, and inhibits GSDMD-NT oligomerization | [230] |
| Cefcapene Pivoxil Hydrochloride Hydrate | A third-generation clinical antibiotic used for the effective treatment of Gram-negative bacterial infections | Bind to the “drug pocket” within the oligomeric interface I $\beta 2$ and $\alpha 3$ regions of GSDMD-NT, forms two hydrogen bonds with Asn58, one with Asp63 and Lys145, and inhibits GSDMD-NT oligomerization                                         | [230] |
| Inhibitor of GSDME<br>MeCbl             | A coenzyme form of vitamin B12                                                                                | MeCbl in base-off conformation coordinates to Cys180 in GSDME, thereby blocking caspase-3/granzyme B-GSDME interaction and GSDME-mediated pyroptosis                                                                                                    | [231] |

DMB: 6,7-dichloro-2-methylsulfonyl-3-N-tert-butylaminoquinoxaline, DSF: disulfiram, DMF: dimethyl fumarate, NSA: necrosulfonamide, SAE: salvanolic acid E, CA: caffeic acid, MeCbl: methylcobalamin.

**Table 7** | Application of GSDMD inhibitors.

| Disease                                                                              | Cells Undergoing Pyroptosis                                                   | Pathological Processes Induced by Pyroptosis                                                                                            | Inhibitor                            | Refs.      |
|--------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|------------|
| Acute myocardial infarction                                                          | Leukocytes                                                                    | Neutrophil production and mobilization                                                                                                  | NSA                                  | [232]      |
| Allergic rhinitis                                                                    | CD11c <sup>+</sup> dendritic cells stimulated with ovalbumin/house dust mites | Th1/Th2/Th17 cell immune imbalance                                                                                                      | NSA                                  | [233]      |
| Sepsis-associated encephalopathy                                                     | /                                                                             | Neuroinflammation and neuronal/synaptic damage result in cognitive deficits                                                             | NSA                                  | [234]      |
| Inflammatory bowel disease/DSS -induced colitis                                      | Macrophage/unclear                                                            | Release proinflammatory cytokines and promote intestinal inflammation                                                                   | NSA                                  | [235]      |
| Bone fracture                                                                        | Osteoblast                                                                    | Inhibition of osteoblast proliferation and differentiation                                                                              | NSA                                  | [236]      |
| Systemic sclerosis                                                                   | Macrophage                                                                    | Inflammation and fibrosis                                                                                                               | DSF and NSA                          | [237]      |
| Developmental sevoflurane neurotoxicity                                              | (Hippocampal) neuron                                                          | Neuronal cell death-induced cognitive defects                                                                                           | DSF and NSA                          | [238]      |
| Peritendinous fibrosis                                                               | Macrophages                                                                   | Pro-inflammatory macrophage-mediated fibrogenesis in tendon-bone injury                                                                 | DSF                                  | [239]      |
| Pristane-induced lupus/Systemic Lupus Erythematosus                                  | Monocyte/macrophage                                                           | Unclear                                                                                                                                 | DSF                                  | [240]      |
| LPS-induced acute respiratory distress syndrome                                      | Pulmonary endothelial cells                                                   | Damage to pulmonary endothelial cells and inflammation                                                                                  | DSF                                  | [241]      |
| Ovarian carcinoma                                                                    | Omental adipocyte                                                             | Induce chemoresistance and macrophage infiltration in advanced stage ovarian carcinoma                                                  | DSF                                  | [242]      |
| Acute kidney injury                                                                  | Renal tubular epithelial cells                                                | Irreversible damage to renal tubular epithelial cells induced by renal ischemia-reperfusion injury                                      | DSF                                  | [243]      |
| LPS-induced sepsis                                                                   | Macrophages and other cells                                                   | Proinflammatory cytokine secretion                                                                                                      | DSF/CuET                             | [128]      |
| Neonatal-onset multisystem inflammatory disease ( <i>Nlrp3</i> <sup>CA/+</sup> mice) | Macrophages                                                                   | Constitutive activation of <i>nlrp3</i> inflammasome and spontaneous cytokine secretion and inflammation caused by both GSDMD and GSDME | CuET                                 | [244]      |
| Multiple sclerosis                                                                   | Myeloid cells (macrophages/microglia), myelin-forming oligodendrocytes        | Axonal injury and inflammatory demyelination                                                                                            | DMF                                  | [93]       |
| Silicosis                                                                            | Silica-induced macrophages                                                    | Chronic lung inflammation and pulmonary fibrosis                                                                                        | DMF                                  | [203]      |
| Gout and hyperuricemia                                                               | MSU-induced neutrophil or macrophage                                          | MSU crystal-induced acute inflammatory response                                                                                         | DMF                                  | [245, 246] |
| Colitis ( <i>Cd177</i> <sup>-/-</sup> mice)                                          | TCR $\gamma$ $\delta$ CD8 $\alpha\alpha$ IELs                                 | TCR $\gamma$ $\delta$ CD8 $\alpha\alpha$ IEL overactivation and intestinal mucosal inflammation                                         | Microbiota-produced fumarate and DMF | [247]      |
| APAP-related drug-induced liver injury                                               | Hepatocytes                                                                   | Promotes deISGylation and degradation of CPS1, and causes ammonia clearance dysfunction                                                 | DMF                                  | [248]      |
| Multiple sclerosis/Experimental autoimmune encephalitis                              | Peripheral myeloid cells                                                      | Neuroinflammation and demyelination, cell infiltration to the central nervous system                                                    | DMF                                  | [183]      |
| Familial mediterranean fever                                                         | /                                                                             | Constitutive activation of the pyrin inflammasomes and recurrent inflammatory responses                                                 | DMF                                  | [183]      |

