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The gasdermin protein family: emerging roles in gastrointestinal health and disease

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Abstract

Since the identification and characterization of gasdermin (GSDM) D as the main effector of inflammatory regulated cell death (or pyroptosis), literature on the GSDM family of pore-forming proteins is rapidly expanding, revealing novel mechanisms regulating their expression and functions that go beyond pyroptosis. Indeed, a growing body of evidence corroborates the importance of GSDMs within the gastrointestinal system, underscoring their critical contributions to the pathophysiology of gastrointestinal cancers, enteric infections and gut mucosal inflammation, such as inflammatory bowel disease. However, with this increase in knowledge, several important and controversial issues have arisen regarding basic GSDM biology and its role(s) during health and disease states. These include critical questions centred around GSDM-dependent lytic versus non-lytic functions, the biological activities of cleaved versus full-length proteins, the differential roles of GSDM-expressing mucosal immune versus epithelial cells, and whether GSDMs promote pathogenic or protective effects during specific disease settings. This Review provides a comprehensive summary and interpretation of the current literature on GSDM biology, specifically focusing on the gastrointestinal tract, highlighting the main controversial issues and their clinical implications, and addressing future areas of research to unravel the specific role(s) of this intriguing, yet enigmatic, family of proteins.

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Author contributions

All authors researched data for and wrote the article. G.P. and N.R. drafted the figures, G.P., A.A. and T.T.P. made substantial contributions to discussion of content, and T.T.P. reviewed/edited the manuscript before submission.

Competing interests

All authors declare no competing interests.

Introduction

Identification of the first gasdermin (GSDM) gene in humans dates back to 2000 (ref.[1]), with five additional paralogue genes identified thereafter[2]. GSDMs were initially associated with a plethora of diseases, including hearing loss[3], asthma[4,5], alopecia[6,7] and cancer[8,9]; however, their specific biological functions remained elusive for over a decade. In 2015, three studies independently described the pore-forming ability of GSDMD, reporting its functional role as the primary effector of pyroptosis[10-12]. The term 'pyroptosis' was first introduced in 2001 to describe a form of regulated cell death, dependent on non-apoptotic caspase-1, that promoted an inflammatory response[13]. The definition of pyroptosis has changed over the last several years, with the assembly of GSDM pores within the cell plasma membrane now considered its hallmark feature[14]. Although this definition has restricted the role of GSDMs as indispensable for pyroptosis, reports demonstrate that they can facilitate other forms of regulated cell death[14] and several non-lytic processes.

The number of studies focusing on the contribution of GSDMs in gastrointestinal pathology is rapidly increasing and GSDMs have been implicated in diseases affecting the gastrointestinal tract, including gastrointestinal-related malignancies, enteric infections and gut mucosal immune-mediated disorders, such as inflammatory bowel disease (IBD). In this Review, we: (1) summarize the current literature about GSDM biology in the gastroenterology field, (2) provide a comprehensive interpretation of these studies, specifically regarding neoplastic, infectious and immune-mediated diseases of the gastrointestinal tract, (3) address the main controversies concerning the role(s) and function(s) of GSDMs in gastrointestinal pathophysiology, and (4) highlight the potential value of GSDMs in the clinical setting as biomarkers and/or therapeutic targets of gastrointestinal-related disorders.

General concepts in GSDM biology

Six paralogue genes are present in humans: *GSDMA, GSDMB, GSDMC, GSDMD*, deafness autosomal dominant (*DFNA*) 5 (also known as *GSDME*) and deafness autosomal recessive (*DFNB*) *59* (also known as pejvakin (*PJVK*)), which share 45% homology in two highly conserved amino-terminal (NT) and carboxy-terminal domains[15]. Beyond the first observations of GSDMD mediating caspase-1-dependent pyroptosis in immune cells, subsequent studies revealed a number of shared and unique features that characterize GSDMs, including their cellular source and/or localization, activation pathways and biological function(s) (summarized in Fig. 1). The general concepts regarding GSDM structure and activation pathways are outlined in Boxes 1, 2, respectively.

Regulated cell death via GSDM pore formation (that is, pyroptosis (Box 2)) is the best-characterized function of GSDMs. The in vitro ability to form pores within liposomal membranes has been demonstrated for the NT domains of GSDMA-E[16], whereas PJVK has lost its pore-forming capabilities, despite retaining a role in inflammation and response to infection[17]. Besides pyroptosis, GSDM pores contribute to other forms of regulated cell death. Caspase-3-mediated GSDME cleavage causes secondary necrosis in apoptotic cells[18]. In neutrophils, cleaved GSDMD participates in inflammatory responses

and/or regulated cell death. Canonical, caspase-1-mediated activation of GSDMD induces neutrophil pyroptosis[19], whereas ELANE-cleaved GSDMD induces lytic neutrophil death during infection[20], thereby exerting a regulatory effect on inflammatory responses or conversely, participating in NETosis[21], possibly by forming nuclear membrane pores and allowing extrusion of DNA fragments that can form neutrophil extracellular traps (NETs)[22]. Furthermore, cathepsin G-cleaved GSDMD promotes inflammatory functions in neutrophils without mediating their death[23]. Proteolytic activation of GSDMs can modulate mitochondrial oxidative stress[24,25], suggesting that they participate in mitophagy. GSDM-dependent mitochondrial permeabilization also promotes regulated cell death: GSDMD-NT induces the release of mitochondrial reactive oxygen species (ROS), which stimulates either pyroptosis (via NLRP3 activation and subsequent GSDMD-mediated pyroptosis)[25] or necroptosis (via mixed lineage kinase domain-like pseudokinase (MLKL) pores)[26]. Finally, in human cells lines, GSDMD-NT and GSDME-NT promote apoptosis by releasing mitochondria-derived caspase 3 (ref.[27]).

GSDMs also regulate the release of intracytoplasmic molecules, which can occur concomitantly with pyroptosis but can also happen in the absence of pyroptosis. GSDM pores are responsible for the unconventional release of IL-1 cytokine family members (for example, IL-1β, IL-18 and IL-33)[2,28-34] and other inflammatory mediators, including ATP and HMGB1 (refs. [35,36]). GSDMD-NT also contributes to IL-1β release from neutrophils by localizing to azurophilic granules and autophagosomes, possibly through vesicle trafficking[37]. Finally, biological functions have been described for some full-length GSDMs (GSDM-FL). In intestinal epithelial cells (IECs), GSDMB-FL translocates to the plasma membrane and regulates proliferation, migration and cellular adhesion in vitro[38], whereas GSDMD-FL participates in the release of IL-1β-containing small extracellular vesicles (sEVs) from IECs ex vivo during dextran sulfate sodium (DSS)-induced colitis, as confirmed in vitro in mouse IECs[39].

Importantly, pyroptosis does not represent an ‘all-or-nothing’ process, and the outcome(s) of GSDM-dependent pore formation can be finely tuned at different levels. Promoter methylation regulates cell-specific GSDM expression[8,40-43], and multiple upstream signalling pathways control GSDM gene expression (Table 1). Proteolysis critically regulates GSDM activity: caspase 3 cleaves GSDMB and GSDMD to produce inactive fragments[44,45], whereas caspase 7 produces inactive GSDMD-NT[44]. Post-translational modification, including phosphorylation[27], oxidation[46], succination[47], palmitoylation[48] and conjugation to itaconate[49], also modulates GSDM activity.

Once GSDM pores are assembled, the imbalanced ionic flow that eventually leads to cell death can be counterbalanced by membrane repair mechanisms through the endosomal sorting complexes required for protein transport (ESCRT) III. The ESCRT machinery, that is activated in response to increases in cytosolic Ca^{2+}, is then recruited to the site of GSDM pores, in which damage is repaired through the budding of pore-containing membranes[50]. This ‘tug-of-war’ between pore formation and membrane repair determines the ultimate fate of the cell (that is, pyroptosis or cell survival). In line with this concept, GSDMD pores are dynamic structures whose open or closed status occurs intermittently, with pore size varying over time[51].

GSDMs in the gastrointestinal tract — call them by their names

GSDMs are implicated in several gastrointestinal disorders, including enteric infections[52], mucosal immune-mediated diseases[2], carcinogenesis and tumour progression[53] (Table 2). Evidence indicates that their contribution can be either dependent on or unrelated to their involvement in inflammation. GSDMs are also involved in two other gastrointestinal-relevant biological processes, coagulation and cell differentiation. In mouse models of sepsis, GSDMD pores in macrophages promoted coagulation via tissue factor release[54] and phosphatidylserine exposure[55]. Additionally, GSDMD-dependent release of neutrophil traps during NETosis can capture platelets and promote clot formation in mice[56]. Whether the role of GSDMD in coagulation might be relevant during hypercoagulatory states associated with gastrointestinal diseases (for example, increased risk of venous thromboembolism during acute severe ulcerative colitis[57]) is conceivable, yet currently unknown. A role for GSDMs in intestinal epithelial differentiation is also postulated based on their differential patterns of expression throughout the human gastrointestinal tract[58]. In the oesophagus and stomach, GSDMA is preferentially expressed in the superficial epithelium, whereas GSDMB is primarily found in the basal region, where stem cells reside. In the oesophagus, GSDMC and GSDMD are predominantly expressed in differentiated and differentiating cells, respectively; conversely, GSDMD is mainly found in differentiated gastric cells[9]. GSDM-dependent regulation of intestinal epithelial differentiation is currently supported by only descriptive observations, however, and functional studies are required to verify this hypothesis.

Current research on GSDMs generally focuses on each family member separately, but the possibility exists that multiple GSDMs are simultaneously involved in a biological process within targeted anatomical regions. For example, *GSDMA* and *GSDMB* expression is observed in the upper gastrointestinal tract, whereas *GSDMB, GSDMC, GSDMD* and *GSDME* are more prevalent in the lower part[1,5,8,9,15,32,59-61]. Although some functions might overlap among GSDM family members, others seem to be decisively different, with even the same result (for example, pyroptosis) mediated by different cellular mechanisms, as discussed below. As such, two approaches can be taken to understand better the roles of GSDMs in the gastrointestinal tract: to evaluate systematically each GSDM separately or to comprehensively consider the family as a whole in the context of a specific biological process. In the following sections, we discuss the unique aspects of each GSDM separately and then address translational studies to frame their functional contributions during specific pathophysiological events.

GSDMA

Genome-wide association studies revealed that polymorphisms in *GSDMA* are associated with susceptibility to skin disorders[62] and IBD[63]; however, little is known about the stimuli and proteases involved in GSDMA activation. Initial observations verified the presence of *GSDMA* in normal gastric and oesophageal human tissues and showed its silencing in gastric and oesophageal cancer cell lines and primary oesophageal and gastric cancer cells, purportedly through promoter methylation[1,8,9], suggesting its potential role as an

oncosuppressor. In addition, TGFβ can upregulate *GSDMA* to induce GSDMA-mediated cell death[8].

The most compelling evidence regarding the function of GSDMA in the epithelium comes from studies on mouse keratinocytes. GSDMA is linked to mitochondrial stress and macroautophagy[24]. A gain-of-function mutation in *Gsdma3* impaired mitochondrial function, with a subsequent increase in ROS that determines autophagy-dependent cell death, indicated by increased LC3-II; similarly, the NT domains of human GSDMA and GSDMD induced LC3-II activation[64]. Some of these results were replicated in HEK293T cells, indicating that such processes could occur in other epithelial cells besides skin cells[24]; however, their relevance in gastrointestinal pathophysiology is unknown. *Streptococcus pyogenes*-derived exotoxin B (SpeB) selectively cleaved GSDMA at its linker region to induce pyroptosis in keratinocytes in vitro[65,66]. *Gsdma1*$^{-/-}$ mice infected with *S. pyogenes* developed less severe skin lesions than wild-type mice but showed more severe systemic infection, suggesting that GSDMA1-mediated pyroptosis might have a role in the development of skin ulcers, but might also be key to confining infection and preventing bacterial dissemination[65]. Conversely, LaRock and colleagues showed that *Gsdma1–3*$^{-/-}$ mice develop larger skin lesions[66]. These two studies disagree on whether SpeB cleaves GSDMA1 or all three mouse GSDMAs, leaving open questions regarding the net effects of GSDMA-mediated pyroptosis upon cutaneous *S. pyogenes* infection. It would be interesting to explore whether GSDMA has a similar role in response to specific gastrointestinal infections in ulcer development.

GSDMB

The roles of GSDMB have remained somewhat elusive until the past few years, as GSDMB shows some peculiarities that distinguish it from other family members. For example, both GSDMB-FL and GSDMB-NT fragments can bind lipid membranes with specific phospholipids (phosphoinositide and glycolipid sulfatide, but not cardiolipin) in contrast to other GSDMs[45]. Another distinctive trait is that *GSDMB* has six splice variants[67]; however, the specific expression and role(s) of each isoform are not yet completely understood. Selective nuclear localization has been reported for isoform 1 (ref.[5]), yet whether GSDMB can function as a transcription factor or has other nuclear functions is not clear. Overexpression of *GSDMB* isoform 1 in airway epithelium correlates with increased levels of mediators implicated in airway remodelling during chronic asthma[5]. Whether the same process contributes to the development of fibrosis in chronic intestinal inflammation is currently unknown.

In vitro experimentation has produced inconsistent results regarding whether GSDMB is activated by inflammatory and/or apoptotic caspases[10,45,68]. Caspase 1 has been found to activate GSDMB-NT, which contributed to the pathogenesis of asthma in a human *GSDMB* knock-in mouse model[69]. Conversely, other studies have found that caspases 1, 3, 6 and 7 mediate proteolytic inactivation of GSDMB[45,68]. Finally, evidence indicates that lymphocyte-derived granzyme A (GZMA) mediates proteolytic activation of GSDMB in human intestinal epithelial cell lines[70].

Increased levels of GSDMB have been documented in patients with IBD[38], and polymorphisms in *GSDMB* have been linked to several chronic inflammatory diseases, including IBD[45]. A role for *GSDMB* in carcinogenesis has also been proposed for several gastrointestinal malignancies, with either pro-tumour or antitumour functions. In oesophageal tumours, *GSDMB* was initially suggested to function as an oncogene[9], but subsequently, expression was found to be reduced in tumours compared with adjacent, uninvolved areas[70]. Furthermore, *GSDMB* in oesophageal tumours can be regulated by promoter methylation[71]. In gastric tumours, GSDMB is either more[72] or less[70] expressed than in adjacent, non-neoplastic tissues. Finally, tissues derived from colorectal cancer (CRC) lesions usually express *GSDMB* at levels comparable to those in tissue from corresponding non-neoplastic areas[70,73]. Interestingly, a differential GSDMB band pattern was observed upon western blotting, comparing involved and non-involved areas from both CRC and diffuse gastric cancer tissues[73]. Collectively, these observations suggest that GSDMB might have different and potentially opposing roles during carcinogenesis.

Regarding function(s), GSDMB-FL promotes non-canonical pyroptosis in THP-1 macrophages by physically interacting with caspase 4 to enhance its proteolytic activity on GSDMD in vitro[68]. Zhou and colleagues described a form of GSDMB-mediated pyroptosis in a colon cancer cell line. Specifically, GZMA, derived from natural killer and/or $CD8^+$ T cells, entered IECs via perforin pores, subsequently generating GSDMB-NT fragments that oligomerized into the plasma membrane and mediated pyroptosis[70]. A subsequent study, adopting a *GSDMB* knock-in mouse model, identified membranes of Gram-negative bacteria as the exclusive target of GZMA-cleaved GSDMB-NT pores[74], sparing host IECs. Finally, non-lytic functions of GSDMB-FL were found in colonocytes: in vitro translocation of GSDMB-FL to the plasma membrane regulated cell proliferation, adhesion and migration through a mechanism involving PDGFA-dependent phosphorylation of focal adhesion kinase[38].

The three-pronged roles of GSDMB in IECs (pyroptotic, bactericidal and pro-restitution) are archetypical of the functional complexity that a single protein can possess within a specific cell. To account for such functional differences, one or a combination of the following explanations can be suggested: (1) GSDMB possesses differential specificity for a precise phospholipid composition within the plasma membrane, enabling it to distinguish between host and bacterial membranes; (2) the functions and membrane-binding capacities of GSDMB vary in an isoform-specific fashion; and (3) partner proteins intervene to facilitate GSDMB docking to membranes, triggering a specific function.

GSDMC

GSDMC has been suggested to have roles as an oncogene in CRC[72] and an oncosuppressor in oesophageal squamous cell carcinoma[9]. *GSDMC* overexpression was observed in CRC tissues compared with normal adjacent areas[75]. In $Apc^{flox/flox}$ mice (a CRC mouse model), *Gsdmc2* and *Gsdmc4* are under negative control of the TGFβ signalling pathway; in these animals, colonic deletion of *Tgfbr2* was associated with upregulated expression of these two *Gsdmc* genes and increased tumour proliferation. *GSDMC* expression in a CRC cell line

correlated with cell proliferation and promoted the growth of cell xenografts implanted in nude mice[75].

Hou and colleagues reported a pro-tumorigenic role for GSDMC in switching regulated cell death from apoptosis to pyroptosis in a breast cancer cell line[76]. Under hypoxic conditions, phosphorylated STAT3 interacted with PDL1 and translocated to the nucleus, where it promoted *GSDMC* transcription. With high expression levels of *GSDMC*, macrophage-derived tumour necrosis factor (TNF) promoted caspase 8-mediated cleavage of GSDMC, whose NT fragments facilitated pyroptosis[76]. It would be interesting to investigate whether such observations can be recapitulated in gastrointestinal cancer cell lines. Furthermore, in a cervical cancer cell line, α-ketoglutarate (αKG) was reported to induce the formation of an intracellular DR6-containing receptosome, which provided a physical platform for the pro-pyroptotic activation of GSDMC, mediated by caspase 8 (ref.[77]). This αKG-induced pyroptosis was also observed in human gastric and CRC cell lines[77].

A role of GSDMC in IEC pyroptosis during a type 2 immune response to helminth infection has also been proposed. HEK293 cells transfected with *Gsdmc2* and infected with *Nippostrongylus brasiliensis* initiated spontaneous cleavage of GSDMC2 and underwent pyroptosis, and mouse intestinal organoids treated with IL-4 or IL-13 showed increased lytic cell death, possibly through a GSDMC-mediated mechanism[78]. Caution should be applied, however, in interpreting these findings as indicating that GSDMC cleavage occurs in organoids, as causal proof of GSDMC-mediated cell death was not shown in this study[78]. Additionally, Zhao and colleagues found that, in the context of type 2 immunity, STAT6 upregulated *Gsdmc2–4* in mouse IECs and GSDMC mediated IL-33 release from goblet cells within intestinal organoids[32].

GSDMD

GSDMD is the primary effector of pyroptosis: its pores cause lytic cell death but also serve as channels for the secretion of inflammatory cytokines[28,30] involved in several inflammatory diseases[79]. ‘Hyperactivation’, described in macrophages[80], dendritic cells[29] and neutrophils[81], is a process in which inflammatory mediators are continuously released concomitantly with GSDMD cleavage, without the cell undergoing lytic death; the stimuli promoting cell hyperactivation seem to differ qualitatively, and not just quantitatively, from those that induce pyroptosis[28], but this issue remains unclear. Membrane repair mechanisms that occur simultaneously with GSDM pore assembly (such as ESCRT[50]) have been suggested to be critical for macrophages to enter the hyperactivation state, but this remains to be proven. Conversely, in neutrophils, GSDMD has been found to be capable of localizing to intracellular organelles selectively and not to the cell’s plasma membrane[37], explaining why the cells can tonically release inflammatory mediators without undergoing pyroptosis. Whether this hyperactivated state might be relevant in gastrointestinal diseases, particularly those characterized by chronic inflammation, such as IBD, is certainly an intriguing possibility.

GSDMD overexpression is observed in IBD[39], and its contribution to disease pathogenesis revolves around its ability to facilitate the release of IL-1 family members. Specifically, GSDMD-associated pores mediate IL-18 secretion[30], whereas chaperoned GSDMD-FL

interacts with NEDD4 to promote the release of sEVs containing IL-1β in a caspase 8-dependent, non-lytic manner[39]. GSDMD pores have also been found to promote IL-33 release from airway epithelial cells[33] and hepatic stellate cells[34] in vitro. Future studies will elucidate if such a mechanism for IL-33 secretion is also pertinent to the luminal gastrointestinal tract. Furthermore, in mice, GSDMD pores regulate exocytosis in intestinal goblet cells by inducing Ca-dependent conformational changes of the cytoskeleton that enable intracellular granules to fuse with the plasma membrane and mucins to be released into the extracellular space without causing pyroptosis[82].

GSDMD has also been suggested to have a role as an oncosuppressor in gastrointestinal malignancies. CRC tissues from 34 patients showed reduced GSDMD levels compared with levels in non-cancerous control tissues, and decreased GSDMD levels correlated with a poor prognosis in patients with CRC ($n = 244$)[83,84]. Gastric cancer cell lines also displayed reduced activity of *GSDMD*, and GSDMD downregulation promoted cell proliferation in vitro[85]. Also, GSDMD was increased in oesophageal squamous cell carcinoma compared to the levels in normal tissue, and metformin induced GSDMD-mediated pyroptosis in oesophageal cancer cell lines[86].

GSDMD can also bind cardiolipin, which is exclusively expressed in the mitochondria of eukaryotic cells and in bacterial walls[87]. Consistently, a role for GSDMD in mitophagy (discussed above) and bacterial killing was observed in vitro. Indeed, recombinant GSDMD-NT showed bactericidal activity against *Listeria monocytogenes, Staphylococcus aureus* and *Escherichia coli*[88]. Furthermore, the release of NETs (discussed above) is dependent on non-canonical GSDMD activation, which can be initiated by either cytosolic bacteria or extracellular-originating lipopolysaccharide (LPS)[22]: NETs have a recognized role in sequestering extracellular pathogens[89], and they also contribute to restraining the dissemination of intracellular bacteria that are released after NETosis[22]. Jorgensen and colleagues described a similar mechanism in pyroptotic macrophages, with the formation of 'pore-induced intracellular traps' (PITs) to sequester intracellular bacteria in vitro[90]. Three studies also showed that caspase 8-mediated proteolysis of GSDMD in macrophages can elicit pyroptosis and promote antimicrobial functions[91-93].

GSDME

In 2017, two groups reported that caspase 3 mediated proteolytic activation of GSDME to induce lytic cell death in vitro[18,94]. As caspase 3 is an apoptotic protease, the possibility exists that GSDME determines the fate of cells when exposed to pro-apoptotic stimuli. In the presence of low GSDME levels, cells progress to apoptosis, whereas high levels can skew cells towards pyroptosis in vitro[95]. Alternatively, Rogers and colleagues showed that GSDME mediated secondary necrosis in vitro; if phagocytes do not promptly remove apoptotic bodies, they can eventually release their intracellular contents via GSDME pores[27]. Caspase 3-activated GSDME can promote apoptosis by targeting mitochondria to facilitate cytochrome *c* release and caspase 3 activation in vitro[27]. Zhang and colleagues found that, in vitro, lymphocyte-derived GZMB cleaved GSDME at the same site as caspase 3 in vitro[96]. Finally, aside from cell death, GSDME is implicated in the secretion of IL-1α (during caspase-8-dependent pyroptosis)[97] and IL-1β (as a complementary and

independent mechanism that can occur without lytic cell death)[98] from macrophages, IL-1β from neutrophils[99], HMGB1 from IECs[36], and IL-18 from gastric cancer cell lines[31].

Increased intestinal *GSDME* has been found in patients with Crohn's disease[59]. In mice treated with trinitrobenzene sulfonic acid (TNBS), caspase 3-dependent GSDME cleavage in IECs induced pyroptosis and the release of inflammatory mediators (that is, HMGB1, IL-1β, TNF and IL-6), thereby promoting inflammation[59]. Furthermore, in the same mouse model, TNF-induced shedding of IECs was GSDME-dependent and under the control of interferon regulating factor 1 (IRF1)[100].

Regarding carcinogenesis, *GSDME* silencing, through methylation of its promoter, was found in CRC[101], with *GSDME* generally considered an oncosuppressor in this setting. Conversely, increased *GSDME* was found in oesophageal squamous cell carcinoma[102] and gastric adenocarcinoma[103] compared with the levels in non-involved areas, with microRNAs contributing to *GSDME* regulation in the context of cancer[104]. The majority of cancer-associated mutations in *GSDME* seem to impair its pore-forming abilities[96]. GSDME can switch regulated cell death from apoptosis to pyroptosis in colorectal[105] and gastric[106] cancer cell lines. Thus, GSDME-mediated cell lysis can reduce tumour burden by killing neoplastic clones[18,94] and promote antitumour functions by immunosurveillance via the release of inflammatory mediators[96]. On the other hand, during experimental colitis, GSDME-dependent release of HMGB1 from IECs promoted carcinogenesis by stimulating cell proliferation[36].

Finally, an in vitro study highlighted the potential importance of GSDME during viral infection[107]. In keratinocytes, the virus-induced shutdown of protein synthesis prompted mitochondrial damage and subsequent caspase 3-dependent GSDME activation, which regulated IL-1α release and pyroptosis[107]. However, whether a similar mechanism might intervene during viral infections of the gastrointestinal tract is currently unknown.