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Table 7 | Continued

| Disease                                                    | Cells Undergoing Pyroptosis    | Pathological Processes Induced by Pyroptosis                                                                                                     | Inhibitor              | Refs.         |
|------------------------------------------------------------|--------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|---------------|
| DSS-induced colitis                                        | Unclear                        | Release proinflammatory cytokines and promote intestinal inflammation                                                                            | NU6300                 | [224]         |
| Subarachnoid hemorrhage/<br>Traumatic brain injury         | Microglial/neuron              | Impair brain function recovery                                                                                                                   | LDC7559                | [249,<br>250] |
| Ang II-induced hypertensive<br>nephropathy                 | Renal tubular epithelial cells | Kidney injury and fibrosis                                                                                                                       | GI-Y1                  | [251]         |
| Atherosclerosis                                            | Atherosclerotic<br>macrophages | Aortic pyroptosis, atherosclerotic lesions,<br>and atherosclerotic plaque development                                                            | GI-Y1/GI-Y2            | [120,<br>226] |
| Multiple sclerosis/Experimental<br>autoimmune encephalitis | Macrophages/unclear            | Lead to immune cell accumulation/<br>activation and demyelination                                                                                | C202-2729              | [227]         |
| Diabetic myocardial infarction                             | /                              | Cardiac remodeling                                                                                                                               | SAE                    | [228]         |
| LPS-induced sepsis                                         | Macrophages                    | Proinflammatory cytokine secretion                                                                                                               | CA                     | [229]         |
| gram-negative-bacteria-<br>induced sepsis                  | /                              | Proinflammatory cytokine secretion                                                                                                               | SCR-1481B1 and<br>CPHH | [230]         |
| B16F10 melanoma                                            | Antigen-presenting cells       | Impairs antigen presentation and<br>suppresses anti-tumor immune responses<br>of CD8 <sup>+</sup> T cells in response to anti-PD-L1<br>treatment | SCR-1481B1 and<br>CPHH | [230]         |

*Nlrp3<sup>CA/+</sup>* mice: mice with gain-of-function mutations in NLRP3, which causes constitutive activation, MSU: monosodium urate, DSS: dextran sodium sulfate, APAP: acetaminophen, *Cd177<sup>-/-</sup>* mice: CD177-deficient mice, IELs: intraepithelial lymphocytes, CPS1: carbamoyl phosphate synthetase-1, DMB: 6,7-dichloro-2-methylsulfonyl-3-N-tert-butylaminoquinoxaline, DSF: disulfiram, CuET: bis (N, N-diethyldithiocarbamate) copper (II) complex, DMF: dimethyl fumarate, NSA: necrosulfonamide, SAE: salvianolic acid E, CA: caffeic acid, CPHH: cefcapene pivoxil hydrochloride hydrate.

survival rates of mice with septic shock induced by lethal doses of LPS.

Further research has revealed the therapeutic potential of DMF in treating MS [93], silicosis [203], gout and hyperuricemia [245, 246], colitis [247], and acetaminophen-related drug-induced liver injury [248] (Table 7).