PJVK

As discussed above, PJVK shows a truncated C-terminal domain with no linker region. Its NT domain does not display pyroptotic capacity in vitro[16], and it is currently unknown whether proteolysis is required to activate PJVK. Mutations in *DFNB59* are linked to hearing loss in humans[108]. PJVK localizes to peroxisomes of inner ear hair cells[109], and in response to a sound-induced increase in ROS levels, PJVK recruits LC3B to induce pexophagy, which protects against noise-induced damage[110]. To date, no role for PJVK in gastrointestinal diseases has been described.

The effect of GSDMs on gastrointestinal-related diseases

Cancer

An attempt can be made to distil the role(s) of GSDMs, in the context of gastrointestinal-related cancers, into the following categories: (1) in contributing to tumour-predisposing conditions, tumour development and dissemination; (2) in the GSDM-dependent death of neoplastic cells; (3) in affecting the tumour microenvironment (TME) upon activation; and

(4) as effectors of antineoplastic therapies. Figure 2 illustrates the main roles of epithelial-derived GSDMs in the context of gastrointestinal cancers.

GSDMs promote inflammation and influence the biology of neoplastic cells.— As GSDM pores commonly prompt local inflammation, their activation can be considered a general predisposing factor to tumour development as chronic inflammation increases the risk of malignant transformation[111-113]. Tan et al. described a mouse model of colitis-associated CRC[36] in which IEC-derived HMGB1, produced and released in a GSDME-dependent manner, induced tumour proliferation[36]. A study in a mouse model indicated a more direct role of GSDMs in controlling the proliferation of neoplastic cells. Specifically, in *Apc*$^{flox/flox}$ mice, GSDMC, once deprived of TGFβ-dependent inhibition, induces tumour cell proliferation in CRC[75]. Conversely, GSDMD downregulation promoted cell proliferation in gastric cancer cell lines[85]. Also, the observation that GSDMB-FL mediates IEC proliferation and migration[38] in vitro hints at its potential contribution to the development of gastrointestinal cancers, similar to breast cancer, in which *GSDMB* expression correlates with tumour growth and dissemination[114]. An analysis of GSDM gene expression in cancer revealed a potential role for the differential regulation of *GSDMA, GSDMB, GSDMD* and *GSDME* in CRC; *GSDME* was suggested to positively regulate cell migration and angiogenesis, as opposed to *GSDMA, GSDMB* and *GSDMD* that might downregulate such processes[115]. Overall, these findings suggest that GSDM-dependent regulation of different cell functions might have an important role in the development and progression of gastrointestinal malignancies.

GSDM-pores lead to the death of neoplastic clones and/or facilitate the release of inflammatory mediators.—Caspase 3 or GZMB can activate GSDME to induce pyroptosis of neoplastic cells. *GSDME* is frequently silenced in CRC cells, and rescuing its expression can reduce tumour burden, suggesting the role of GSDME as an oncosuppressor[105,106]. This concept seems to contradict the contribution, as mentioned above, of GSDME pores, to the pathogenesis of colitis-associated cancer[36]. Chronic GSDME activity in otherwise normal IECs might be a stimulus for carcinogenesis, whereas abrupt pyroptosis of neoplastic cells might exert an overall tumour-suppressive effect. Croes and colleagues demonstrated that *Gsdme* deletion in both azoxymethane-treated and *Apc*$^{1638N/+}$ mice did not affect CRC development[116]. The possibility exists that, in mouse models of sporadic CRC, for tumours to evade immunosurveillance, neoplastic cells might need *GSDME* to be silenced. As such, in *Gsdme*-deficient mice, susceptibility to carcinogenesis would not be increased as, even in wild-type mice, *Gsdme* is already functionally absent in their tumours. Also, GSDME levels are increased in oesophageal tumour tissues compared with their levels in healthy adjacent tissues, and its gene expression correlates with a better prognosis[102], which might depend on caspase 3-mediated GSDME cleavage that induces pyroptosis and subsequent clearance of neoplastic cells.

In line with this concept, Zhou and colleagues proposed that GZMA-dependent GSDMB cleavage promotes pyroptotic clearance of neoplastic cells in CRC[70]. Conversely, a study using the azoxymethane-DSS mouse model found that *Gzma* deletion protects from colitis-associated tumorigenesis by reducing the release of pro-inflammatory cytokines from

intestinal macrophages[117]. However, as mice lack a *GSDMB* orthologue, this observation is difficult to reconcile with the findings of Zhou et al.[70]. Possibly, GZMA exerts different effects on the inflamed intestinal mucosa as on the neoplastic epithelium. Finally, αKG-dependent GSDMC-mediated pyroptosis was reported in gastric and colon cancer cell lines[77], but the translational relevance of these findings has yet to be defined.

GSDM-mediated pyroptosis profoundly affects the tumour microenvironment.—Pyroptosis of neoplastic clones can reduce the tumour burden; however, its antitumorigenic effects also include a profound influence on the TME, which is critical in modulating the biology of cancer cells (Box 3). As opposed to 'immunologically silent' apoptosis, pyroptosis is a form of inflammatory cell death[52] in which inflammatory mediators, released either through GSDM pores or as a result of membrane rupture, recruit and activate immune cells with antitumour functions. Zhang and colleagues elegantly showed that GZMB, derived from natural killer and/or cytotoxic T cells, induce GSDME-mediated pyroptosis, which in turn, promotes antineoplastic functions within the TME, whereas *Gsdme* overexpression in cancer cells reduces tumour growth[96]. In *Gsdme*-expressing tumours, tumour-associated macrophages (TAMs) and tumour-infiltrating lymphocytes (TILs) exhibited enhanced immunosurveillance functions, and tumour growth in NOD SCID gamma mice (lacking mature lymphocytes) was more rapid than in wild-type mice, regardless of GSDME presence, suggesting that TILs are necessary for the in vivo antitumour effects of GSDME[96]. Intriguingly, there is also an inherent presence of GSDME-dependent tumour-suppressor function, as xenografted cancer cells devoid of *Gsdme* grew faster in NOD SCID gamma mice than *Gsdme*-expressing cells[96]. Although the researchers in this study reported similar phenotypic observations with a number of other cancer cell lines, their functional and mechanistic studies in vivo utilized a breast cancer model. Hence, caution should be applied in extrapolating this information to gastrointestinal models. In a pan-cancer analysis, increased *GSDME* in stomach adenocarcinoma correlated with infiltration of tumour-associated fibroblasts[103]. Finally, PDL1-dependent, GSDMC-mediated pyroptosis occurred in nude mice xenografted with breast cancer cell lines and was under the control of macrophage-derived TNF[76]. However, this form of GSDMC-mediated pyroptosis in tumour-hypoxic regions supports tumour development and dampens TME antitumour functions[76]. Collectively, these observations demonstrate that TAMs and TILs critically mediate both pro-tumour and antitumour functions through GSDMC and GSDME cleavage, respectively.

Pyroptosis can also occur in tumour-infiltrating immune cells, usually through inflammasome-dependent GSDM activation, as opposed to the observations in neoplastic cells. The contribution of immune cell-derived GSDMs to gastrointestinal malignancies is controversial and has been extensively discussed elsewhere[52,53]. For example, IL-1β and IL-18 (two of the main GSDM-dependent cytokines secreted by macrophages) possess both antitumorigenic and pro-tumorigenic functions, as shown in mouse models of CRC[52], suggesting that their effects are probably time-dependent and cell-specific. As a general rule, the net effect of GSDM activation in infiltrating immune cells of the TME depends on whether it ultimately leads to the prevalence of cells primed towards either immunosurveillance or tolerogenic functions.

GSDMs as effectors of antineoplastic drugs that can modulate their efficacy.—GSDMs mediate some of the effects of anticancer therapies and might also represent potential therapeutic targets. Indeed, several known chemotherapeutic agents induce pyroptosis of neoplastic cells, primarily via GSDME. For example, cisplatin induces GSDME-dependent pyroptosis in oesophageal squamous cell cancer in vitro, with concomitant STAT3β expression enhancing this effect by disrupting the mitochondrial respiratory chain and enhancing ROS levels[118]. Similarly, lobaplatin promoted GSDME-mediated lytic cell death in a CRC cell line[105], and 5-FU triggered caspase 3-dependent GSDME cleavage to mediate pyroptosis in gastric cancer cell lines[106]. Photodynamic therapy also induces caspase 3-dependent and caspase 8-dependent GSDME-mediated pyroptosis in oesophageal squamous cell carcinoma[119]. GSDME was shown to regulate gut radiosensitivity, as radiation can drive caspase 3-dependent GSDME-mediated pyroptosis in IECs[119]. In a homograft mouse model, *Gsdme*$^{-/-}$ CRC cells were less sensitive to radiotherapy, whereas *Gsdme* expression enhanced the number of tumour-infiltrating natural killer cells[120]. Furthermore, radiotherapy was reported to induce GSDME-mediated pyroptosis in a CRC cell line[120]. It could be speculated that such GSDM-dependent mechanisms of action might also be responsible for some of the adverse events associated with antineoplastic treatment, primarily through the induction of pyroptosis in high *GSDME*-expressing normal cells. Proposed strategies to specifically exploit GSDMs in antineoplastic treatment are discussed below.

GSDMs interfere with all phases of cancer, from development to treatment.—Taken together, the contribution of the GSDM family to the pathogenesis of gastrointestinal-related cancers is complex, and their activation can indeed give rise to dichotomous effects. Inflammation sustained by GSDM-dependent processes in IECs can be considered a general tumorigenic stimulus, whereas induction of inflammasome-dependent pathways in immune cells, culminating in GSDM activation, can result in more ambiguous effects. On the other hand, GSDME-mediated pyroptosis of neoplastic clones seemingly has a net tumour-suppressive effect via direct reduction of tumour burden and promotion of TME-dependent antineoplastic functions, whereas the effects of GSDMC-mediated pyroptosis on the TME promote tumour growth. Stimulation of pyroptotic cell death is one of the mechanisms by which antineoplastic treatments exert their functions; however, low GSDM expression might potentially mediate resistance to specific mechanisms of action of antineoplastic agents. Finally, non-pyroptotic GSDM-dependent functions, mostly related to control of the cell cycle, might also contribute to tumour growth and dissemination.

Enteric infections

Evidence shows that GSDMs are activated in response to food-borne pathogens, including *Salmonella, Shigella* and *Yersinia* bacteria. Figure 3a shows the functions performed by gut mucosa-derived GSDMs in response to local infection. In this regard, two main issues need to be considered: (1) activation of GSDMs in intestinal haematopoietic versus epithelial cells, and (2) their response to extracellular versus intracellular pathogens.

GSDMs as key effectors of antibacterial functions in haematopoietic cells.—Inflammasome activation was crucial in inducing GSDMD-mediated pyroptosis and

IL-1β release in bone marrow-derived mouse macrophages upon infection with *E. coli, Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Shigella flexneri*[11]. An in vitro bactericidal effect of GSDMD against several bacteria, including *E. coli*, was also found[16,88], but its biological relevance in vivo has yet to be determined[16]. It was proposed that GSDMD-dependent formation of PITs contributes to controlling intracellular infections, with cellular debris trapping intracellular pathogens, such as *S. Typhimurium*, which neutrophils could subsequently kill via efferocytosis[90]. Thurston and colleagues reported that intracellular growth of *S.* Typhimurium was restricted by caspase 1 and caspase 11 activity in mouse macrophages, independent of GSDMD activity[121]. Conversely, Zuo and colleagues reported that GSDMD-mediated pyroptosis of macrophages in the caecum of mice infected with *S. Typhimurium* protected against bacterial dissemination. The researchers observed that *S.* Typhimurium-derived *Salmonella* plasmid virulence C inhibited pyroptosis, which correlated with reduced intestinal inflammation, but increased bacterial dissemination and liver damage[122]. GSDM-mediated cell lysis might be a redundant mechanism to further disrupt the niche for intracellular pathogens and, at the same time, promote immune cell recruitment via the release of inflammatory mediators to fight against infecting microorganisms. Extracellular pathogens also activate GSDMD-mediated defence responses. In vitro, the Shiga toxin 2-LPS complex from extracellular enterohaemorrhagic *E. coli* (EHEC) activated GSDMD in macrophages via the non-canonical inflammasome pathway to induce mitochondrial damage, ROS production, and subsequent pyroptosis and release of IL-1β[25]. However, the in vivo pathophysiological consequences of such a mechanism have not yet been explored. Notably, other studies found that Shiga toxin alone (that is, not in complex with LPS)[123] and subtilase cytotoxin[124] (another EHEC-derived virulence factor) suppressed caspase 11-dependent inflammasome activation in mouse macrophages.

Studies on yersiniosis have uncovered another relevant GSDMD pathway. *Yersinia pseudotuberculosis*-derived *Yersinia* outer protein J (YopJ) blocked TAK1 to trigger receptor-interacting serine–threonine-protein kinase 1 (RIPK1)-dependent, caspase 8-mediated GSDMD and GSDME cleavage in mouse macrophages[91,92]; however, in this scenario, a pathophysiological role has not been identified for GSDME in macrophages[92]. Yet, human macrophages are resistant to cell death induced by TAK1 inhibition, suggesting that they might have evolved a mechanism to escape this form of cell death[92]. Caspase 8-dependent GSDMD cleavage has been proven to provide anti-*Yersinia* defence in vivo; furthermore, concomitant caspase 3-mediated GSDMD inactivation also occurs, which is protective against *Y. paratuberculosis* infection in vivo, suggesting that tight control over GSDMD activity is fundamental during *Yersinia* infection[93].

An important role in fighting infections has also been described for neutrophil-derived GSDMs. Caspase 11-dependent, GSDMD-mediated NETosis control *Salmonella* ΔsifA (a mainly cytosolic strain) infection in mice[22]; conversely, canonical inflammasome pathways, via caspase 1-mediated cleavage, reportedly control GSDMD-dependent pyroptosis and IL-1β secretion in neutrophils during *S.* Typhimurium infection in mice, but the in vivo implications of such observations have not yet been explored[19]. *Gsdmd*$^{-/-}$ mice are protected against *E. coli* infection, as they exhibit enhanced bactericidal activity attributed to a reduction in GSDMD-dependent neutrophil death[20]. It is plausible that GSDMD-

dependent neutrophil death dampens the host's defence against extracellular pathogens by reducing the number of active neutrophils recruited to the infection site, whereas in the presence of bacteria capable of intracellular replication, NETosis has a positive effect. Finally, during *Yersinia* infection, RIPK1 (besides triggering GSDMD-mediated pyroptosis in macrophages) activates neutrophil-derived GSDME to mediate pyroptosis and IL-1β release, notably without NET exclusion. In this model, IL-1β blockade increased bacterial burden in the spleen and liver of *Gsdme*$^{-/-}$ mice[99].

Epithelial-derived GSDMs grapple between bactericidal and pyroptotic functions.—Hansen and colleagues reported that in IECs, GSDMB-NT cleaved by natural killer cell-derived GZMA selectively formed pores in membranes of Gram-negative bacteria, thereby exhibiting direct bactericidal capabilities, sparing host cells[74]. The researchers found that the Gram-negative bacterium, *S. flexneri*, has evolved a mechanism to escape GSDMB-mediated killing through bacteria-derived ubiquitin ligase IpaH7.8 that promoted GSDMB ubiquitination and subsequent proteasomal degradation[74]. Likewise, *S. flexneri*-derived IpaH7.8 targeted human GSDMD in IECs to promote its proteasomal degradation. GSDMD mediated IEC pyroptosis with protection against infection, but no direct targeting against the pathogen was detected[125]. Such observations suggest that different GSDMs might have evolved to exert simultaneous yet separate and complementary functions in IECs during intracellular infections.

Specific functions have also been described for epithelial GSDMD in response to *Salmonella* infection. Activation of the NAIP–NLRC4 inflammasome prompted contraction, pyroptotic cell death and extrusion of IECs. In mice, GSDMD sublytic pore formation was required to mediate epithelial contractions that allowed packing of IECs in specific foci, but their expulsion did not appear to be GSDMD-dependent, with controversial findings regarding the ability of GSDMD to mediate pyroptosis in this setting[126,127]. IEC expulsion is a crucial, epithelial-inherent process to restrain *S.* Typhimurium infection that depends on NAIP–NLRC4 (ref.[128]) and caspase 11 (ref.[129]), and coincides with other defence responses[130]. Evidence suggests that extruding cells display both apoptotic and pyroptotic features[131]. The observation that GSDMD is proteolytically activated during such processes, which is in line with the concept that inflammasome pathways activate GSDMs, reinforces the concept that different defence mechanisms are contemporaneously active during infection and interact with each other, but also provokes the need to investigate further how these processes are orchestrated by the cell. Enteropathogenic *E. coli* also induces GSDMD-mediated IEC pyroptosis; however, whether such a process promotes or is protective against *E. coli* infection has not yet been explored[132].

Do GSDMs contribute to shaping the gut microbiota?—Extensive studies have shown a relationship between inflammasome activation and the gut microbiota (reviewed elsewhere[133]), but the specific effect of GSDMs has only been investigated in one study. *Gsdmd*$^{-/-}$ mice fed a high-fat diet displayed more severe colonic inflammation, gut dysbiosis and systemic endotoxaemia than *Gsdmd*-expressing controls fed the same diet, and GSDMD-NT exerted direct bactericidal effects on bacteria of the Proteobacteria phylum[134]. Furthermore, GSDMD was important for mucus secretion from goblet cells in mice,

and *Gsdmd* deficiency was associated with increased bacterial attachment to the colonic epithelium, rendering mice more susceptible to *Citrobacter rodentium* infection[82].

Role of GSDMs during infections with non-bacterial pathogens.—Studies investigating the contribution of GSDMs to non-bacterial gastrointestinal infections are sparse. In macrophages in vitro, *Entamoeba histolytica* independently induced activation of both caspase 1, which promoted pro-IL-1β maturation, and caspase 4, and mediated proteolytic GSDMD activation, resulting in macrophage hyperactivation[135,136]. Currently, a role of GSDMC in worm infection has been proposed. In a mouse model of *Nippostrongylus brasiliensis* infection, hyperactivated tuft cells recruited type 2 innate lymphoid cells, which upregulated *Gsdmc2* in IECs, leading to their lytic death[78]. Similarly, Zhao and colleagues confirmed the importance of GSDMC in helminth infection, showing that *Gsdmc1–4*$^{\Delta IEC}$ mice did not develop hyperplasia of goblet and tuft cells and displayed reduced responses against *Heligmosomoides polygyrus* owing to the lack of GSDMC-dependent IL-33 secretion[32].

Evidence is scarce regarding the role of GSDMs in various viral infections, wherein pyroptosis is suggested to exert either detrimental or favourable functions[137]. In mouse IECs, rotavirus infection elicited NLRP9B-dependent GSDMD activation and subsequent IL-18 release upon pyroptosis, with purportedly protective effects[138]. Conversely, a pathogenic role for GSDMD was proposed in norovirus gastrointestinal infection in mice by inhibiting NLRP3-dependent, GSDMD-mediated pyroptosis of macrophages[139]. GSDMs were also implicated in the response against enterovirus 71 (EV71). Caspase 3-mediated activation of GSDME occurred in epithelial cells infected with EV71 in vitro, and *Gsdme*$^{-/-}$ mice showed less severe disease symptoms than controls[140]. Conversely, GSDMD was cleaved in vitro by the viral protease 3C to form an inactive NT fragment[141]. NLRP3 inflammasome[142] and caspase 1 (ref.[143]) contribute to the defence against EV71; it is plausible that they serve to produce mature IL-1β and IL-18, whose secretion is augmented during EV71 infection[143], but they cannot generate active GSDMD-NT owing to viral counteractive mechanisms in play. GSDM activation also occurs concomitantly with fungal infections, particularly *Candida albicans* and *Aspergillus fumigatus* infections[144]. However, whether this is relevant in the gastrointestinal system has not yet been explored.

GSDM-dependent pore formation is paramount in controlling infections.—Overall, evidence suggests that GSDM pores have important roles in controlling bacterial infections of the gastrointestinal tract via different mechanisms. This evidence is in line with evolution studies showing that, in the subkingdom Metazoa, GSDMs evolved under the selective pressure of responding to infections[17,145,146]. First, GSDMs exert a direct bactericidal effect (that is, pore formation within bacterial membranes) against both intracellular and extracellular microorganisms in vitro; however, the in vivo relevance of this direct killing was established only for the intracellular pathogen, *S. flexneri*[74]. Second, pyroptosis of infected cells disrupts the niche for intracellular pathogen replication. Third, GSDM pores are implicated in other mechanisms that restrict pathogen replication and dissemination, such as the formation of NETs and PITs. Finally, lytic cell death mediates local inflammatory damage but might also be critical for immune cell recruitment to contain

the infection and prevent systemic dissemination. Evidence supports the involvement of GSDMs in non-bacterial infections, but their precise functions and roles have yet to be elucidated in the gastrointestinal system.

Inflammatory bowel disease

GSDMs are gaining increasing attention in the IBD field as key mediators of host allostatic responses that sustain chronic intestinal inflammation, as summarized in Fig. 3b. Several single-nucleotide polymorphisms (SNPs), mostly in GSDMB[38,45,147], but also in *GSDMA*[63,148], *GSDMD*[147] and *GSDME*[147], are associated with the genetic susceptibility to IBD. Furthermore, GSDMs participate in innate responses in immune and epithelial cells and have reciprocal influences on the host gut microbiota.

IEC-derived GSDMs at the crossroads of repair and pyroptosis during intestinal inflammation.—Rana and colleagues reported that GSDMB was increased in IBD, particularly in ulcerative colitis, and made the compelling observation that GSDMB-FL translocation to the plasma membrane promoted epithelial restitution in vitro without causing pyroptosis[38], supporting an overall role for GSDMB-dependent wound repair. Notably, dichotomous roles have been ascribed to GSDMB in IECs, particularly GZMA-dependent pyroptosis and epithelial proliferation. It could be speculated that GSDMB is crucial to determining IEC fate and that its function depends on the specific pathway that is activated. In addition, it has yet to be established if GSDMB cleavage contributes to IBD pathogenesis by enhancing intestinal inflammation and if the full-length protein encoded by *GSDMB* variants preferentially facilitates, or deters, cleavage. Preliminary data suggest that GSDMB-mediated IEC pyroptosis might promote intestinal inflammation in Crohn's disease and that disease-associated *GSDMB* SNPs might facilitate or prevent cleavage, with pathogenic or protective effects, respectively[149]. These competing functions might underlie the different pathogeneses associated with ulcerative colitis and Crohn's disease and/or the different anatomical regions affected within the gastrointestinal tract, as Crohn's disease is most commonly found in the terminal ileum and ulcerative colitis is restricted to the colonic mucosa. Future studies should clarify these issues.

GSDMC has been implicated in the promotion of colonic inflammation; however, DSS-induced colitis in $Gsdmc^{\Delta IEC}$ mice did not show differences in the severity of inflammation compared with that in wild-type controls, possibly due to low baseline expression of GSDMC in controls. Conversely, when wild-type and *Gsdmc*-deficient mice were pre-infected with *H. polygyrus* (which normally upregulates GSDMC expression in IECs), $Gsdmc^{\Delta IEC}$ mice displayed enhanced recovery. These results support the concept that epithelial GSDMC might promote the pathogenesis of IBD, but only if additional environmental factors intervene to upregulate its expression, therefore suggesting that intestinal pathogens might affect whether and how specific GSDMs contribute to IBD pathophysiology. Furthermore, $Il10^{-/-}$ colitic mice displayed increased expression and cleavage of GSDMC and *Gsdmc* deficiency in this model significantly ($P < 0.001$ for weight loss, $P < 0.01$ for colon length) mitigated DSS-induced colitis[32], providing further support for a pathogenic role of GSDMC in IBD.

GSDME has also been suggested to have a role in the pathogenesis of Crohn's disease. Tan and colleagues observed that *Gsdme*$^{\Delta IEC}$ mice were protected from TNBS-induced colitis[59]. Mechanistically, caspase 3-mediated GSDME cleavage determined pore formation and pyroptotic cell death associated with HMGB1 release, which actively contributed to intestinal inflammation, as the administration of anti-HMGB1 antibodies alleviated colitis in wild-type mice. HMGB1 blockade was not sufficient to completely recapitulate the effects of *Gsdme* deficiency on histological evaluation, suggesting that other GSDME-dependent functions might also contribute to colitis in this model[59]. In the same mouse model, the researchers also found that TNF stimulation induced GSDME-mediated pyroptosis in IECs by caspase 3-mediated cleavage under the control of IRF1 (ref.[100]).

The controversial role(s) of GSDMD in the pathogenesis of IBD.—Although several studies have detected increased levels of *GSDMD* in patients with IBD, animal studies have found conflicting results concerning its contribution to intestinal inflammation. Specifically, *Gsdmd*$^{-/-}$ mice were either protected from DSS-induced colitis[39] or displayed a more severe phenotype than wild-type control mice[150], with controversy also surrounding whether epithelial or immune-derived GSDMD exerts a prominent role during intestinal inflammation. Using a model of acute DSS-induced colitis, Bulek and colleagues showed non-pyroptotic release of sEVs containing IL-1β (via chaperoned GSDMD-FL)[39] from IECs ex vivo, whereas another group observed pyroptotic release of IL-18, which promoted colitis by driving goblet cell loss in vivo[30]. A specific role of GSDMD was also found in a mouse model of ileitis[151], in which caspase 8 promoted ileal inflammation by inducing GSDMD-dependent IEC death, independently from MLKL-mediated necroptosis (which also contributed to the development of intestinal inflammation in this model)[151]. This study suggested a role for GSDMD-mediated pyroptosis in ileitis and underscored the importance of crosstalk between different programmes of regulated cell death during intestinal inflammation. Collectively, these data strongly support the concept that GSDMD activation in IECs mediates intestinal inflammation.