**7.3.3 Necrosulfonamide (NSA).** NSA has been shown to be the first specific inhibitor of GSDMD and directly binds to Cys191 and inhibits oligomerization of GSDMD dimers but does not affect GSDMD cleavage or the initial dimerization of GSDMD [195] (Table 6).

In addition to sepsis [195, 253], NSA has potential in the treatment of acute myocardial infarction [232], allergic rhinitis [233], sepsis-associated encephalopathy [234], inflammatory bowel disease [235], bone fracture [236], systemic sclerosis [237], and developmental sevoflurane neurotoxicity [238] (Table 7).

**7.3.4 NU6300.** NU6300 is a highly specific inhibitor of GSDMD [224] (Table 6) that can mitigate GSDMD-mediated inflammation *in vitro* and *in vivo*. Mechanistically, NU6300 covalently interacts with Cys191 of GSDMD with a vinyl sulfone moiety, effectively blocking GSDMD cleavage and impairing the palmitoylation of FL-GSDMD and GSDMD-NT in THP-1 cells. Furthermore, NU6300

potently inhibits the earlier steps in the NLRP3 inflammasome, indicating a unique feedback inhibition effect on the NLRP3 inflammasome-GSDMD pathway upon GSDMD targeting.

Despite its effects on alleviating DSS-induced colitis and LPS-induced sepsis in mice, the pharmacologic profile of NU6300 is hindered by a narrow safety window for cardiotoxicity and low exposure in the blood [224] (Table 7).

**7.3.5 LDC7559.** Primarily, LDC7559 was identified as the modulators of NETosis. LDC7559 blocks the activity of GSDMD-NT and in turn efficiently inhibits GSDMD-mediated generation of neutrophil extracellular traps as well as pyroptosis [171] (Table 6). In addition, LDC7559 has been shown to suppress neuronal pyroptosis and inhibit microglial activation after subarachnoid hemorrhage [249], which also exhibits neuroprotective effects in mice with traumatic brain injury [250] (Table 7).

However, a study published in 2021 reached a different conclusion, indicating that LDC7559 does not affect GSDMD-dependent pyroptosis [254]. According to this report, LDC7559 or the more potent designed analog, NA-11, inhibits NETosis by inhibiting the NOX2-dependent oxidative burst in neutrophils. Mechanistically, these small compounds function by activating the glycolytic enzyme, phosphofructokinase-1

liver type, and dampening flux through the pentose phosphate pathway to limit cellular NADPH production. As a result, LDC7559 and NA-11 restricts the availability of NADPH, which is crucial for an effective oxidative burst.

**7.3.6 GI-Y1 and GI-Y2.** GI-Y1 was identified as a selective GSDMD inhibitor with cardioprotective effects utilizing hGSDMD-NT as the template of molecular docking. GI-Y1 functions by targeting the Arg7 residue on GSDMD, which is crucial for the lipid-binding and pore-forming activity of GSDMD-NT. GI-Y1 inhibits GSDMD pore formation and suppresses pyroptosis by binding to GSDMD [225] (Table 6). Further studies showed that GI-Y1 also effectively alleviates the progression of atherosclerosis [120] and hypertensive nephropathy [251] (Table 7).

Furthermore, using full-length GSDMD as the template, a novel inhibitor with stronger inhibitory activity, GI-Y2, was screened out. GI-Y2 functions by targeting the Arg10 residue on GSDMD, which reduces the membrane binding activity of GSDMD-NT. GI-Y2 seems to be an effective drug for atherosclerosis, which inhibits ox-LDL-induced macrophage pyroptosis *in vitro* and exhibits a therapeutic effect on the formation of atherosclerotic plaques *in vivo* [226].

**7.3.7 C202-2729.** Based on *in silico* docking studies of compounds against the reported structure of GSDMD, C202-2729, which directly binds to GSDMD-NT, is reported to block GSDMD activation and prevent membrane translocation of GSDMD-NT. Moreover, in a mouse model of sepsis and EAE, this small molecule demonstrates strong anti-inflammatory effects [227].

**7.3.8 Salvianolic acid E.** High-throughput screening identified Danhong injection as a pyroptosis inhibitor, which directly blocks GSDMD-NT oligomerization and pore formation. Danhong injection inhibits cellular pyroptosis in BMDMs and exhibits protective effects in both mouse model of sepsis and diabetic myocardial infarction (Table 7). Further research identifies salvianolic acid E as the major active component, which has a strong affinity for mouse GSDMD Cys192 [228] (Table 6).