Aside from IECs, GSDM-expressing myeloid cells also contribute to the pathogenesis of IBD. Ma and colleagues showed that selective deletion of *Gsdmd* in myeloid cells exacerbated DSS-induced colitis in mice, independent of gut microbiota[150]. The researchers did not detect a difference in colitis phenotype between *Gsdmd*$^{-/-}$ and *Gsdmd*$^{\Delta IEC}$ mice[150], which suggests that the main contribution to colitis comes from non-epithelial GSDMD. Conversely, two groups found that selective deletion of upstream regulators of GSDMD in macrophages, namely NEK[152] and TRIM2 (ref.[153]), conferred protection against experimental colitis in mice by restricting GSDMD-mediated pyroptosis.

The global effects of GSDMD activation during the pathogenesis of IBD seem to be multifaceted and probably depend on the specific pathway(s) and timing of activation. Although some of the results described above are difficult to reconcile, three main conclusions can be drawn: (1) GSDMD has an active role in colitis and ileitis, (2) IEC-derived GSDMD seems to promote intestinal inflammation via both cell death and secretion of inflammatory mediators, and (3) GSDMD activation in macrophages might have a subtle and context-dependent role in intestinal inflammation.

GSDMs orchestrate inflammation in the gastrointestinal tract.—Overall, it is plausible that GSDMs exert dichotomous roles during IBD pathogenesis. In IECs, GSDM-mediated pyroptosis and/or release of inflammatory mediators promote intestinal inflammation[30,32,36,59,151]. Furthermore, the crosstalk between pyroptosis and other programmes of regulated cell death involving GSDMs is highly probable. So far, a purported protective role for IECs is described only for GSDMB[38]. It would be interesting to explore if other GSDMs share similar functions, or if this phenomenon is unique to GSDMB-FL, perhaps through its ability to bind lipid membranes. Regarding GSDMD in myeloid cells, the ambiguous results for its contribution to intestinal inflammation[150,152,153] suggest that fine-tuning and regulatory mechanisms are in play and are probably more relevant than GSDMD activation. In this regard, one interesting question pertains to the potential role of cell hyperactivation in chronic intestinal inflammation. As pyroptotic cells release inflammatory mediators but eventually die, therefore ceasing their secretory activity, it is conceivable that hyperactivated cells contribute to the self-sustaining, non-resolving inflammation observed in conditions such as IBD. Future research should focus on the various physiological and pathophysiological stimuli that determine which cells express a specific GSDM, what particular function is specific for each GSDM, and, importantly, what is the net outcome of their activation in both IECs and myeloid cells.

Clinical insights into GSDMs in the gastrointestinal tract

The body of knowledge on GSDMs in gastrointestinal health and disease is rapidly expanding; as such, the logical extension of these findings is to leverage such discoveries into developing clinically useful tools for the management of patient care. Two main areas of such application can be identified: (1) using GSDMs as biomarkers for patient monitoring and/or precision medicine, and (2) exploiting GSDM biology to design effective therapeutics.

GSDMs as biomarkers for disease states

Several studies have shown a correlation between the expression of one or more GSDM and clinical outcome(s) in patients with cancer. Two main approaches are proposed: evaluating the expression of specific GSDMs and attempting to build multi-parametric scores including pyroptosis-related genes[154]. In rectal adenocarcinoma, high *GSDMD* expression correlated with a better prognosis and enhanced infiltration and activation of immune cells in the TME[155]. Differences in GSDMD immunolocalization patterns in epithelial cells of 178 patients with CRC correlated with both prognosis (cytoplasmic GSDMD associated with favourable outcomes and nuclear GSDMD with deeper infiltration depth) and TME composition (membranous GSDMD correlated with enrichment of $CD68^+$ macrophages and $CD8^+$ T cells and nuclear GSDMD with $CD3^+$ cells)[156]. A specific methylation pattern in *GSDME* (involving two CpG islands) accurately discriminated CRC from normal colon, suggesting its potential use as a biomarker for CRC detection through blood-based liquid biopsy screening[157]. In signet ring cell gastric carcinoma, expression of intracellular *MUC20* variant 2 correlated with a reduction of apoptosis and GSDME-mediated pyroptosis in cancer cells and with resistance to cisplatin and paclitaxel[158]. In HER2-positive breast cancer, *GSDMB* expression correlated with a poor prognosis and reduced response to anti-

HER2 therapy[159]. As HER2 can also be overexpressed in gastric tumours and trastuzumab (anti-HER2 monoclonal antibody) is used for the treatment of HER2-positive gastric and gastroesophageal cancers[160], it is conceivable that such associations might also be present in gastrointestinal malignancies. Zeng and colleagues identified a signature of five pyroptosis-related genes that predicted the prognosis in 65 patients with oesophageal adenocarcinoma. Specifically, regarding GSDMs, high *GSDMB* correlated with improved survival[71].

Besides cancer, little is known regarding the potential application(s) of GSDMs as biomarkers in other diseases, namely, infections and immune-mediated diseases. For instance, elevated levels of GSDMD-NT in circulating plasma microparticles were observed in 26 patients who were critically ill with sepsis, but no significant correlations were found with clinical and clinically relevant biochemical parameters[161]. Regarding gastrointestinal pathologies, the first issue to address would be whether GSDM levels should be investigated explicitly within the gut mucosa or if systemic serum levels might also serve the same purpose. The potential applications span from predicting disease course to precision medicine to tailor specific treatment modalities. Given that most GSDMs require proteolysis to be activated, the levels of NT fragments are likely to be more relevant as biomarkers. An alternative argument could be made for GSDMB, as both full-length and cleaved NT forms are biologically active and exert opposing functions in IECs. In this context, it might be more beneficial to measure the ratio between NT and full-length forms to assess which process prevails (that is, pore-forming or wound healing function(s)). Furthermore, increasing evidence indicates that multiple GSDMs are probably simultaneously expressed in the same tissues and perhaps even within the same cells. Thus, the relative expression of each GSDM compared to other family members might also correlate with clinically meaningful outcomes.

Therapeutic potential of GSDMs in cancer

Anticipation is mounting regarding the potential to manipulate GSDM-mediated pyroptosis for the treatment of specific malignancies. In general, pyroptosis could be exploited in two ways: inducing proteolytic activation of endogenous GSDMs or selectively introducing exogenous GSDMs into cancer cells.

The possibility of combining different therapies to enhance the antitumour effects of GSDM-mediated pyroptosis is intriguing, with preliminary evidence supporting this potential concept. Decitabine restored *GSDME* expression in CRC cells via demethylation of its promoter[94], thereby enhancing the efficacy of chemotherapeutics that act via a GSDME-dependent mechanism. The combination of BIX 01294 (a methyltransferase inhibitor) and cisplatin induced autophagy-dependent GSDME-mediated pyroptosis in gastric cancer cell lines transferred into nude mice[162]. Furthermore, the PLK1 inhibitor, BI2536, synergized with cisplatin to induce GSDME-mediated pyroptosis in mice xenografted with human oesophageal squamous cell carcinoma cells[102]. LPS enhanced GSDMD expression in HT-29 cells, making them more sensitive to oxaliplatin-induced pyroptosis[84]. Tumour grafts of mouse colon carcinoma cells that expressed human GSDMB showed an increased response to anti-PD1 therapy[70], suggesting a link between pyroptosis and immune checkpoints that might be exploited for future therapies against colon cancer.

Other pharmacological modulators not routinely used for oncology treatments might also be employed to enhance GSDM-mediated pyroptosis in cancer. For instance, famotidine, an antihistamine H2 receptor, induced GSDME pore assembly in gastric cancer cells, with subsequent IL-18 release and pyroptotic cell death[31].

It should also be noted that chemotherapy-induced GSDM-dependent pyroptosis might be responsible for some of the adverse events associated with antineoplastic treatment. For example, doxorubicin and cisplatin, which are used for the treatment of gastric cancer and CRC, respectively, induced caspase 3-dependent GSDME-mediated pyroptosis of kidney tubular epithelial cells, both in human cell cultures and in mice, which partially accounts for the nephrotoxicity associated with such therapies[163]; similarly, cisplatin induced GSDME-dependent weight loss and tissue damage in mice[94]. Experiments in mice suggest that the vitamin D–vitamin D receptor pathway might mitigate such pyroptosis-related adverse effects[164,165]. Furthermore, excessive pyroptosis of neoplastic cells resulted in the development of detrimental systemic inflammatory syndromes, such as that described for chimeric antigen receptor T cell therapy that activated GSDME-mediated pyroptosis of tumour cells, but also GSDMD-mediated pyroptosis of macrophages leading to cytokine release syndrome in a mouse model[166].

In this regard, selective targeting of neoplastic cells could prove to be extremely beneficial to maximize treatment effectiveness and, at the same time, reduce potential deleterious adverse effects. Xiao and colleagues proposed a sophisticated approach based on a designed nanoprodrug that enhanced the cancer-targeted distribution of paclitaxel, which then activated GSDME-mediated pyroptosis via chemophototherapy in mice[167]. In this study, the researchers showed that the efficacy of this approach was dependent on the ability of GSDME-mediated pyroptosis to evoke an antitumour response in the TME, presenting the advantage of avoiding potential adverse effects related to diffuse GSDME-dependent cell lysis. A different strategy was proposed by Liu and colleagues, who used an adenovirus to deliver apoptin, a protein of the chicken anaemia virus, to target HCT116 cells, a human CRC cell line. This strategy induced GSDME-dependent pyroptosis via the mitochondrial pathway through caspase 3 and caspase 9 activation, and in a tumour xenograft model in mice, significantly reduced tumour growth ($P < 0.0001$ for both tumour volume and weight)[168].

A different argument can be made for those malignancies in which GSDMs seemingly exert tumour-promoting functions. For example, the delivery of a monoclonal antibody targeting GSDMB in HER2-positive breast cancer cells was proposed as a therapeutic strategy. This approach led to reduced GSDMB-dependent cell migration and enhanced sensitivity to trastuzumab (anti-HER2 therapy) in vitro, as well as reduced tumour growth and lung metastases in xenograft mouse models[169]. Similarly, preliminary findings showed that GSDMB mediated resistance to anti-HER2 therapy, which upregulated GSDMB expression in HER2-expressing breast and gastric cancer cells[170]. This phenomenon occurred through a mechanism that involved the interaction of GSDMB-NT and LC3B (an autophagy protein), which upregulated protective autophagy-dependent functions[170]. Interestingly, concomitant blockade of autophagy pathways increased the efficacy of anti-HER2 therapy in a mouse model of xenograft breast cancer[170].

Direct delivery of GSDMs has also been proposed for the treatment of malignancies. Wang et al. developed a biorthogonal system that allowed the targeted intracellular release of active GSDMA3 (GSDMA3-NT) in breast cancer cells[171]. In this study, GSDMA3-mediated pyroptosis in about 15% of 4T1 cells comprising the tumour graft was sufficient to induce clearance of the entire mass, further reinforcing the concept that the anticancer effects of pyroptosis, besides lytic death of cancer cells, is conferred through immune activation to mount specific antineoplastic responses[171].

Therapeutic potential of GSDMs in non-malignant diseases

Pharmacological modulation of GSDM activity during intestinal inflammation, either owing to infection or immune-mediated conditions, also represents a tantalizing possibility. Chemical inhibitors and activators of GSDMs are extensively reviewed elsewhere[172], and their discussion goes beyond the scope of the present Review. However, briefly, small molecules can target specific residues of GSDMs to modulate their cleavage[47] or oligomerization[173,174]. In this regard, it is worth mentioning that, in a preliminary study utilizing a mouse model of LPS-induced sepsis, disulfiram, a drug approved for the treatment of alcohol addiction that inhibits GSDMD oligomerization, either prevented or delayed lethality when administered before or right after LPS injection, respectively[174]. Furthermore, a study showed that the administration of disulfiram-loaded lactoferrin nanoparticles in mice had a protective effect against DSS-induced colitis; curiously, however, the researchers did not investigate GSDM cleavage and pore formation[175]. Caution should be applied when interpreting the results from this study as, although a trend towards increased efficacy was observed with disulfiram-loaded particles, it did not reach statistical significance when compared with treatment with free nanoparticles, therefore leaving some ambiguity as to whether disulfiram would provide a therapeutic benefit in IBD.

Overall, GSDMD inhibition seems to exert its therapeutic effects by alleviating pyroptosis-dependent inflammation. Nonetheless, considering the importance of appropriate inflammatory responses in counteracting local infections and the more than often contradictory results regarding the roles of GSDMs in IBD, caution should also be applied in assuming that the mere inhibition of GSDMs results in the dampening of intestinal inflammation. The bactericidal effects of GSDMs also need to be considered, especially with intracellular pathogens. In this regard, GSDM inhibition might be detrimental, as it might impair the host’s defence against infection with pathogens. As such, it is tempting to speculate that an appropriate strategy would be to either induce activation of endogenous GSDMs skewed towards promoting bacterial killing or introduce exogenous GSDMs engineered to target bacterial wall selectively. An alternative approach could be to induce the repair processes conferred by GSDMB-FL by enhancing its role in epithelial restitution. Indeed, expression of GSDMB and its translocation to the plasma membrane might serve as an effective strategy to promote mucosal healing, particularly in pathologies that damage the gut mucosa, such as IBD. The potential of methotrexate, an immunomodulator approved for the treatment of Crohn’s disease[176,177], in achieving such success was discussed in an editorial published in 2022 (ref.[178]).

Conclusions

In less than a decade, GSDMs have progressively taken centre stage regarding their contribution(s) to innate immune responses, particularly within mucosal surfaces at the interface between the host and external environment, such as in the gastrointestinal tract. However, many key questions remain unanswered, and further research is needed to clarify the role of GSDMs and their contributions to gastrointestinal health and disease (Box 4). Although the present Review is restricted to discussing the role of GSDMs in the luminal gastrointestinal tract, there is strong evidence also showing their contributions to the pathophysiology of hepatic[179-182] and, potentially, pancreatic[183,184] diseases.

The cellular mechanisms that activate and control GSDMs are one of the most prominent issues that should be investigated more thoroughly. A better understanding of the signals that regulate cell fate upon GSDM proteolytic activation (that is, whether they do or do not undergo lytic cell death) is greatly needed. Furthermore, it will be necessary to discover whether such signals differ qualitatively or quantitatively. As functional and mechanistic evidence indicates that GSDMs can also exert roles beyond pore formation, characterizing these processes and elucidating the specific stimuli that control them will also be essential. In this regard, a prominent line of future research should be to investigate the post-translational modifications that regulate GSDM activation and subsequent functions.

Indeed, individual GSDMs can be selectively upregulated, and their functions do not seem to overlap completely with other family members. Different GSDMs are simultaneously expressed by IECs and/or immune cells during active disease phases. In this context, specific GSDM-inducing signals will need to be identified. Additionally, it should be considered that the same stimulus might control different GSDMs in a dose-dependent manner (as described for UVB stimulation of keratinocytes that upregulate GSDMC at lower doses[61], whereas increasing irradiation activates GSDMD and GSDME-mediated pyroptosis[185,186]). Furthermore, it would be interesting to investigate whether the expression of not j ust a single GSDM but also the relative expression among different GSDMs is relevant to the biology and phenotype of GSDM-expressing cells.

Finally, although GSDMs have been traditionally described as inactive in their full-length forms, evidence for biological activities for at least some of the GSDMs (namely, GSDMB-FL and GSDMD-FL in IECs) has been uncovered. Unanswered questions remain as to whether other GSDMs also share these characteristics and which specific stimuli control the activity of their full-length and cleaved forms. Mechanisms that promote cell proliferation can, in parallel, shut down the cellular functions associated with the GSDM pyroptotic machinery, and vice versa, with the contribution of other GSDMs, aside from GSDMB, implicated in regulating the cell cycle. Indeed, the dichotomy of functions reported, specifically for GSDMB-FL versus GSDMB-NT in IECs, is a fascinating conundrum. It is conceivable that diverging pathways for GSDMB activation exist, and alterations in signals emanating from intracellular and extracellular environments are responsible for switching the momentum of epithelial GSDMB from pyroptotic to pro-restitution functions and vice versa. A deeper understanding of such mechanisms and their regulation will be paramount

in further dissecting the role of GSDMs, in general, and in leveraging this information to design future therapeutics for GSDM-dependent disease processes.

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Glossary

Apoptosis
Form of regulated cell death under the control of executioner caspases that results in the formation of intact vesicles (apoptotic bodies).

Enterovirus 71
Enterovirus with faecal-oral transmission that is the primary causative agent of ‘hand, foot and mouth disease’.

Inflammatory bowel disease
Chronic inflammatory condition with a relapsing-remitting course that affects (primarily) the gastrointestinal tract; it arises from dysregulated interactions between the host immune system and its gut microbiome, and its main clinical phenotype is represented by Crohn’s disease and ulcerative colitis.

Macroautophagy
Catabolic process characterized by the formation of intracellular vesicles (‘autophagosomes’) and aiming at the degradation of cellular components through the lysosomal system.

Mitophagy
Selective form of autophagy targeting damaged mitochondria.

Necroptosis
Form of regulated cell death mediated by mixed lineage kinase domain-like pseudokinase pores, whose activation depends on RIPK3 (and RIPK1).

NETosis
Form of regulated cell death restricted to neutrophils that results in the extrusion of neutrophil extracellular traps (NETs).

Pexophagy
Removal of damaged peroxisomes via autophagy.

Pyroptosis
Form of regulated cell death that depends on the formation of GSDM pores on the cell plasma membrane; it is often (but not always) a consequence of inflammatory caspase activation, and is usually associated with the release of inflammatory mediators into the extracellular space.

Receptosome
Intracellular vesicle that is formed via concentrative, receptor-mediated endocytosis, possibly with the ligand-receptor complex still bound to the membrane.

Regulated cell death
Form of cell death dependent on the activation of specific signal transducers and terminal effectors; it is susceptible to chemical and/or pharmacological modulations (including both apoptosis and a form of inflammatory cell death).

***Salmonella enterica* subsp. *enterica* serovar Typhimurium**
Facultative intracellular pathogen that causes food-borne infectious colitis in mice; it is used as a model of human typhoid fever (caused by *S.* Typhi).

Secondary necrosis
Event following end-stage apoptosis in vitro or in vivo when phagocytic clearance of apoptotic bodies fails resulting in plasma membrane rupture and release of cytoplasmic contents.

Yersiniosis
Food-borne gastrointestinal infection with pathogens localizing to intestinal lymphoid tissues and mesenteric lymph nodes: it is clinically characterized by abdominal pain and diarrhoea and is usually caused by *Yersinia enterocolitica*; *Y. pseudotuberculosis* is commonly used in mouse models of yersiniosis but rarely causes yersiniosis in humans.

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Box 1

Structure of gasdermins

Each gasdermin (GSDM) presents with highly conserved amino-terminal (NT) and carboxy-terminal domains[16], connected through a linker region unique to each GSDM[195], except for pejvakin (PJVK), whose NT domain is directly connected to a shorter C-terminal domain[16]. The C-terminal domain is almost exclusively composed of α-helices to assume a globular conformation, and it is thought to be responsible for the inhibited state of full-length GSDMs by completely masking the NT hydrophobic pocket that binds lipids[16]. Three interface regions have been identified in GSDMA3, involving the α1 and α4 helices (both crucial in lipid binding) and the β1–β2 loop on the NT domain[187]. Once inhibition of the C-terminal domain is released, the NT domain binds to the negatively charged lipids of the plasma membrane and undergoes extensive conformational changes, forms homo-oligomers and inserts within the membrane[188]. The release of inhibitory states is usually achieved via proteolytic cleavage in the linker region to allow dissociation of the C-terminal domain. However, some mutations affecting the sequence of the C-terminal domain can abolish its inhibitory function and allow full-length GSDM to form pores[16]. Notably, in the absence of lipid membranes, the C-terminal and NT domains remain associated even when the linker region is cleaved[16]. The β3–β4–β5 and β7–α4–β8 regions of GSDMA3-NT form transmembrane β-hairpins. Each GSDMA3-NT subunit contributes four β-strands to the formation of the pore; 27 NT fragments comprise the entire pore, characterized by a β-barrel transmembrane region and a globular cytosolic rim, and whose inner diameter measures 18 nm[187]. For GSDMD, the β1–β2 loop mediates lipid binding, and a dominant 33-fold symmetry has been reported with an inner diameter of 21.5nm[189]. Evidence suggests that GSDMD pore structure is dynamic, alternating between open and closed states and regulating pore size[51].

Box 2

The inflammasome and pyroptosis

Inflammasomes are multimeric cytosolic complexes expressed in immune and non-immune cells; they assemble in response to pathogen-associated and damage-associated molecular patterns, which are recognized by intracellular sensors (reviewed elsewhere[196]), and orchestrate several cell responses. These sensors interact (either directly or via an adaptor protein) with pro-caspase 1 (ref.[196]). In the canonical pathway, pro-caspase 1 undergoes dimerization and auto-proteolysis, generating active caspase 1 (ref.[197]), which serves as a protease for several cytosolic molecules, including full-length GSDMs[12,198], pro-IL-1β and pro-IL-18 (ref.[199]). GSDMD–amino-terminal (GSDMD-NT) assembles into membrane-inserted pores enabling the secretion of inflammatory mediators[28], and it can eventually determine an alteration of the osmotic gradient across the plasma membrane, ultimately leading to membrane disruption and pyroptotic cell lysis. Lactate dehydrogenase, being a higher molecular weight protein, cannot pass through GSDM pores, so its presence in the extracellular space is considered a marker of complete membrane rupture and cell death. In the non-canonical pathway, caspases 4 and 5 (caspase 11 in mice) are directly activated by lipopolysaccharides. They form dimers that process the maturation of GSDMD-NT, after which GSDMD pores enable the influx of K^+ ions, which activates the NLRP3 inflammasome[10,11,200]. The currently accepted definition of pyroptosis is: “a form of regulated cell death that critically depends on the formation of plasma membrane pores by members of the gasdermin protein family, often (but not always) as a consequence of inflammatory caspase activation”[14].

The morphological features of pyroptosis in vitro are represented by: (1) chromatin condensation with an intact nucleus, (2) mild cell swelling, (3) membrane blebbing and formation of pyroptotic bodies, and (4) osmotic lysis disrupting membrane integrity[201]. Research has shown that GSDM pores can also participate in other forms of regulated cell death (such as secondary necrosis), highlighting that active crosstalk exists between different cell death pathways[202].

Box 3

The immune tumour microenvironment – overview and insights for the potential role of GSDMs

A solid tumour consists of two main components: neoplastic cells and the tumour microenvironment (TME), which exerts a critical role in carcinogenesis. The TME comprises immune cells, stromal cells, blood vessels, extracellular matrix and extracellular vesicles. Both innate and adaptive immunity have paramount roles in the TME, as they can either promote or suppress tumour growth, depending on the specific cell population residing in the TME and their activation pathways[203]. The TME usually polarizes monocytes towards an M2 phenotype, which mediates immunosuppressive functions, thereby promoting carcinogenesis[204], whereas M1 macrophages have antitumour functions[205]. Dendritic cells typically present neoplastic antigens to trigger antitumour responses, but they can also be co-opted to promote immunosuppression[206]. Among tumour-infiltrating lymphocytes, regulatory T (T_{reg}) cells dampen immune responses and support tumour growth[207], whereas $CD4^+$ T helper (T_H) cells[208], $CD8^+$ cytotoxic T (CTL) cells[209] and natural killer cells[210] are responsible for antitumour functions. Specifically, CTL cells serve as sentinels that detect tumour antigens and kill neoplastic cells. Accessory signals are also required for $CD8^+$ T cells to attack neoplastic clones; conversely, other signals — such as those involving the immune checkpoints PD1 and CTLA-4 — can induce the ‘exhaustion’ of $CD8^+$ T cells[211]. Gasdermin B (GSDMB) and GSDME have been identified as effectors of CTL-induced cell death in neoplastic epithelial cells[70,96]. Overall, bidirectional crosstalk exists between neoplastic cells and the TME. Tumour cells attempt to promote a ‘permissive’ environment with immunosuppressive functions, whereas the TME can either support carcinogenesis or be activated to exert immunosurveillance functions that restrict tumour growth. The balance between the two determines whether a malignancy develops and progresses, and the modulation of these interactions surely represents an intriguing target for antineoplastic therapies.