**7.3.9 Caffeic acid.** By forming hydrogen bonds with residues located at or near the  $\alpha 1$  helix and  $\beta 1$ - $\beta 2$  loop of GSDMD-NT (including Asp22, Lys52, Tyr55, and Arg54), caffeic acid blocks GSDMD activation (Table 6) and alleviates LPS-induced sepsis in mice [229] (Table 7).

**7.3.10 SCR-1481B1 and cefcapene pivoxil hydrochloride hydrate.** SCR-1481B1 and cefcapene pivoxil hydrochloride hydrate can specifically inhibit GSDMD-NT oligomerization and pyroptosis in a dose-dependent manner by directly targeting key residues at the oligomerization interface I of the GSDMD-NT fragment, including Asn58, Asp63, and Lys145 (Table 6) [230]. SCR-1481B1 and cefcapene pivoxil hydrochloride hydrate

are intrinsically classified as angiogenesis inhibitors and antibiotics, respectively. Thus, SCR-1481B1 and cefcapene pivoxil hydrochloride hydrate may exhibit a synergistic dual-target therapeutic effect (Table 7). In mouse models of tumors, SCR-1481B1 exerts anti-tumor effect by regulating pyroptosis and tumor angiogenesis. In mouse models of sepsis, cefcapene pivoxil hydrochloride hydrate functions by targeting GSDMD and bacterial eradication [230]. Importantly, according to the *in vivo* toxicity assessment, no cytotoxicity or adverse effects were detected in mice treated with these two drugs, which indicates SCR-1481B1 and cefcapene pivoxil hydrochloride hydrate are safe inhibitors.

**7.3.11 VHH<sub>GSDMD-1</sub> and VHH<sub>GSDMD-2</sub>.** The antagonistic GSDMD nanobodies, VHH<sub>GSDMD-1</sub> and VHH<sub>GSDMD-2</sub>, specifically target human GSDMD-NT and inhibit GSDMD pore formation [255]. These nanobodies do not affect GSDMD expression and cleavage nor do the nanobodies interfere with inflammasome assembly. Mechanistically, through binding to an epitope residing in the oligomerization interface of GSDMD-NTD, these nanobodies stabilize monomeric GSDMD-NTD and block the side-to-side assembly of individual NTDs. When administered extracellularly, these nanobodies might enter pyroptotic cells through initial transient GSDMD pores, thereby rescuing cells from pro-inflammatory pyroptosis.

## 7.4 Delivery system for GSDM-targeted drugs

The development of advanced drug delivery systems is imperative to enhance the therapeutic efficacy of GSDM-targeted drugs and overcome the inherent limitations. An ideal delivery system for GSDM-targeted drugs should offer superior cytocompatibility, minimal systemic toxicity or side effects, extended drug retention time, precise targeted delivery, and ease of administration via both intravenous and oral routes, while also maximizing drug utilization (Figure 8). These criteria are essential for optimizing treatment outcomes and multiple delivery systems are being engineered to meet these standards.

For example, to enhance the bioavailability of the hydrophobic DSF, bovine serum albumin (BSA) is utilized to produce safe and long-lasting nanoparticles (BSA@DSF NPs) [243]. To effectively deliver DSF to pulmonary vascular endothelial cells, biocompatible liposomes incorporating "CGSPGWVRC", a lung endothelial cell-targeted peptide, are produced to load the drug [241]. To minimize the side effects of DMF and prolong retention time in the body, poly lactic-hydroxyglycolic acid (PLGA) nanoparticles are used to encapsulate DMF, producing PLGA-DMF [246]. To minimize the cytostatic effects of free NSA and effectively deliver the hydrophobic NSA to phagocytic cells, porous nanoparticles, such as mesoporous silica nanoparticles (MSNs) and porous crosslinked cyclodextrin carriers (CD-NPs), have been utilized to load NSA [256]. Because butyrate