Box 4

Future directions and outstanding questions

Current evidence regarding the characterization and function(s) of gasdermins (GSDMs) has shed light upon the main roles of this intriguing family of proteins, particularly in the gastrointestinal tract; however, it has also opened the door to several new questions. Conflicting results concerning the contribution and effects of GSDM activity in specific diseases should be framed following these general observations: (1) the formation of GSDM pores does not necessarily result in pyroptotic cell death, (2) GSDM-driven inflammation can promote different effects, depending on its duration and the specific cell population(s) undergoing pyroptosis (that is, immune or non-immune cells), and (3) some GSDMs also possess functions beyond pyroptosis and pore formation, particularly in their full-length forms. These considerations suggest that a more systematic approach should be taken when investigating GSDMs, accounting for several aspects of their inherent biology (Fig. 1). Three main areas of research are warranted for future investigation of GSDMs in the gastrointestinal tract:

- Basic science:
  - Relative expression of different GSDMs in the same tissues and/or cells
  - Non-lytic and pore-independent functions of GSDMs
  - Specificity of activation pathways in terms of which GSDM is upregulated or downregulated and which GSDM-dependent function is controlled
  - GSDM interactions/cooperation with other GSDM family members and/or with partner proteins
- Translational studies:
  - Functional phenotype(s) depending on pyroptotic and non-pyroptotic functions of GSDMs
  - Functional phenotype(s) depending on GSDMs expressed by immune and non-immune cells
  - Functional phenotype(s) depending on GSDMs with time-restricted and anatomically restricted activities and on GSDMs with uncontrolled activity
- Clinical research:
  - Potential use of GSDMs as diagnostic and prognostic and/or predictive biomarkers: gene expression in cancer, and the consideration of measuring ratios of proteins (amino-terminal and full-length forms; N-terminal to full-length ratio)

- Potential use of GSDMs as therapeutic targets: pharmacological modulation of expression and activity, the introduction of exogenous GSDMs, and synergy with other therapies (for example, checkpoint inhibitors)

Key points

- The gasdermin (GSDM) family of lipid-binding proteins is involved in several biological processes, with its five members (GSDMA to GSDME) serving as major factors during gastrointestinal health and disease.
- GSDMs are primarily known as mediators of pyroptosis; however, evidence supports other roles, including the non-lytic release of inflammatory cytokines, regulation of vital cell functions and targeted bactericidal effects.
- The contribution of each GSDM during gastrointestinal health and disease is unequivocal, albeit ambiguous, with reports of both promotion of and protection from gastrointestinal cancers, infections and immune-mediated disorders.
- Preliminary evidence suggests potential applications for GSDMs in clinical gastrointestinal practice, with future investigation warranted to leverage their use as predictive or prognostic biomarkers and/or specific therapeutic targets.
- Knowledge gaps and controversies exist in GSDM biology regarding their pyroptotic and non-pyroptotic functions and associated signalling pathways, the biological activities of full-length forms, and their roles in immune and non-immune cells.

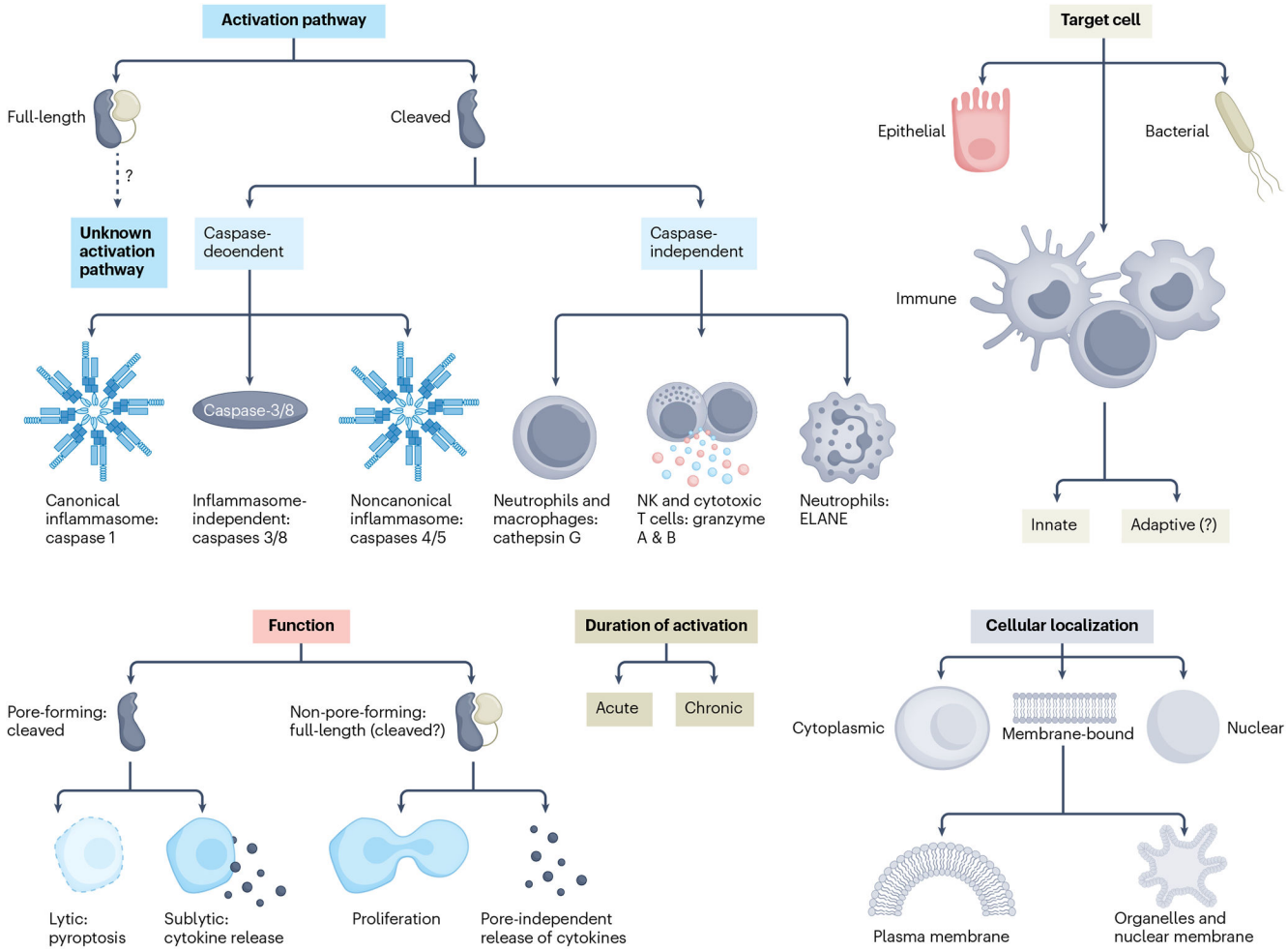

Fig. 1 |. Important features of gasdermins.

Schematic representation of the most important features of gasdermins (GSDMs). The activation pathways of full-length GSDMs are not fully understood. Two major pathways have been identified for cleaved GSDMs: caspase-dependent (with or without the involvement of the inflammasome) and caspase-independent (requiring different proteases). GSDMs can target either human cells (immune and non-immune) and/or bacterial cells. GSDM functions can be schematically classified as pore-forming (that is, pyroptosis and cytokine release) and non-pore-forming (including cell proliferation and pore-independent cytokine release). GSDM activation can occur acutely (resulting in either pyroptosis or definitive membrane repair) or chronically (with continuous pore formation counterbalanced by repair mechanisms). Within eukaryotic cells, GSDMs can be found in different locations: intracytoplasmic or nuclear, or bound to lipid membranes (that is, plasma or organelle membranes). ELANE, neutrophil elastase; NK, natural killer.

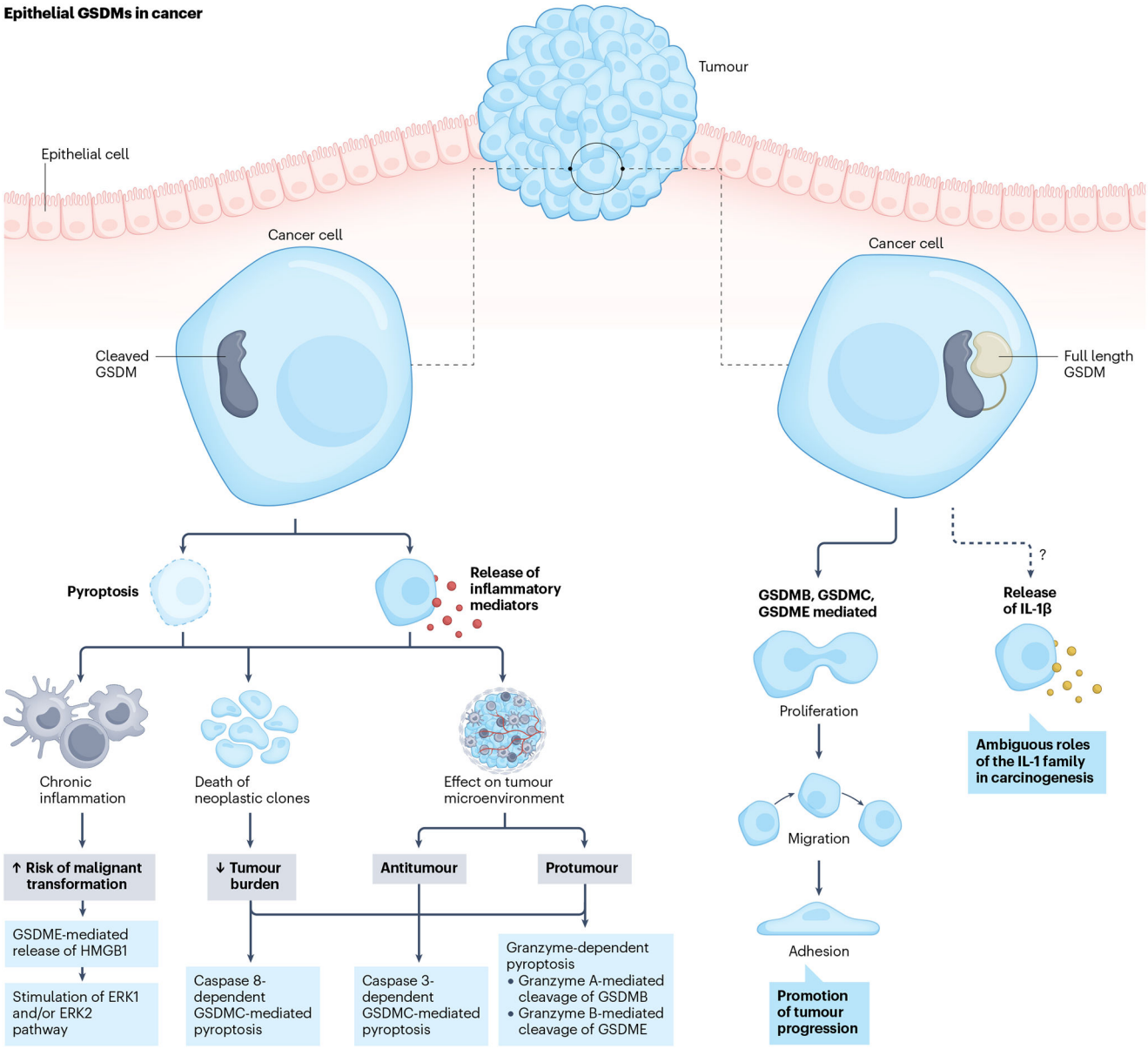

Fig. 2 |. Role and contribution of epithelial-derived gasdermins in gastrointestinal cancers. Cleaved gasdermins (GSDMs) contribute to gastrointestinal carcinogenesis by mediating pyroptotic cell death and/or sublytic release of inflammatory mediators. Chronic intestinal inflammation, secondary to GSDM-dependent release of intracellular mediators, is a pro-carcinogenic stimulus that perpetuates tumorigenesis. GSDM-mediated pyroptosis of cancer cells can exert a direct antitumour effect by killing neoplastic clones, thereby reducing the tumour burden. Finally, the GSDM-dependent release of intracellular mediators from neoplastic cells shapes the tumour microenvironment to promote either antitumour or pro-tumour functions in relation to the specific leukocyte subpopulations that are recruited and activated. Full-length GSDMs can also affect the pathogenesis of cancer. GSDMB, GSDMC and GSDME are involved in the proliferation, migration and adhesion of intestinal epithelial cells, all of which are critical components for the development and growth of malignancies.

Lastly, as IL-1β has been reported to play a role in the pathogenesis of gastrointestinal cancer, it is possible that the specific form of IL-1β release (and potentially, of other cytokines belonging to the IL-1 superfamily) mediated by GSDMs might also contribute to carcinogenesis. HMGB1, high-mobility group box protein 1.