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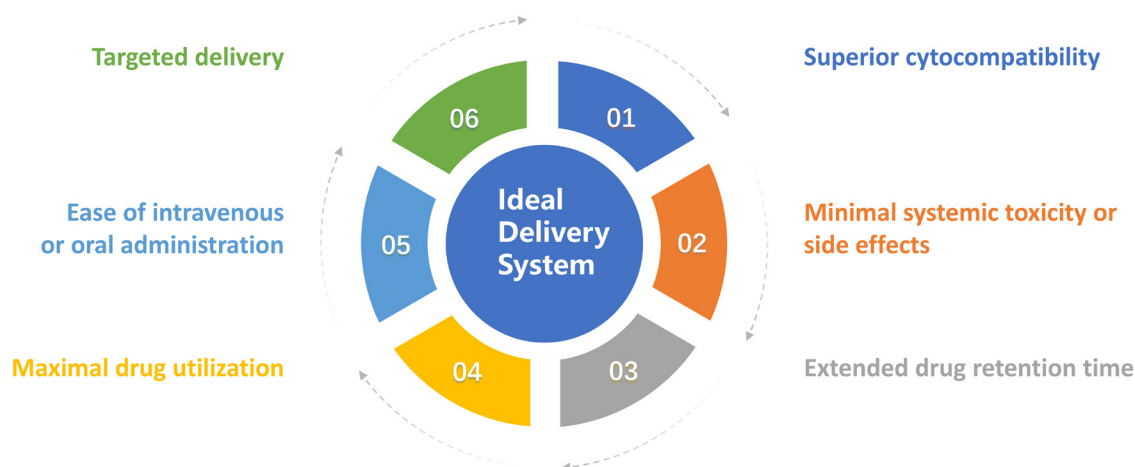


Figure 8 | Objectives of drug delivery system enhancement.

is recognized for the ability to be rapidly and extensively absorbed by enterocytes, 4-octyl-itaconate (4-OI) was encapsulated within butyrate-modified liposomes, producing 4-OI/Blipo to achieve targeted delivery to IECs [124]. DMF was encapsulated with R8-cardiolipin-containing nanoliposomes to precisely release drugs into pyroptotic cells, forming a self-adaptive pyroptosis-responsive liposomes ("RC-NL@DMF"). This liposome targets GSDME-NT-positive pyroptotic cells and exhibits good therapeutic efficacies to alleviate autoimmune inflammatory damage in mice with zymosan-induced arthritis and dextran sulfate sodium-induced inflammatory bowel disease.

### 7.5 Strategies for developing pyroptosis-based anti-tumor drug system

The anti-tumor drug system based on GSDM-mediated pyroptosis generally consists of three principal components (a pyroptosis inducer, a delivery system, and a "trigger" that facilitates the release or activation of a pyroptosis inducer; Table 8).

When these components are precisely delivered to cancer cells, most "triggers" activate the pyroptosis inducer to generate toxic ROS, which induces ROS-dependent pyroptosis [257-264]. This process can be achieved and regulated through various therapies, including photodynamic therapy (PDT) [257-259], sonodynamic therapy (SDT) [260], chemodynamic therapy (CDT) [261, 262], and ion-interference therapy (IIT) [263, 264], all of which are capable of producing ROS to trigger pyroptosis.

Other "triggers" can directly promote pyroptosis by facilitating the release of exogenous GSDM [145, 216] or driving the expression of GSDM within tumor cells [266]. Furthermore, some "triggers" may also release PAMPs or attenuated pathogens, which subsequently activate GSDM cleavage [216, 265], leading to pyroptosis.

These diverse mechanisms demonstrate the great potential of GSDM-mediated pyroptosis as a potent strategy for targeted cancer therapy.

## 8. CONCLUSION AND FUTURE PROSPECTS

Since the discovery of the pyroptotic function of the GSDM family, an increasing number of reports have emerged on the role of GSDMs in regulating important biological processes and contributing to disease progression. Thus, it is essential to determine the specific mechanisms underlying GSDM activation and regulation.

The key to activating GSDMs is to release the pore-forming fragment, which can be achieved through proteolytic cleavage in the linker region or PTMs/residue mutants that impair autoinhibition. Upon activation, pyroptotic cells undergo PMR with involvement of NINJ1, leading to lytic death. The released cellular contents, in addition to the known pro-inflammatory effects, may also function in wound healing.

Three ways to modulate GSDM pore formation are described in this review. First, PTMs affect GSDM activation and pore formation by influencing GSDM auto-inhibition, caspase-mediated enzymatic activation, translocation, and oligomerization. Second,  $\text{Ca}^{2+}$  influx regulates the formation and dynamics of GSDMD pores through calcium-driven phosphoinositide metabolism. Third, ASM- and ESCRT-mediated membrane repair compete with GSDM-mediated membrane pore formation to rescue cells from pyroptosis.

Because GSDMs have an important role in inflammatory diseases, infectious diseases, neurologic disorders, and cancer, researchers are focused on the development of GSDM-specific agonists/inhibitors and have made significant breakthroughs. However, constrained by the intrinsic physicochemical properties of these drugs and the unclear pathogenesis of pyroptosis-related diseases,

**Table 8** | Strategies for anti-tumor drug design based on GSDM-mediated pyroptosis.

| Anti-tumor Drug System                    | Target | Feature                                                                | Pyroptosis inducer                                                                          | Delivery system                                                                         | "Trigger"-mediated preparation for pyroptosis                                                                                                                                                                             | Pyroptosis mechanism                                                                                                                                                                                                                                                             | Refs. |
|-------------------------------------------|--------|------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| PDT<br>DBCO-<br>pYCK6 +<br>SAIE-N3        | GSDME  | Enrichment of PSs on cancer cell membranes via bioorthogonal reactions | SAIE-N3 (type-I AIEgens PSs)                                                                | DBCO-pYCK6: alkaline phosphatase and cholecystokinin-2 receptor dual-targeting peptide) | DBCO-pYCK6 can selectively and rapidly self-assemble on the cancer cell membrane, then bioorthogonally enrich SAIE-N3.                                                                                                    | Upon light irradiation, the membrane-anchored SAIE-N3 effectively generates ROS, which induces GSDME-mediated pyroptosis                                                                                                                                                         | [257] |
| Copper-bacteriochlorin nanosheet (Cu-TBB) | GSDMD  | Spatiotemporal selective PDT                                           | Cu <sup>+</sup> , TBB: 10,15,20-tetra(p-benzoato) bacteriochlorin                           | /                                                                                       | Cu-TBB disassembles due to the reaction between Cu <sup>2+</sup> and the overexpressed GSH in TME, leading to the specific release of Cu <sup>+</sup> and PSs within tumors                                               | Cu <sup>+</sup> drives cascade reactions that produces O <sub>2</sub> <sup>•-</sup> and highly toxic ·OH in cells. TBB can also generate O <sub>2</sub> <sup>•-</sup> and <sup>1</sup> O <sub>2</sub> upon 750-nm laser irradiation. These ROS activate ROS-dependent pyroptosis | [258] |
| TD@COFs                                   | GSDME  | Hypoxia-responsive, imaging-guided cancer immunotherapy                | T-2TM (AIEgen)+DAC (DNA methyltransferase inhibitor)                                        | Azido-based COFs                                                                        | Following hypoxia-triggered degradation of COFs, the fluorescence and photodynamic therapy properties of AIEgen are significantly enhanced, along with controlled release of DAC                                          | DAC upregulates GSDME expression, while the PDT-mediated ROS generation activates the expression of caspase-3, which subsequently cleaves GSDME and executes pyroptosis in tumor cells                                                                                           | [259] |
| SDT CuTA-Ce6                              | GSDME  | TME-driven enzyme cascade reaction, exogenous US-mediated SDT          | CuTA, Ce6 (Chlorin e6)                                                                      | /                                                                                       | In response to the overexpressed GSH and hydrogen peroxide in TME, CuTA-Ce6 nanoneedles are activated through self-destructive degradation, destroying the antioxidant defense system of tumor cells                      | The CuTA nanoneedles with four kinds of enzyme simulation activity (SOD, CAT, POD, and GSH-Px), and the sonosensitizer, Ce6, exhibits an exceptional capacity to generate ROS storm (·OH and <sup>1</sup> O <sub>2</sub> ), which promotes pyroptosis                            | [260] |
| CDT CSC <sub>CPT/SNAP</sub>               | GSDME  | Cascade-amplified pyroptosis inducer, long-lasting immunotherapy       | CPT (ROS producer), SNAP (NO donor)                                                         | CD (cyclodextrin)-Fc (ferrocene) based assembly                                         | CSCCPT/SNAP achieves simultaneous release of CPT and NO through cleavage of disulfide bond and binding to GSH, respectively                                                                                               | Pyroptosis is activated by both endogenous mtROS induced by CPT and exogenous NO released by SNAP: owing to endogenous RNS generated through the rapid reaction between NO and mtROS, which has a longer lifetime; pyroptosis is enhanced and amplified                          | [261] |
| LPZ                                       | GSDMD  | Membrane-anchoring strategy                                            | LGG: <i>lactobacillus rhamnosus</i> GG and PZ: polyamine oxidase-integrated SOD-like ZIF-67 | /                                                                                       | LPZ adheres to cancer cell membranes through interaction between the SpaC pilin subunit of LGG and the mucin of cancer cells. LGG produces lactic acid and gradually creates an optimal catalytic microenvironment for PZ | PZ enables the continuous production of H <sub>2</sub> O <sub>2</sub> within tumors and the bursts of ROS on the cancer cell membrane and within the tumor cells, triggering pyroptosis and eventually killing LGG                                                               | [262] |