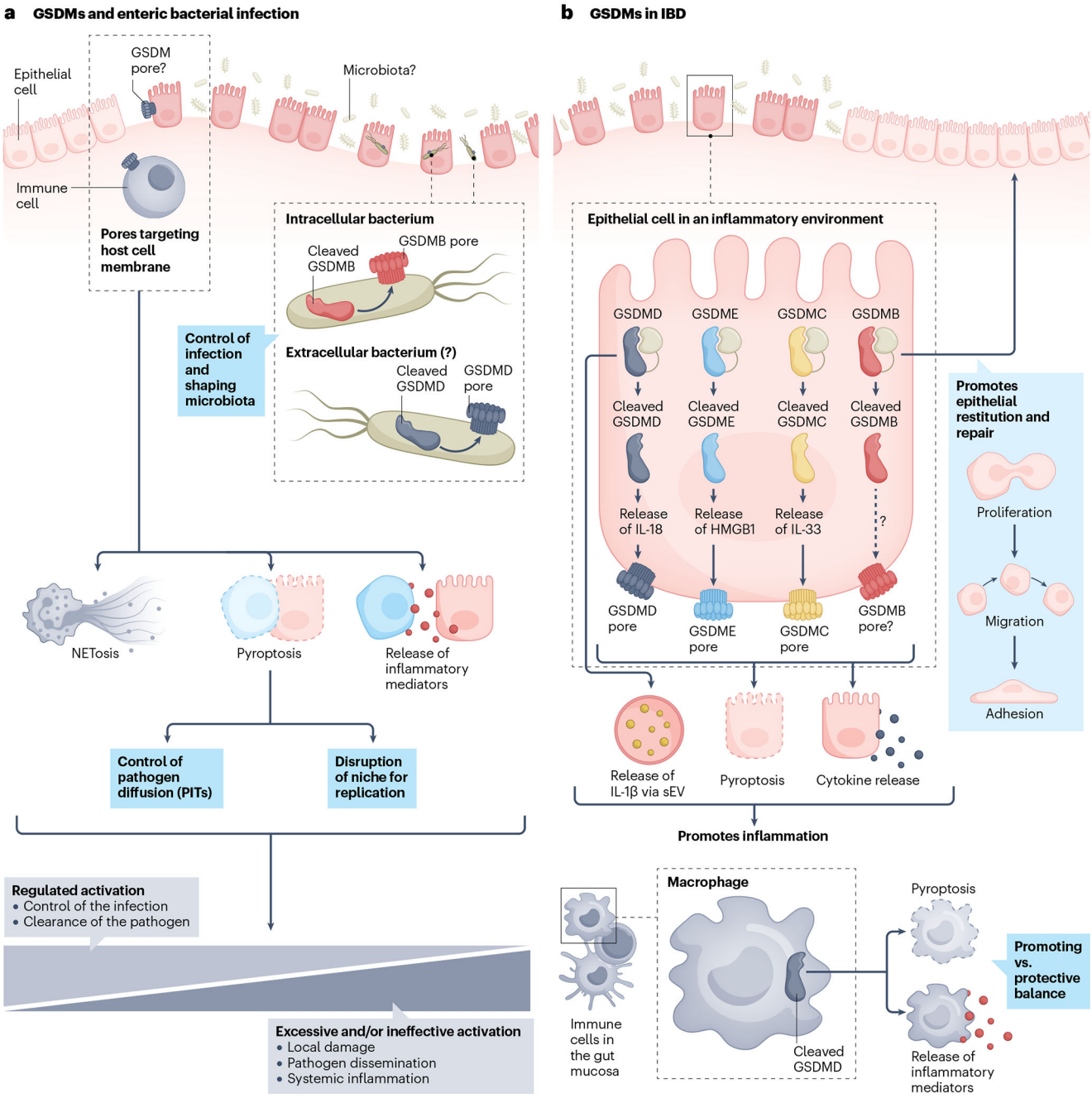

Fig. 3 | Role and contribution of gasdermins in gastrointestinal inflammation.

a, Gasdermins (GSDMs) facilitate host–bacteria interactions within the gut. GSDMD-amino-terminal (GSDMD-NT) mediates NETosis, through which neutrophils extrude neutrophil extracellular traps to confine extracellular bacteria. Pyroptosis of cells infected with intracellular bacteria disrupts the niche for their replication. Furthermore, when undergoing GSDMD-mediated pyroptosis, infected macrophages can form pore-induced intracellular traps (PITs), which further help to restrain intracellular pathogens. Finally, the GSDM-dependent release of mediators is involved in eliciting and regulating local and potentially systemic inflammatory responses associated with infections. GSDM pore

formation is a tightly regulated process during infection. Controlled activation can support battling an infection, whereas excessive activation can have detrimental effects. GSDM pores can also target bacterial walls, as described for GSDMB and GSDMD, with intracellular and extracellular pathogens, respectively. **b**, During the course of inflammatory bowel disease (IBD), GSDMs expressed in both epithelial and immune cells are pivotal in regulating inflammation. In intestinal epithelial cells (IECs), full-length (FL) GSDMB promotes epithelial repair. Conversely, GSDMD-FL mediates the release of IL-1β that fuels intestinal inflammation. Furthermore, GSDM-dependent pyroptosis of IECs is pathogenic in IBD, causing loss of mucosal integrity (by killing epithelial cells) and mediating the release of inflammatory mediators. Macrophage-derived cleaved GSDMD has an ambiguous role, described as both promoting and protecting from colitis. HMGB1, high-mobility group box protein 1; sEVs, small extracellular vesicles.

Table 1 |

General biology of the gasdermin family

GSDMs (aliases) and location on chromosomes (human and mouse)	Structure	Lipid binding capacity	Cellular localization	Proteolytic modulator(s)	Signalling pathways	Biological functions	Refs.
GSDMA (*GSDM1, FKSG9*) chr17q21.1 *Gsdma1–3* chr11D	NT and CT domains connected through a linker region	Cardiolipin, phosphatidic acid, phosphatidylserine, phosphoinositide	Plasma membrane; cytoplasm; mitochondria	SpeB; unknown human activator?	Upregulated by TGFβ1-LMO1, TNF Interactions with Hsp90–Hsp70–Hop, Trap1–mtHsp75 complexes (GSDMA3)[a]	Pyroptosis Mitochondrial homeostasis Autophagy	8,9,16,24,62,64-66,187,188
GSDMB (*GSDML*) chr17q21.1	NT and CT domains connected through a linker region	For both NT and FL: phosphatidic acid, phosphatidylglycerol sulfatide, phosphoinositide	Plasma membrane; cytoplasm; nucleus	GZMA; caspase 1 (activation, uncertain); caspases 1, 3, 6, 7 (inactivation, uncertain); caspases 4, 9 (unknown effect)	Upregulated by IFNγ (FL, cytoplasmic); upregulated by methotrexate (FL localized to plasma membrane) Upregulates transcription of TGFβ1, 5-LOX and MMP-9 (isoform 1, nuclear) FAK phosphorylation (downstream effector of GSDMB-FL) Interacts with caspase 4 (FL)	Pyroptosis (NT domain) Adjuvant in GSDMD pyroptosis (FL) Cell proliferation, migration, adhesion (FL) Bactericidal (NT domain) Promotes tissue remodelling	5,9,16,38,45,68-70,74,189
GSDMC (*MLZE*) chr8q24.21 *Gsdmc1–4* chr15D1	NT and CT domains connected through a linker region	Uncharacterized	Plasma membrane	Caspase 8	Downregulated by TGFβ[a] Upregulated by STAT3-PDL1 Upregulated by STAT6 (Gsdmc2–4), IL-4, IL-13 (GSDMC2–4)[a] ERK and JNK pathways (downstream effectors)	Pyroptosis Control of cell cycle?	9,16,32,75,76,190

GSDMs (aliases) and location on chromosomes (human and mouse)	Structure	Lipid binding capacity	Cellular localization	Proteolytic modulator(s)	Signalling pathways	Biological functions	Refs.
GSDMD (*GSDMDC1, FNA5L, FKSG10*) chr8q24.3 *Gsdmd* chr15D3	NT and CT domains connected through linker region	Cardiolipin, phosphatidic acid, phosphoinositide	Plasma membrane; cytoplasm; nuclear membrane; mitochondria; neutrophilic granules; autophagosome	Canonical inflammasome (caspase 1); non-canonical inflammasome (caspases 4 and 5); RIPK1–FADD–caspase 8; caspase 3; ELANE; cathepsin G	IRF1 and IRF2 (transcription regulation)[a] Forms complex with Hsp90–Cdc73–NEDD4–caspase 8[a]	Pyroptosis NETosis; mitophagy Sublytic release of inflammatory mediators Release of inflammatory mediators in microvesicles (FL)[a] Bactericidal effect?	9-12,16,21-23,25,28,33,3,39,51,84,86,91-93,99,186,191-194
DFNA5 or *GSDME* (*ICERE-1*) chr7p15.3 *Dfna5* or *Gsdme* chr6B2.3	NT and CT domains connected through a linker region	Cardiolipin, phosphatidylserine, phosphoinositide	Plasma membrane; cytoplasm; mitochondria	Caspase 3 (and caspase 8); GZMB	IRF1 (transcription regulation)[a] TNF stimulates caspase 3-mediated pyroptosis[a]	Pyroptosis Sublytic release of inflammatory mediators Autophagy	36,94-96,98,99,106,116,131
DFNB59 or *PJVK* (*GSDMF?*) chr2q31.2 *Pjvk* or *Dfbn59* chr2.3	NT, shorter CT, no linker region	Uncharacterized	Peroxisomes	Unknown, not required?	Unknown	Pexophagy	15,16,109,110,195

All data refer to human GSDMs, unless otherwise specified. Cdc, cell division cycle; CT, carboxy-terminal; DFNA5, deafness autosomal dominant 5; FADD, FAS-associated death domain protein; ELANE, neutrophil elastase; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; FL, full-length; GSDM, gasdermin; GZMA, granzyme A; GZMB, granzyme B; Hop, Hsp70/Hsp90 organizing protein; Hsp, heat-shock protein; IFNγ, interferon-γ; IRF, interferon regulatory factor; JNK, c-Jun amino-terminal kinase; LMO1, LIM domain only 1; MMP-9, matrix metallopeptidase 9; mtHsp75, mitochondrial Hsp 75; NEDD, neural precursor cell-expressed developmentally downregulated gene; NT, amino-terminal; PJVK, pejvakin; RIPK1, receptor-interacting serine–threonine protein kinase 1; SpeB, streptococcal pyrogenic exotoxin B; STAT, signal transducer and activator of transcription; TGFβ1, transforming growth factor-β1; TNF, tumour necrosis factor; Trap1, TNF receptor associated protein 1; 5-LOX, 5-lipoxygenase.

[a]Evidence only available in mice.

Table 2 |

Summary of the functions of gasdermins in gastrointestinal diseases

GSDM	Cancer	Infectious diseases	Immune-mediated diseases	Refs.
GSDMA	Silencing associated with gastric cancer Promotes TGFβ-associated death of neoplastic cells?	Unknown — role described for *Streptococcus pyogenes* infection in keratinocytes	SNPs associated with susceptibility to IBD	1,8,9,63,65,196
GSDMB	GZMA-dependent clearance of neoplastic cells Potential role for GMZA-dependent pyroptosis in boosting antitumour immunity	Bactericidal effect against intracellular pathogens	SNPs associated with susceptibility to IBD Promotes epithelial restitution (GSDMB-FL) Potential pro-inflammatory role with GMZA-dependent pyroptosis	1,8,9,38,63,69,70,74,196
GSDMC	Increased expression in CRC Promotes growth of CRC cells when TGFβ control fails[a] Potential role as oncosuppressor in oesophageal squamous cell carcinoma Can switch apoptosis to pyroptosis in neoplastic cells (caspase 8-dependent cleavage)	Potential role in helminth infection[a]	Potential role in type 2 immune responses[a]	1,8,9,32,75-78
GSDMD	Downregulation promotes cell proliferation in gastric cancer cell lines	Pyroptosis of macrophages and IECs infected by intracellular bacteria (protective or pathogenic, depending on the model)[a] NET formation (response against intracellular and extracellular pathogens) PIT formation (response against intracellular pathogens) Potential direct bactericidal effect against extracellular pathogens Potential role in shaping gut microbiome[a] Response against parasites (macrophage hyperactivation)[a] Pyroptosis of macrophages and IECs infected by virus (protective or pathogenic, depending on the model)[a]	Controversial role (promoting or protective) in colitis[a] Mediates IL-1β and IL-18 release from IECs[a] Mediates cytokine release and pyroptosis in macrophages	1,8,9,20,22,30,39,82,85,88,90-93,125,134-136,138,139,150,191
GSDME	Silencing and loss-of-function mutations associated with CRC development Increased expression in oesophageal cancer compared to expression in adjacent tissues Caspase 3 and GZMB-dependent clearance of neoplastic cells GZMB-dependent pyroptosis boosts antitumour immunity	Unknown	Promotes intestinal inflammation via HMGB1 release[a] TNF-induced caspase 3-dependent pyroptosis in IECs[a]	18,36,59,95,96,100-102,105,106

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GSDM	Cancer	Infectious diseases	Immune-mediated diseases	Refs.
	Promotes the development of colitis-associated cancer via HMGB1 release[a]			

All data refer to human GSDMs, unless otherwise specified. CRC, colorectal cancer; FL, full-length; GSDM, gasdermin; GZMA, granzyme A; GZMB, granzyme B; HMGB1, high-mobility group box 1; IBD, inflammatory bowel disease; IECs, intestinal epithelial cells; NET, neutrophil extracellular trap; PIT, pore-induced intracellular traps; SNPs, single nucleotide polymorphisms; TGFβ, transforming growth factor-β; TNF, tumour necrosis factor.

[a]Evidence only available in mice.