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Table 8 | Continued

| Anti-tumor Drug System | Target                                                                    | Feature      | Pyroptosis inducer                                                                                      | Delivery system                                                                  | "Trigger"-mediated preparation for pyroptosis                       | Pyroptosis mechanism                                                                                                                                             | Refs.                                                                                                                                                                                                                                                                                                   |
|------------------------|---------------------------------------------------------------------------|--------------|---------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|---------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| IIT                    | CSE@PP                                                                    | GSDMD        | Biowindow persistent luminescence (PersL), PH-responsive degradation properties                         | H <sub>2</sub> S and Ca <sup>2+</sup>                                            | Polyethylene glycol-poly lactic acid-co-glycolic acid (PEG-PLGA/PP) | Upon degradation within the tumor, CSE@PP constantly releases H <sub>2</sub> S and Ca <sup>2+</sup> , while PersL gradually fades away                           | Released H <sub>2</sub> S and Ca <sup>2+</sup> lead to mitochondrial dysfunction and inactivation of ROS scavenging enzymes, which synergistically facilitate intracellular oxidative stress, thereby inducing caspase-1/GSDMD dependent pyroptosis [263]                                               |
|                        | Phospholipid-coated sodium citrate nanoparticles (PSCT NPs)               | GSDMC, GSDMD | Joint effect of both caspase-8/GSDMC and caspase-1/GSDMD pathway                                        | Sodium citrate                                                                   | Phospholipid-coated sodium citrate nanoparticles                    | After getting into tumor cells through endocytosis, a large amount of citrate ions and Na <sup>+</sup> ions are released into the cytoplasm                      | Citrate ions and Na <sup>+</sup> lead to an increase in intracellular osmotic pressure and a surge in ROS, activating the caspase-1/GSDMD pathway. Excess citrate disrupts the TCA cycle, leading to accumulation of $\alpha$ -KG and activating the $\alpha$ -KG-induced caspase-8/GSDMC pathway [264] |
|                        | Lmo@RBC                                                                   | GSDMC        | Lower systemic inflammatory response, preference for hypoxic TME and high accumulation in tumor         | <i>Listeria monocytogenes</i> without virulence factors                          | Red blood cell membrane                                             | /                                                                                                                                                                | High accumulation of Lmo@RBC in the tumor promotes NADPH oxidase-mediated ROS release and caspase-8 activation, triggering tumor cell pyroptosis [265]                                                                                                                                                  |
|                        | VNP-GD+EI-NP                                                              | GSDMD        | Delivery system based on attenuated <i>Salmonella typhimurium</i>                                       | GSDMD, Ca <sup>2+</sup> chelator BAPTA-AM, bacterial flagellum of VNP20009       | VNP20009, biodegradable dextran nanoparticle                        | Upon activation by the overexpressed GSH in TME, VNP-GD effectively shuttles GSDMD from the protein cage to the cytoplasm of tumor cells                         | GSDMD was cleaved by bacterial flagellum-activated caspase-1, which activates pyroptosis. The calcium chelator EI-NP block Ca <sup>2+</sup> influx-triggered ESCRT III-dependent membrane repair, further promoting pyroptosis [216]                                                                    |
|                        | Phenylalanine Trifluoroborate (Phe-BF3) +NP-GA3                           | GSDMA        | A bioorthogonal chemical system                                                                         | GSDMA3                                                                           | TES-linked NP                                                       | The cancer imaging probe, Phe-BF3, desilicates and cleaves the designed silica ether-containing linker to modulate the specific release of GSDMA3 in tumor cells | GSDMA3 (N + C) was released from NP-GSDMA3, which was capable of activating GSDMA3-mediated pyroptosis [145]                                                                                                                                                                                            |
|                        | HRV2 IRESS-GSDMD <sup>p.D275E/E295G</sup> -F1L <sup>CT</sup> circRNA-LNPs | GSDMD        | Targeting EIF4G2 <sup>+/PTBP1</sup> pan-adenocarcinomas, Targeting mitochondria to inhibit tumor growth | GSDMDENG-F1L <sup>CT</sup> (hGSDMD <sup>p.D275E/E295G</sup> -F1L <sup>CT</sup> ) | Lipid nanoparticles                                                 | Cyclic RNA drives tumor-specific expression of GSDMD mutant, hGSDMDENG-F1L <sup>CT</sup> , which can be cleaved and activated by mitochondrial peptidase PMPCB   | GSDMD-NT targets mitochondria and leads to mtDNA and mtROS leakage, which in turn activates MLKL pathway and mitophagy [266]                                                                                                                                                                            |

AlEgen: aggregation-induced emission luminogens, PSs: photosensitizers, ROS: reactive oxygen species, COF: covalent organic framework, GSH: glutathione, TME: tumor microenvironment, NO: nitric oxide, RNS: reactive nitrogen species, mtDNA: mitochondrial DNA, PDT: photodynamic therapy, SDT: sonodynamic therapy, CDT: chemodynamic therapy, IIT: ion-interference therapy.

most of these agonists/inhibitors are still in the preclinical stage, and more research is needed to apply these drugs in clinical practice. In addition to the pyroptotic function of GSDMs, the non-pyroptotic functions in physiologic and pathologic processes also deserve attention.

As the structures of GSDMA [71]/B [19, 20]/D [70, 72] are successively reported and with advances in the study of GSDM as well as progress in drug design, breakthroughs in the clinical application of GSDM-targeted drug development are likely to be achieved.

#### ABBREVIATIONS

GSDM, gasdermin; NT, N-terminal; CT, C-terminal; NTD, NT domain; FL, full-length; PARPi, poly ADP-ribose polymerase inhibitors; DAMPs, damage-associated molecular patterns; GZMA, granzyme A; GAS, group A *Streptococcus*; GZMB, granzyme B; ROS, reactive oxygen species; mito-ROS, mitochondrial ROS; IL, interleukin; IECs, intestinal epithelial cells; EVs, extracellular vesicle; LDH, lactate dehydrogenase; HMGB1, high mobility group box-1; LPS, lipopolysaccharides; 11,12-EET, 11,12-epoxyeicosatrienoic acid; PMR, plasma membrane rupture; NINJ1, ninjurin-1; TM, transmembrane; EAE, experimental autoimmune encephalomyelitis; CNS, central nervous system; PD, Parkinson's disease; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; ZIKV, Zika virus; DN, diabetic nephropathy; DCM, diabetic cardiomyopathy; bECs, brain endothelial cells; MTB, *Mycobacterium tuberculosis*; TAK1, TGF $\beta$ -activated kinase 1; IKK $\beta$ , I $\kappa$ B kinase  $\beta$ ; PEP, protein expression profiling; TME, tumor microenvironment; CCP, cancer cell pyroptosis; PDAC, pancreatic ductal adenocarcinoma; ESCRT, endosomal sorting complexes required for transport; NETosis, neutrophil extracellular trap formation; PTM, post-translational modification; BMDM, bone marrow-derived macrophages; THP-1, a human monocytic cell line; bGSDM, bacteria GSDM; 4-OI, 4-octyl itaconate; PP1, protein phosphatase 1; NSA, necrosulfonamide; IVDD, intervertebral disc degeneration; MCDs, Mn-containing nanodots; DAG, diacylglycerol; PLC, phospholipase C; PI3K, phosphoinositide 3-kinase; ASM, acid sphingomyelinase; ER, the endoplasmic reticulum; RTN4, reticulon 4; MYO6, myosin 6; DMB, 6,7-dichloro-2-methylsulfonyl-3-N-tert-butylaminoquinoxaline; MeCbl, methylcobalamin; DSF, disulfiram; DTC, diethylthiocarbamate; CuET, bis(N,N-diethylthiocarbamate)copper(II) complex; DMF, dimethyl fumarate; MSU, monosodium urate; IEL, intraepithelial lymphocyte; BSA, bovine serum albumin; PLGA, poly(lactic-hydroxyglycolic acid); aa, amino acids

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#### CONFLICTS OF INTEREST

The authors declare that they have no competing financial interests.

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